Methods and issues in cheese authenticity studies: a workshop.

L'autenticità dei formaggi: dagli approcci analitici alle politiche per la tutela di consumatori e produttori

3/9/2009 – 5/9/2009

Hotel de la Ville
Avellino

Proceedings
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Atti

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Foreword

Authenticity and traceability are key issues for the protection of traditional food products, and EU and member states have developed quality branding schemes and specific regulations to this end. Recent scandals connected to frauds related to origin, authenticity, safety, and labelling of some PDO and non-PDO cheeses have weakened consumers' confidence in quality branding schemes and caused severe economical damage to producers. The objective of this meeting is to present to researchers, public quality control agencies, and cheese maker organizations, an overview of analytical, economic and technical issues on cheese authenticity and traceability.

Introduzione

L'autenticità e la rintracciabilità sono due aspetti chiave nella produzione degli alimenti tradizionali e la UE e gli stati membri hanno adottato sistemi di marchi e regolamenti per tutelare i prodotti tipici. I recenti scandali legati a frodi relative all'origine dichiarata, all'autenticità, alla sicurezza e all'etichettatura di alcuni formaggi, DOP e non, hanno minato la fiducia dei consumatori nei marchi a tutela della qualità e causato danni significativi ai produttori. L'obiettivo di questo convegno è presentare a ricercatori e tecnici impegnati nella tutela e promozione della qualità dei formaggi una visione complessiva dei problemi analitici, tecnici, economici e legislativi sull'autenticità e rintracciabilità dei formaggi.

Programme

Thursday, September 3rd, 2009

14.00 Registration
Poster set-up
Opening session. "Cheese and honey: romance for the senses".
Welcome addresses:
16.00 Prof. R. Coppola, Director, ISA-CNR, Avellino. Sen. C. Sibilia, Presidente Provincia di Avellino, Dr. G. Nappi, Assessore all'Agricoltura, Regione Campania.
Opening lecture:
16.15 Classical and innovative process markers in honey. - Prof. E. Marconi, DISTAAM, Università degli Studi del Molise, Campobasso
Guided tasting session: Dr. M. Sarnataro, ONAF
16.30 The pasta filata cheeses of Campania meet honeys of Irpinia
18.00 Pecorino: terroir, autochthonous breeds and honeys
19.00 Minor cheese productions from Campania. A rediscovery of ancient cheeses paired with honeys from Irpinia
20.30 Welcome party

Friday, September 4th, 2009

Session 1. Invited speakers

08.30 Registration
Welcome addresses:
09.30 Prof. R. Coppola, Director, ISA-CNR, Prof. E. Parente, Dip. Biologia DBAF, Università degli Studi della Basilicata, Dr. A. Bertani, Dip. Agrolimentare CNR.
Invited speakers:
10.00 Factors that affect the quality of cheese - Prof. P.F. Fox, University College Cork, Cork (p 17)
10.30 Classification and diversity of cheese - Prof. P. McSweeney, Dept. of Food and Nutritional Sciences, University College Cork, Cork (p 18)
11.00 Coffee break and poster session
11.30 Protein and peptide composition analysed by CE, HPLC and LC-MS - Prof. Y. Ardö, Dept. of Food Science, University of Copenhagen (p 19)
12.00 Microbial fingerprinting and authenticity of cheese - Dr. D. Ercolini, Dip. Scienza degli Alimenti, Università degli Studi di Napoli "Federico II", Napoli (p 20)
12.30 Spectroscopic methods for cheese quality and authenticity - Prof. L. Mannina[1], M. Delfini[2], D. Capitani[3], M. E. Di Cocco[2], F. Sciubba[2] E. Brosio [2], R. Gianferri[2]. [1]Dipartimento STAAM, Università degli Studi del

Molise, Campobasso. [2]Dipartimento di Chimica, Università "La Sapienza" di Roma. [3]Istituto di Metodologie Chimiche, CNR, Roma (p 21)

13.00 **Discussion**
13.30 **Lunch**
15.00 Immunochemical methods for cheese authenticity studies – Dr. R. Pizzano[1], Dr. M. A. Nicolai[2], Dr. C. Manzo[1], Prof. F. Addeo[12], [1]ISA-CNR, Avellino; [2] Dip. Scienza degli Alimenti, Università degli Studi di Napoli "Federico II", Napoli (p 22)
15.30 Small molecules for the evaluation of cheese authenticity - Dr. M.C. Messia, DISTAAM, Università degli Studi del Molise, Campobasso (p 23)
16.00 **Coffee break and poster session**
17.00 Statistical methods for cheese authenticity studies – Prof. E. Parente, Dr. A. Ricciardi, Dr. T. Zotta, Dip. Biologia, Università degli Studi della Basilicata, Potenza (p 24)
17.30 The role of the Italian Central Inspectorate for Food Quality in the protection of food authenticity - Dr. F. Fuselli, Ispettorato Centrale per il Controllo della Qualità dei Prodotti Agroalimentari, Roma (p 26)
18.00 **General discussion and conclusions.**

Saturday, September 5th, 2009

Session 2. Oral communications

9.00 Traceability of artisan raw milk cheeses of the Marche region: a preliminary approach. V. Babini, S. Santarelli, L. Aquilanti, A. Osimani, C. Garofalo, G. Silvestri, E. Zannini, F. Clementi. SAIFET, Università Politecnica delle Marche, Ancona (p 28)
9.10 Branched-chain fatty acids: a potential parameter for assessing authenticity of PDO mountain cheeses. G. Battelli[1], I. De Noni[2]. [1]Istituto di Scienze delle Produzioni Alimentari del Consiglio Nazionale delle Ricerche (ISPA-CNR) Milano, [2]DiSTAM, Università degli Studi di Milano, Milano (p 29)
9.20 Analytical traceability of Parmigiano Reggiano cheese. F. Camin[1], R. Larcher[1], D. Bertoldi[1], L. Bontempo[1], L. Ziller[1], G. Nicolini[1], M. Nocetti[2]. [1]FEM-IASMA, San Michele all'Adige (TN), [2]Consorzio Formaggio Parmigiano-Reggiano, Reggio Emilia (p 30)
9.30 Non volatile hydrocarbons: promising tools for dairy products traceability. G. Contarini, M. Povolo, V. Pelizzola, CRA-Centro di Ricerca per le Produzioni Foraggere e Lattiero-Casearie, Lodi (p 31)

9.40 Proteolytic oligopeptides as molecular markers for cheese authenticity. A. Dossena, V. Cavatorta, S. Sforza, G. Galaverna, R. Marchelli, Dip. di Chimica Organica e Industriale, Università degli Studi di Parma, Parma (p 32)

9.50 Evaluation of dairy products' authenticity by aromatic and nutritional compounds. V. Fedele, S. Claps, L. Sepe, A. R. Caputo, M. A. Di Napoli. CRA-ZOE Unità di Ricerca per la Zootecnia Estensiva, Bella (PZ) (p 33)

10.00 Sensory conformity of Asiago cheese PDO. A. Marangon, M. Cappellari, Veneto Agricoltura - Istituto per la Qualità e le Tecnologie Agro-alimentari, Thiene (VI) (p 34)

10.10 Pyroglutamyl-γ3-casein as marker of proteolysis and ripening period of Grana Padano and Parmigiano Reggiano cheeses. F. Masotti, J. Hogenboom, I. De Noni, L. Pellegrino. DiSTAM, Università degli Studi di Milano, Milano (p 35)

10.20 Microbiological and chemical characterization of Fruhe cheese: a new dairy product eligible for a PDO certification. M.A. Murgia, N.P. Mangia, G. Garau, F. Fancello, P. Deiana. DISAABA – Università degli Studi di Sassari, Sassari (p 36)

10.30 Discussion

11.00 Coffee break

11.30 Round table: Cheese authenticity and traceability: technical, economical, sociological and legislative aspects (in Italian).

Coordinated by:
Ms. M. Borea, RAI

Invited speakers:
Sen. C. Sibilia, Presidente Provincia di Avellino
Prof. R. Coppola, ISA-CNR Avellino
Dr. A. Tartaglia, Regione Campania
Dr. L. Perozzi, Segretario generale CCIAA Avellino
Prof. G. Cannata, Magnifico Rettore Università degli Studi del Molise
Prof. A. Di Porto, Università degli Studi di Roma "La Sapienza"
Dr. M. Ferraro, Dirigente Coldiretti, Roma
Dr. M. Zema, CSQA, Bari
Dr. V. Amendolara, Ismecert
Dr. L. Bertozzi, Consorzio di Tutela del Parmigiano Reggiano
Dr. L. Pevere, Consorzio di Tutela Montasio
Dr. B. Bartemucci, Ispettorato Centrale Controllo Qualità degli Alimenti
Dr. R. Rubino, ANFOSC
Dr. M. Sarnataro, ONAF
Dr. A. Limoni, Commissario Istituto Zooprofilattico per il Mezzogiorno
Dr. V. D'Amato, Presidente Ordine dei Veterinari di Avellino

Posters

1. Identification and characterization of yeast strains isolated from samples of Water Buffalo Mozzarella cheese and natural whey cultures. M. Aponte, O. Pepe, G. Blaiotta. Dip. Scienza degli Alimenti, Università degli Studi di Napoli Federico II, Portici (NA) (p 38)
2. Genotypic and technological diversity of *Leuconostoc mesenteroides*, *Lactobacillus paracasei* and *Brevibacterium linens* strains isolated from the artisanal 'Pecorino di Filiano' cheese. M.G. Bonomo, M. A. Sico, G. Salzano. Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali, Università degli Studi della Basilicata, Potenza (p 39)
3. Use of colorimetric methods for the analysis of the type of rennet used in traditional cheese production. K. Carbone, B. Ferri, D. Settineri. Centro di ricerca per la produzione delle carni e il miglioramento genetico (CRA-PCM), Monterotondo, Roma (p 40)
4. The lactic microflora isolated from traditional, raw milk Italian cheeses: a tool to define cheese authenticity? D. Carminati, B. Bonvini, M.E. Formasari, A. Perrone, M. Remagni, L. Rossetti, M. Zago, G. Giraffa. Centro di ricerca per le produzioni foraggere e lattiero-casearie (CRA-FLC), Lodi (p 41)
5. Diversity of Caciocavallo cheese produced in different regions of Southern Italy. T. Di Renzo, M.L. Grasso, S. Niro, A. Reale, P. Tremonte, G. Panfili, E. Sorrentino, R. Coppola, M. Succi. Dipartimento di Scienze e Tecnologie Agro-alimentari, Ambientali e Microbiologiche, Università degli Studi del Molise, Campobasso (p 42)
6. The flavour profile of Mozzarella cheese: natural vs. defined starter cultures. T. Di Renzo[1], A. Di Luccia[2], M. Succi[1], A. Reale[1], P. Tremonte[1], A. Trani[2], V. Capilongo[1], R. Coppola[1], E. Sorrentino[1]. [1]Dipartimento di Scienze e Tecnologie Agro-alimentari, Ambientali e Microbiologiche, Università degli Studi del Molise, Campobasso. [2]Dipartimento di Progettazione e Gestione dei Sistemi Agro-zootecnici e forestali (PROGESA), Università degli Studi di Bari, Bari (p 43)
7. ELISA for monitoring the freshness of river buffalo curd. R. Gagliardi[1], Y.T.R. Proroga[1], A. Barbato[1], A. Trani[2], M. Faccia[2], A. Di Luccia[2]. [1]Dipartimento Ispezione degli Alimenti Istituto Zooprofilattico del Mezzogiorno, Portici (NA). [2]Dipartimento di Progettazione e Gestione dei Sistemi Agro-zootecnici e forestali (PROGESA), Università degli Studi di Bari, Bari (p 44)
8. Biochemical characterization of "Laticauda" ovine hard cheese to value and to safeguard a traditional dairy product of "Sannita" bio-territory. D. Matassino[1], N. Castellano[1], G. Gigante[1], F. Inglese[1], L. Rillo[1], F. Romagnuolo[1], A. Trani[2], G. Varricchio[1], A. Di Luccia[2]. [1]ConSDABI. NFP.-FAO- Center of Omic Science for the Nutritional Quality and Excellence - Piano Cappelle Benevento. [2]PROGESA - Università degli Studi di Bari, Bari (p 45)

Programma

Giovedì, 3 Settembre, 2009

14.00 **Registrazione**
Consegna dei poster
Sessione introduttiva. "Formaggio e miele: corrispondenza di odorosi sensi".
Inaugurazione dei lavori congressuali
16.00 Prof. R. Coppola, Direttore, ISA-CNR, Avellino. Sen. C. Sibilia, Presidente Provincia di Avellino, Dr. G. Nappi, Assessore all'Agricoltura, Regione Campania.
Intervento introduttivo
16.15 Marcatori di qualità tradizionali e innovativi per il miele – Prof. E. Marconi, DISTAAM, Università degli Studi del Molise, Campobasso
Degustazione guidata. A cura di Dr. M. Sarnataro, ONAF
16.30 Le paste filate dell'Appennino Campano incontrano i mieli della provincia di Avellino
18.00 Pecorino, territorio, razze autoctone campane e mieli
19.00 Le produzioni "minori" della Campania. Gusti di antichi formaggi ritrovati, proposti in abbinamento a mieli irpini
20.30 **Party di benvenuto**.

Venerdì, 4 Settembre 4, 2009

Sessione 1. Relazioni invitate

08.30 **Registrazione**
Interventi introduttivi
09.30 Prof. R. Coppola, Direttore, ISA-CNR, Prof. E. Parente, Dip. Biologia DBAF, Università degli Studi della Basilicata, Dr. A. Bertani, Direttore Dip. Agrolimentare CNR.
Relazioni invitate
10.00 I fattori che influenzano la qualità dei formaggi - Prof. P.F. Fox, University College Cork, Cork (p 17)
10.30 Classificazione e diversità dei formaggi - Prof. P. McSweeney, Dept. of Food and Nutritional Sciences, University College Cork, Cork (p 18)
11.00 **Coffee break e sessione poster**
11.30 Composizione in proteine e peptidi dei formaggi determinata mediante CE, HPLC e LC-MS - Prof. Y. Ardö, Dept. of Food Science, University of Copenhagen (p 19)
12.00 Fingerprinting delle comunità microbiche e autenticità dei formaggi - Dr. D. Ercolini, Dip. Scienza degli Alimenti, Università degli Studi di Napoli "Federico II", Napoli (p 20)

12.30 Metodi spettroscopici per la qualità e l'autenticità dei formaggi. Prof. L. Mannina[1], M. Delfini[2], D. Capitani[3], M. E. Di Cocco[2], F. Sciubba[2] E. Brosio [2], R. Gianferri[2]. [1]Dipartimento STAAM, Università degli Studi del Molise, Campobasso. [2]Dipartimento di Chimica, Università "La Sapienza" di Roma, Roma. [3]Istituto di Metodologie Chimiche, CNR, Roma (p 21)

13.00 **Discussione**

13.30 **Colazione di lavoro**

15.00 Metodi immunochimici per l'autenticità dei formaggi – Dr. R. Pizzano[1], Dr. M. A. Nicolai[2], Dr. C. Manzo[1], Prof. F. Addeo[12], [1]ISA-CNR, Avellino; [2] Dip. Scienza degli Alimenti, Università degli Studi di Napoli "Federico II", Napoli (p 22)

15.30 Profiling di piccole molecole per l'autenticità dei formaggi - Dr. M.C. Messia, DISTAAM, Università degli Studi del Molise, Campobasso (p 23)

16.00 **Coffee break e sessione poster**

17.00 Metodi statistici multivariati e autenticità dei formaggi – Prof. E. Parente, Dr. A. Ricciardi, Dr. T. Zotta, Dip. Biologia, Università degli Studi della Basilicata, Potenza (p 24)

17.30 Il ruolo dell'ICQ nella difesa del patrimonio agroalimentare italiano, con particolare riferimento al settore lattiero-caseario - Dr. F. Fuselli, Ispettorato Centrale per il Controllo della Qualità dei Prodotti Agro-alimentari, Roma (p 26)

18.00 **Discussione e conclusioni**

Sabato, 5 Settembre 2009

Sessione 2. Comunicazioni orali

9.00 Tracciabilità dei formaggi artigianali da latte crudo della regione Marche: un approccio preliminare. V. Babini, S. Santarelli, L. Aquilanti, A. Osimani, C. Garofalo, G. Silvestri, E. Zannini, F. Clementi. SAIFET, Università Politecnica delle Marche, Ancona (p 28)

9.10 Acidi grassi ramificati: un potenziale parametro per la valutazione dell'autenticità dei formaggi DOP di montagna. G. Battelli[1], I. De Noni[2]. [1]Istituto di Scienze delle Produzioni Alimentari del Consiglio Nazionale delle Ricerche (ISPA-CNR) Milano, [2]DiSTAM, Università degli Studi di Milano, Milano (p 29)

9.20 Tracciabilità analitica del Parmigiano Reggiano. F. Camin[1], R. Larcher[1], D. Bertoldi[1], L. Bontempo[1], L. Ziller[1], G. Nicolini[1], M. Nocetti[2]. [1]FEM-IASMA, San Michele all'Adige (TN), [2]Consorzio Formaggio Parmigiano-Reggiano, Reggio Emilia (p 30)

9.30 Idrocarburi non-volatili: strumenti promettenti per la tracciabilità dei prodotti lattiero-caseari. G. Contarini, M. Povolo, V. Pelizzola, CRA-

Centro di Ricerca per le Produzioni Foraggere e Lattiero-Casearie, Lodi (p 31)

9.40 Oligopeptidi come marcatori per l'autenticità dei formaggi. A. Dossena, V. Cavatorta, S. Sforza, G. Galaverna, R. Marchelli, Dip. di Chimica Organica e Industriale, Università degli Studi di Parma, Parma (p 32)

9.50 Valutazione dell'autenticità dei prodotti lattiero-caseari mediante composti aromatici e ad alto valore nutrizionale. V. Fedele, S. Claps, L. Sepe, A.R. Caputo, M.A. Di Napoli. CRA-ZOE, Unità di Ricerca per la Zootecnia Estensiva, Bella (PZ) (p 33)

10.00 Conformità sensoriale dell'Asiago DOP. A. Marangon, M. Cappellari, Veneto Agricoltura - Istituto per la Qualità e le Tecnologie Agroalimentari, Thiene (VI) (p 34)

10.10 La piroglutamil-γ3-caseina come marcatore della proteolisi e della durata della stagionatura in Grana Padano e Parmigiano Reggiano. F. Masotti, J. Hogenboom, I. De Noni, L. Pellegrino. DiSTAM, Università degli Studi di Milano, Milano (p 35)

10.20 Caratterizzazione microbiologica e chimica del formaggio Fruhe: un nuovo prodotto eleggibile per il riconoscimento DOP. M.A. Murgia, N.P. Mangia, G. Garau, F. Fancello, P. Deiana. DISAABA – Università degli Studi di Sassari, Sassari (p 36)

10.30 **Discussione**

11.00 **Coffee break**

11.30 **Tavola rotonda**: Autenticità e rintracciabilità dei formaggi: aspetti tecnici, economici, legislativi e sociologici (in Italiano).

Coordina: Dr. M. Borea, R.A.I.

Partecipano:

Sen. C. Sibilia, Presidente Provincia di Avellino
Prof. R. Coppola, ISA-CNR Avellino
Dr. A. Tartaglia, Regione Campania
Dr. L. Perozzi, Segretario generale CCIAA Avellino
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Prof. A. Di Porto, Università degli Studi di Roma "La Sapienza"
Dr. M. Ferraro, Dirigente Coldiretti, Roma
Dr. M. Zema, CSQA, Bari
Dr. V. Amendolara, Ismecert
Dr. L. Bertozzi, Consorzio di Tutela del Parmigiano Reggiano,
Dr. L. Pevere, Consorzio di Tutela Montasio,
Dr. B. Bartemucci, Ispettorato Centrale Controllo Qualità degli Alimenti
Dr. R. Rubino, ANFOSC
Dr. M. Sarnataro, ONAF
Dr. A. Limone, Commissario Istituto Zooprofilattico per il Mezzogiorno
Dr. V. D'Amato, Presidente Ordine dei Veterinari di Avellino

Poster

ABSTRACTS

INVITED SPEAKERS

RELATORI INVITATI

Factors that affect the quality of cheese
I fattori che influenzano la qualità dei formaggi

Patrick F. FOX*

Department of Food & Nutritional Sciences, University College Cork, Cork, Ireland

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Cheese, of which there are at least 1000 varieties, is a complex and variable biochemical system. The manufacture of cheese involves coagulation of the protein of milk by limited proteolysis, isoelectric precipitation or heat-acid precipitation; if the milk contains fat, it is incorporated into the coagulum. The acid and acid-heat varieties are consumed when fresh and their quality depends primarily on the composition of the curd, which depends on the chemical, microbial and organoleptic quality of the milk. If these are good and if the manufacturing protocol is controlled carefully, the fresh cheese will be of good quality and if refrigerated, will change little during the customary short storage period. However, rennet-coagulated cheeses, which represent about 75% of all cheese, are ripened/matured for a period ranging from ~3 weeks to > 2 years, during which the characteristic flavour, body, texture and functionality of the cheese develop. Ripening involves a complicated cascade of biological and biochemical events which are affected by many compositional, processing and environmental factors. The ripening agents in cheese are: (i) the coagulant (rennet), some of which is retained in the cheese curd, (ii) the starter culture (or adventitious milk microflora) used to acidify the curd, and, after the cells have died, their intracellular enzymes, (iii) the non-starter microflora, mainly mesophilic lactobacilli, which grow during ripening, and their intracellular enzymes, (iv) indigenous milk enzymes, and (v) secondary cultures (moulds and bacteria) used for some varieties. The key to good cheesemaking is to control and co-ordinate the activity of these agents, which is affected by (i) the composition of the curd, which is affected by the composition of the cheese milk and the manufacturing protocol, especially cooking temperature, pH and agitation, (ii) quality of the rennet, (iii) starter culture, (iv) the microflora of the milk and whether it is pasteurised or not, (v) treatment of the curd, especially cooking and salting, (vi) duration and temperature of ripening. The principal biochemical changes in cheese during ripening are: (i) glycolysis of lactose and catabolism of lactic and citrate acids, (ii) proteolysis and amino acid transformations and (iii) lipolysis and catabolism of fatty acids.

Cheese production is essentially a biochemical process, the principal steps of which are known and controllable and, therefore, it should be possible to produce top quality cheese consistently, but in practise, this is not achieved; I will attempt to explain why not.

Classification and diversity of cheese
Classificazione e diversità dei formaggi

Paul L.H. McSWEENEY*

Department of Food and Nutritional Sciences, University College Cork, Cork, Ireland

*corresponding author: p.mcsweeney@ucc.ie

A great diversity of cheeses is produced from the same raw materials: milk (bovine, ovine, caprine or buffalo), coagulant, starter cultures and NaCl. Cheesemaking has a history dating back many thousand years and a wide range of technologies are used in their manufacture resulting in many different varieties. No definitive list of cheeses is available but it is likely that the number of varieties produced worldwide greatly exceeds 1000; however, the similarities between certain cheeses suggest that they should be considered variants rather than true varieties. Because of the considerable international trade that exists in cheese, attempts have been made to classify these many varieties into more homogeneous groups. Commonly used classification schemes are based on cheese texture (hard, semi-hard, soft), which in turn is greatly affected by moisture content and processing operations. However, such schemes often result in cheeses with very different characteristics being grouped together. Attempts have also been made to classify cheeses based on indices of ripening, although such schemes have not achieved widespread use. An alternative classification scheme for cheese and cheese-like products will be discussed which groups natural cheeses into approximately 12 superfamilies based initially on the way by which milk is coagulated and then on characteristic ripening agents or manufacturing technology. Although not without inconsistencies, this scheme provides a useful and logical basis for the classification of cheeses into relatively homogeneous superfamilies.

Protein and peptide composition of cheese analysed by CE, HPLC and LC-MS
Composizione in proteine e peptidi del formaggio determinata mediante CE, HPLC e LC-MS

Ylva ARDÖ*

Department of Food Science, University of Copenhagen, 1958 Frederiksberg, Denmark

*corresponding author: ya@life.ku.dk

Each cheese variety has its characteristic pattern of breakdown of casein to peptides and amino acids. Important mechanisms behind it may be revealed by analysing casein components by capillary electrophoresis (CE), pH 4.6 soluble peptides by reverse phase liquid chromatography mass spectrometry (RP-LC-MS) and amino acid composition by RP-HPLC. CE measures the first attacks on casein, comprising rennet hydrolysis of α_{S1}-casein into α_{S1}-I-casein, plasmin hydrolysis of β-casein into the γ-caseins and specific microbial hydrolysis. Para-κ-casein, α_{S1}-casein with 8 and 9 phosphate groups (P), respectively, the corresponding α_{S1}-I-casein (8P) and (9P), β-casein (A1, A2 and B), and the corresponding four γ-caseins may be separated. α_{S2}-casein is represented by at least four rather small peaks corresponding to different number of P. By identifying peptides using LC-MS, the specific activities in a cheese variety are possible to map. Typically, the peptides identified are breakdown products of the plasmin produced β-casein (f1-28) and (f28-107) and α_{S2}-casein (f1-21) as well as of the rennet produced α_{S1}-casein (f1-23). Peptides that are less sensitive to further hydrolysis depending on the specific microflora of the cheese variety will accumulate, e.g. highly basic peptides, phosphopeptides or proline rich peptides. Amino acid analysis by RP-HPLC shows the characteristic composition of a cheese as the result of amino acid release from defined parts of casein and the specific microbial use and formation of amino acids. The 20 amino acids of casein may be analysed as well as GABA and AABA from decarboxylation of glutamic acid and ornithine and citrulline from deamination of arginine.

Microbial fingerprinting and authenticity of cheese
Fingerprinting delle comunità microbiche e autenticità dei formaggi

Danilo ERCOLINI*

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Many different dairy products can be found on the market and all have different microbiological, sensorial and textural traits. Fingerprinting techniques can be applied to cheeses in order to detect the bacteria occurring in that particular environment and can be applied to both DNA directly extracted from a cheese matrix and to templates obtained after microbial growth. The fingerprint gives a "picture" of the microbiota of the cheese or of an intermediate of production and can be taken into account as a specific trait of that sample just like other biochemical, structural or sensorial properties. Polymerase chain reaction – denaturing gradient gel electrophoresis (PCR-DGGE) fingerprinting of cheeses has been experimented by several authors and as it turned out, it can provide information on the structure of the microbial populations associated to that cheese, on the traditional vs industrial mode of manufacture, and in some cases it can also be linked to the geographical origin of a dairy product. In addition, the use of selected or natural starter cultures can be also highlighted by the fingerprinting approach. By tracing the origin and mode of production of traditional cheeses, microbiological fingerprints can represent one of the possible tools for the evaluation of the product authenticity

Spectroscopic methods for cheese quality and authenticity
Metodi spettroscopici per la qualità e l'autenticità dei formaggi

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Food quality and safety assessment are of paramount importance in the food trade. A central concern of the European Union (EU) is to ensure a high level of protection of human health and consumers' interests in relation to food, taking into account diversity including traditional products, whilst ensuring the effective functioning of the internal market. Of the employed techniques used to characterize food products NMR spectroscopy seems to be one of the most promising approach.
Here, we report on an NMR protocol, based on both low-resolution and high-resolution NMR techniques, to characterise Mozzarella di Bufala Campana cheese and Grana Padano cheese.
Low-resolution NMR relaxometry is concerned with the quantitative determination of the most abundant components in a sample by evaluating the decay rates and amplitudes of the NMR signal. The main advantage of low-resolution NMR is that it does not require any pre-treatment of the sample to be investigated, and its capacity for studying physical structures that are sensitive to manufacture and mechanical manipulation, and which are unstable during storage, as in stretched curd cheese. Furthermore, once developed, the standard protocols based on the rapid measurements obtained by low-resolution NMR equipments can be easily transferred to on-line quality control applications. On the other hand, high-resolution NMR concerns with the elucidation of the chemical structure of molecules in solution. The use of high-resolution NMR has many positive aspects: among others, it allows a thorough analysis of all components in the solution extracted from the sample, unknown or unexpected substances can be identified, and quantitative data are obtained relatively to a single standard without the need for calibration curves. Because of this, all measured concentrations are estimated with comparable errors, allowing statistical treatment of the experimental data to be more meaningful.

Immunochemical methods for cheese authenticity studies
Metodi immunochimici per l'autenticità dei formaggi

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Antibody-based techniques were successfully employed in developing methods for quality control of milk and cheese. In comparison to conventional analysis, mainly using chromatographic and electrophoretic separations, immunochemistry can provide analysts and researchers with rapid, economical, highly sensitive and specific methods, that are relatively simple to perform and interpret. On account of the inherent complexity of the protein and the peptide fraction of dairy products, highly selective immunoreagents were required. According to antipeptide antibody technology, properly designed peptides, mimicking specified structures included in the protein fraction of dairy products, were used as model antigens. Tailor-made antibodies were obtained either for the detection of specific protein components or for recognizing protein adducts raised by technological processes. Different reagent configurations and immunoassay formats were devised for a number of analytical applications, ranging from monitoring of molecular markers to tracing of milk processing in cheese manufacture.

Small molecules for the evaluation of cheese authenticity
Profiling di piccole molecole per l'autenticità dei formaggi

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The individuality and the typicality of cheese derive from the intrinsic characteristics of raw milk and an harmonious balance of microbial and enzymatic processes which together concur to determine the physico-chemical and sensory quality of the final product.
The proteolytic and lipolytic activity behaviour during cheese ripening can be monitored analyzing some molecular markers such as small molecules and/or degradation products.
Free L and D-amino acids, hydroxy fatty acids, Maillard reaction compounds, lysinoalanine and others can be successfully used to verify cheese authenticity. In this context, the development of reliable detection methods which are able to determine small molecules changes during cheese making and ripening is of great relevance. In addition, the knowledge/use of these molecules can significantly improves cheese quality and valorizes cheeses, with a protected designation of origin (PDO) and protected geographical indication (PGI) (EEC Council Regulation 2081/92 of 14 July 1992).

Statistical methods for cheese authenticity studies
Metodi statistici multivariati e autenticità dei formaggi

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Consumers' confidence in quality cheese branding and in the authenticity of PDO and PGI cheese has been recently shaken by frauds and by carryover of environmental contaminants in PDO cheeses. Authenticity of cheese depends on several aspects all of which are related to cheese perceived quality or market price: geographical origin, milk source, mode of production, duration of ripening, lack of contaminants, etc. Except in very simple cases in which measurement of a single biomarker or of gross composition parameters may suffice for fraud detection, evaluation of authenticity of PDO and PGI cheeses requires complex analytical approaches which usually generate large data matrices. This is especially the case with several modern spectroscopic (NIR, FTIR, MIR, NMR, FS), chromatographic (RP-HPLC of soluble nitrogen, GC-MS or LC-MS of volatile or soluble products of proteolysis, glicolysis and lipolysis), electrophoretic (UREA-PAGE or SDS-PAGE of insoluble nitrogen), sensor array (electronic noses), microbiological (fingerprinting of cheese microbial communities by tRFLP, PCR-DGGE/TGGE, PCR-SSCP, LH-PCR, etc.) and sensory techniques.
The presentation and interpretation of the these data and the discrimination of samples requires almost invariably extensive pre-treatment of raw data and chemo metric approaches. Chemometrics "is the science of relating measurements made on a chemical system or process to the state of the system via application of mathematical or statistical methods". Application of multivariate statistical approaches for the treatment of analytical data is now well established in dairy science.
In this presentation examples and case studies of application of data pre-treatment and descriptive and inferential tools for cheese authenticity studies will be presented.
Principal Component Analysis (PCA), Multidimensional scaling (MDS) and Hierarchical (HCA) and Non-Hierarchical Cluster analysis (NHCA) are the most frequently used descriptive tools. Both PCA and MDS can be used to reduce the dimensionality of the data set and represent the relationships by objects (cheeses) and variables in a 2 dimensional space. Preliminary information on variables which have the largest influence on the variance of the data set can be also obtained. Although both these techniques are relatively robust and easy to use and interpret, care is needed in standardization of data and in the choice of the distance/proximity matrix to factor or to use in dimensioning. Both HCA

and NHCA can be used together with PCA and MDS in attempt to identify natural groups of objects in the data set and investigate their properties. Kohonen Artificial Neural Networks are a type of unsupervised network that is becoming increasingly popular in processing and representing large amount of data. When more than a data matrix is available for each sample, more complex descriptive techniques, such as Canonical Correspondence analysis can be used to represent the results from different techniques in a single space.

Descriptive techniques are usually used as a preliminary tool in the analysis of data. However, to build prediction models which enable the user to discriminate samples on the basis of factors relevant to authenticity, inferential tools are necessary. Multiple linear regression is of little use in the prediction of cheese age or quality from the complex, collinear data sets generated by most fingerprinting techniques. Although Principal Component Regression (which uses components extracted from the x data set to predict properties (y data set) of cheese samples, Partial Least Square Regression (PLS) tools are by far more efficient and have been frequently used in cheese chemometric studies. Discriminant analysis (DA) has been traditionally used to discriminate cheese samples on the base of origin. However, supervised Artificial Neural Networks and regression and classification trees are becoming increasingly important in this area.

Although a vast array of chemometric techniques is now available for the presentation and interpretation of cheese authenticity data, their use require specific training and even if relatively user friendly software tools are available for some analytical techniques (electronic noses are an example), more effort is still needed to spread the use of multivariate statistical tools to prevent frauds and mislabelling of cheese.

The role of the Italian Central Inspectorate for Food Quality in the protection of food authenticity
Il ruolo dell'ICQ nella difesa del patrimonio agroalimentare italiano, con particolare riferimento al settore lattiero-caseario

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This presentation describes institutional tasks, goals, role and activity of the Central inspectorate of feed and food quality control, related to the protection of such products against unfair and fraudulent practices, in order to ensure trust of consumer and fair producers, with a special attention paid to milk and dairy products.
ICQ is presented, as well as its functions and the results of both inspective and analytical activity, in general and in the milk and dairy field in particular.
It is underlined the role of the ICQ not only as an official inspection body, but in promoting initiatives aimed at the correct and up-to-date development of the milk and dairy sector.
Some analytical issues, not yet solved, are finally highlighted, in order to promote the discussion and to get advices.

ORAL COMUNICATIONS

COMUNICAZIONI ORALI

Traceability of artisan raw milk cheeses of the Marche region: a preliminary approach
Tracciabilità dei formaggi artigianali da latte crudo della regione Marche: un approccio preliminare

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The aim of the study was to distinguish artisan raw milk cheeses (Pecorino, Caciotta, Caprino and Caserotto), which are typically manufactured in the Marche region, from pasteurized milk competitors, available on the market with the same denomination. Raw milk cheeses were collected from four local dairy farms, while pasteurized milk cheeses were picked up at the retail level from supermarkets. All the samples were subjected to: (i) pH and aw measurements; (ii) determination of the main compositional parameters (NaCl, lipids, proteins, rancidity, peroxides); (iii) viable counting of lactobacilli, mesophilic lactococci, thermophilic cocci, total coliforms, Escherichia coli, Staphylococcus aureus, Salmonella spp. and Listeria monocytogenes; (iii) PCR-DGGE analysis of the bacterial DNA extracted directly from the food matrices and the bulk cells harvested from the MRS and M17 dilution plates; (iv) solid-phase microextraction coupled with gas chromatography (GC-SPME). Physico-chemical and microbiological data were subjected to principal component analysis (PCA). Except for Caserotto, which was characterized by a short ripening period, a high hygienic standard of both the artisan and industrial manufactures was verified. The polyphasic PCR-DGGE approach, combined with traditional plating methodology, revealed slight differences in the microbial composition of raw and pasteurized milk cheeses, while a neat diversification was highlighted by SPME-GC. PCA allowed the artisan manufactures to be definitely distinguished from the industrial competitors. Given the increasing importance of food traceability for safety, quality and typicalness issues, the polyphasic approach proposed may represent a suitable tool for tracing traditional dairy specialties, as those considered in the present study.

Branched-chain fatty acids: a potential parameter for assessing authenticity of PDO mountain cheeses.
Acidi grassi ramificati: un potenziale parametro per la valutazione dell'autenticità dei formaggi DOP di montagna

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During the last decades several analytical approaches have been developed for assessing typicalness and authenticity of PDO mountain cheeses. As milk fat composition is very sensitive to changes in cow feeding, the study of fatty acid profile can give information about cow farming. In particular presence of branched-chain fatty acids (BCFAs) has been associated with the activity of rumen bacteria, especially under certain feeding practices. On this basis the levels of different BCFAs were investigated in four PDO mountain cheeses (Toma, Raschera, Bitto, Formai de Mut) and PDO Grana Padano cheese. The results demonstrated that the highest levels of 13-methyl-tridecanoic acid (i-C14) were recorded in fat of cheeses produced with milk from cows grazed or fed forage. Similar levels were found in cheese also during winter if cows were fed hay and haylage. Use of maize silage in cow diet significantly (about 60 %) reduced the amount of i-C14 in cheese fat. Overall, level of BCFAs and particularly of i-C14 allowed to distinguish mountain cheeses from lowland cheeses and to assess the compliance to PDO rules not allowing the use of maize-silage for cow feeding.

Analytical traceability of Parmigiano Reggiano cheese
Tracciabilità analitica del Parmigiano Reggiano

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In collaboration with the Consortium for Parmigiano Reggiano cheese, we investigated the possibility of creating a traceability model based on analytical data, capable of verifying the authenticity of commercial cheeses. 76 samples of Parmigiano Reggiano representative of the Consortium's production and 64 samples from its three main commercial competitors, from Italy (17), the Czech republic (18) and Lithuania (29), were considered. We analysed the stable isotope ratios of H, C, N and S in the extracted casein of the cheeses using Isotopic Ratio Mass Spectrometers connected to an Elemental Analyser and a Pyroliser, as well as elemental composition after cheese digestion using an Inductively Coupled Plasma–Mass Spectrometer. The statistical model (Canonical Discriminant Analysis), structured on the basis of the most significant analytical parameters (H, C, N, S stable isotope ratios and Li, P, K, Ca, Fe, Cu, Zn, Se, Rb, Sr, Mo, Cs, Sm, Gd, Dy, Yb, Re, U), was 100% effective in discriminating origin. The model can be proposed as a suitable tool for detection of mislabelling and for consumer protection.

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Non volatile hydrocarbons: promising tools for dairy products traceability
Idrocarburi non-volatili: strumenti promettenti per la tracciabilità dei prodotti lattiero-caseari

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During the last few years, traceability of dairy products was became of great interest, at all levels of the production process, for researchers, consumers, industries and policy markers. Several researches have been carried out to distinguish mountain products, obtained from animals feeding fresh pasture, from those deriving from intensive breeding systems. Volatile compounds, terpenes in particular, were widely investigated in milk and cheese produced by cows grazing on pasture, and they were often recognized as effective biochemical markers of origin.
In order to verify if other molecules could be of interest to evaluate dairy product traceability, our research was focused on the investigation of the non volatile hydrocarbons of the neutral lipid fraction. Dairy products obtained from animals feeding either on montain pasture on under intensive breeding systems, located in Padana Plain, were analysed. Hydrocarbons were separated by silica gel column chromatography and analysed by GC/MS.
The main compounds detected, in all the samples analysed, belonged to the following chemical classes: n-alkanes, isoprenoid hydrocarbons, esters. In particular, 1-phytene, 2-phytene, neophytadiene and some esters of phytol with long chain fatty acids allowed a good discrimination of the different feeding systems.
This method could find an interesting application in the control of the cheese origin, particularly when, within the same cheese type, two different productions are present and the mountain product, having a higher economical value, needs to be protected.

Proteolytic oligopeptides as molecular markers for cheese authenticity
Oligopeptidi come marcatori per l'autenticità dei formaggi

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During the production and the ageing process of cheeses, caseins undergo an extensive degradation, due to the endoprotesases and exoproteases of the starter, either natural or added, of the rennet and of raw milk. Thus, the nitrogen fraction of the aged cheeses is composed by a mixture of native caseins, high, medium and low molecular mass peptides and free amino acids. By using originally developed extraction techniques and LC/MS methodologies, a detailed study of the oligopeptide (<10 kDa) fraction of several cheeses has been performed. The most abundant oligopeptides can be rapidly and reliably identified and semiquantified in cheese samples, also allowing to determine their evolution starting from the production and during the ageing time. The starter mixture, the technology and the ageing time are strongly linked to a characteristic enzymatic activity which in turn leads to a typical composition of the peptide fraction, which can be considered as a characteristic "fingerprint" of the product. In a typical example, several peptides identified in Parmigiano-Reggiano at different ageing times can be reliably linked to the dominant lactic acid bacteria in the different periods. Moreover, the evolution of the peptide pattern during the ageing process allowed to precisely assess the age of the cheeses. In a different study, the different technologies used for preparing Asiago cheeses and the different production locations could be identified. As far as authenticity is concerned, cows' milk fraudulently added to sheep milk can be traced by identifying specific proteolytic peptides arising from cows' casein degradation.

Evaluation of dairy products' authenticity by aromatic and nutritional compounds
Valutazione dell'autenticità dei prodotti lattiero-caseari mediante composti aromatici e ad alto valore nutrizionale.

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The natural pasture is an alimentary factor able to mark with its secondary metabolites milk and cheese. This report summarizes the principal results of researches carried out in the last ten years at CRA-ZOE (Bella) on the effects of feeding system and plants ingested by animals on milk and cheese Volatile Organic Compounds (VOC) and nutritional secondary metabolites. VOC content and profile of milk from grazing animals were different to that from indoor animals. In milk from grazing animals were detected, meanly, 9000 ng/l of sesquiterpenes, characterised by α-pinene, p-cymene, α+γ-terpineol, camphene and sabinene, vs. 1000 ng/l in milk from zero-grazing animals, not showing the same compounds. Common grasses, such as Periennal ryegrass and Orchad grass, showed a poorer profile and a lower amount of aromatic compounds, compared to Rubiaceae, Gentianaceae and Compositae. This different richness affected VOC content and profile. For example, an increase from 11 to 35% of Sweet Woodruff in the diet caused a 2.5 time increase of terpenes content and of an unidentified terpenes from 32 to 47% in milk. Also the CLA and vitamin content, and the degree of antioxidant protection (DAP) were affected by feeding system. Milk from grazing animals showed higher CLA (+72%), α-tocopherol (+53%) and DAP (+38%) than milk from zero-grazing animals.
These results suggested the possibility to use secondary metabolites to evaluate the authenticity of dairy products and to improve their quality.

Sensory conformity of Asiago cheese PDO
Conformità sensoriale dell'Asiago DOP

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Within the "Quality Project of Asiago Cheese", promoted by the "Consorzio di Tutela del Formaggio Asiago", together with "Veneto Agricoltura, laboratorio di analisi sensoriale dell'Istituto per la Qualità e le Tecnologie Agroalimentari di Thiene (VI)" and CSQA Thiene (VI), there is a study in progress with the objective of adding sensory analysis requirements (in addition to chemical analysis) to Asiago Cheese specifications. This sensory analysis will be performed by the cheese factories and verified by auditors.
The Quality Project of Asiago cheese is born as a result of twenty years of activity and collaboration between the Consorzio di Tutela and Veneto Agricoltura.
Many studies on the chemico-physical and sensory characteristics of Asiago Pressato and Asiago Allevo (the two typologies of Asiago cheese) have been conducted during this period in order to describe the products for characterization, quality control, and consumer protection purposes.
Sensory characteristics described in the specifications have been evaluated following ISO 12933:2003 "Sensory analysis – Methodology – General guidance for establishing a sensory profile", and a specific method for cheese "Guide to the smell, aroma and taste evaluation of hard and semi-hard cheeses.
Before starting evaluation of the cheese, Asiago producers attended a training course on sensory analysis in order to educate them about the technique.
The object of the sensory analysis was to identify which cheeses were not in accordance with specifications.
A panel of assessors, trained on cheeses, evaluated colour, eyes and flavour on a numerical scale using standard references and considering conformity limits.
In parallel with the trained panel, the same characteristics and the same cheeses were evaluated by Asiago cheese producers.
Therefore it was possible to correlate their results with those of the trained panel, in order to find a useful method to apply within each dairy, to meet all the requirements of PDO certification.

Pyroglutamyl-γ$_3$-casein as marker of proteolysis extent and ripening period of Grana Padano and Parmigiano Reggiano cheeses
La piroglutamil-γ$_3$-caseina come marcatore della proteolisi e della durata della stagionatura in Grana Padano e Parmigiano Reggiano

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Grana Padano (G.P.) and Parmigiano Reggiano (P.R.) are extra-hard, long ripened PDO cheeses and, during maturation, they undergo to extensive proteolysis. Different chemical indices related to proteolysis and ageing period have been investigated in G.P. (n=155) and P.R. (n=27) cheese samples aged from 7 to 38 months, by means of ion exchange chromatography and capillary electrophoresis (CE). The levels of intact caseins, γ-caseins and free amino acids were measured but showed low correlation with the ripening period as they varied among samples of the same age. A peptide, identified by means of HPLC/electrospray ionization-mass spectrometry as pyroglutamyl-γ3-CN (pγ3-CN), resulted from cyclization of N-terminal GLU of γ3-CN into pyroglutamic acid. According to CE electropherograms, this peptide accumulated as a function of cheese age. Based on the peak area ratio of pγ3-CN and γ3-CN, an algorithm suitable to determine the cheese age was proposed and adopted to verify the typicality and the ripening period of the two G.P. categories "G.P. ripened over 16 months" and "G.P. Riserva" (i.e. ripened over 20 months).

Microbiological and chemical characterization of Fruhe cheese: a new dairy product eligible for a PDO certification
Caratterizzazione microbiologica e chimica del formaggio Fruhe: un nuovo prodotto eleggibile per il riconoscimento DOP

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Fruhe is the name of a fresh cheese traditionally manufactured in Sardinia (Italy) from sheep or goat milk. Fruhe is a fermented curd characterized by a compact coagulum and manufactured from whole milk, raw or heat-treated, deriving from Sardinian farms. In this study twenty samples (10 artisanal and 10 industrial) of Fruhe, manufactured from sheep and goat milk using different cheese-making technologies, have been investigated for their chemical and microbiological composition. Microbiological analyses showed that total mesophilic bacterial counts were between $1x10^6$ and $2,70\ x10^9$ CFU/gr. Lactobacilli and lactic streptococci were constantly present in a 1:1 ratio and in a few samples they reached significant numbers ($\sim 1,00\ x10^9$ CFU/gr) while more generally the size of these populations was more limited ($\sim 1,00\ x10^6$ CFU/gr). LAB recovered from the samples investigated were *Lactococcus lactis* subsp. *lactis*, *Streptococcus thermophilus*, *Lactobacillus helveticus*, *Lactobacillus plantarum, Enterococcus faecium* and *Enterococcus faecalis*.
Chemical analyses showed a certain variability between industrial and artisanal samples. In general, Fruhe samples were all characterized by a substantial amount of essential free amino acids as well as glutamic acid, glycine, alanine, tyrosine, histidine, proline and α and γ-amino butyric acid. The amount of free fatty acids was quite variable depending on the sample. More generally, caproic, caprylic and caprinic acids were more abundant in Fruhe made with goat milk.
The diversity of chemical parameters recorded in the different Fruhe samples examined suggests the need for a standardization of the milk and technological procedures employed in order to get a dairy product with more constant characteristics.

POSTERS

Identification and characterization of yeast strains isolated from samples of Water Buffalo Mozzarella cheese and natural whey cultures
Identificazione e caratterizzazione di ceppi di lieviti isolati da campioni di Mozzarella di Bufala Campana e da sieroinnesti.

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In recent years, yeasts have been increasingly considered as important agents in the maturation process of cheeses, but their contribution to cheese quality remains quite unclear. In the present study, sixty yeast strains were isolated from samples of natural whey cultures and Water-buffalo Mozzarella, a popular 'pasta filata' cheese from Southern Italy.
All strains were identified by means of 5.8S-ITS RFLP analysis, and D1/D2 domain of 26S rDNA sequencing. Yeast species most frequently isolated were *Kluyveromices* (*K.*) *marxianus* (38.3%) and *Saccharomyces* (*S.*) *cerevisiae* (21.6%). Nevertheless, the occurrence of these two species in the samples is widely unequal, since were detected in 8 and 6 out of 16 farms, respectively. *Candida butiry/aaseri*, *Candida pararugosa*, *Candida sorbophila, Clavispora lusitaniae, Pichia barkeri, Pichia cactophila, Pichia norvegensis* and *Pichia pastoris* were recovered as well.
All strains were phenotypically characterized by considering features of technological interest. All species showed the ability to assimilate glucose, lactose and galactose while the ability to ferment these carbohydrates was limited to minor number of isolates. In detail, the ability to ferment lactose, in agreement with many authors, was restrict to the isolates of the species *K. marxianus*. A large majority of cultures exhibited lipolytic activity, with the exception of *S. cerevisiae* strains that showed a weak capability. All strains of *K. marxianus* were able to assimilate lactate, while 11 out of 13 strains of S. cerevisiae exhibited this trait. Less common appeared the ability to assimilate citrate: only 13 out 23 and 3 out 13 stains of *K. marxianus* and *S. cerevisiae*, respectively proved to be able.

Genotypic and technological diversity of *Leuconostoc mesenteroides, Lactobacillus paracasei* and *Brevibacterium linens* strains isolated from the artisanal 'Pecorino di Filiano' cheese

Diversità genotipica e tecnologica di ceppi di *Leuconostoc mesenteroides, Lactobacillus paracasei* and *Brevibacterium linens* isolati da 'Pecorino di Filiano' artigianale

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Seventeen strains of *Leuconostoc* (*Leuc.*) *mesenteroides*, thirty-three of *Lactobacillus* (*Lb.*) *paracasei* and twenty-two of *Brevibacterium* (*B.*) *linens*, isolated from the traditional 'Pecorino di Filiano' cheese of Basilicata region, were characterized on the basis of their technological properties (proteolytic and lipolytic activities, diacetyl production) and typed at strain level to study the genetic variability and distinguish closely related isolates. A middle-low level of proteolytic activity was observed in 41% of *Leuc. mesenteroides* and in 45% of *Lb. paracasei*; moreover, 23% of *B. linens* strains showed a middle-low proteolyitc ability, while a small group of this species (9%) had an high proteolytic level. The lipolytic activity of strains was determined at three different temperatures. The highest lipolytic activity was detected at 20°C, especially for *Lb. paracasei* and *B. linens* strains with 18% and 32% respectively; while 35% of *Leuc. mesenteroides* showed a slightly lower lipolysis. The evaluation of diacetyl production revealed that the most of *Leuc. mesenteroides* and *Lb. paracasei* strains (76% and 88% respectively) were able to produce diacetyl, while only 23% of *B. linens* showed this ability. Moreover, strains diversity was investigated by RAPD analysis. Results showed a high degree of heterogeneity among strains of all species tested. The results of this study suggest that the strains possess metabolic activities that affect the sensory quality of the cheese in which they grow during ripening, confirming the complexity of the microbiota of 'Pecorino di Filiano' cheese, which influences the typical sensory properties of this product.

Use of colorimetric methods for the analysis of the type of rennet used in traditional cheese production
Uso di metodi colorimetrici per l'analisi del tipo di caglio usato nella produzione di formaggi tradizionali

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The exceptional characteristics and qualities of some Italian cheeses are due to the particular place of production and to the methods used to produce them. These characteristics are mostly due to the use of different types of coagulants. Among these, kid and lamb rennet pastes stand out. It is well known that cheeses made with these kinds of rennet show specific sensory characteristic distinguishable from the cheeses made with commercial calf rennet, due to their own proteolytic activity and to the presence of lipolytic enzymes, completely absent in the latter. Given the importance of rennet in the quality and authenticity of traditional cheeses, the object of the present work was to study the effect of the type of rennet (kid and calf) on the quality of the Marzolina, a goat's raw milk cheese traditionally made using kid rennet paste. For this purpose, the CIELab system was used to evaluate its colorimetric profile. The reflectance spectra were similar for both cheese groups, whereas colorimetric indexes were significantly different. These data, taken together with the compositional one (i.e. moisture and protein content), indicate that the chemical changes occurring as a result of employing different coagulants may be due to the different proteolytic activity of them, being higher in the case of calf rennet. This effect may produce changes in the intricate microstructure of the cheese, breaking down the extent of casein-water interactions in it, reducing the micelle solvatation due to imbibed water, which translates itself in a significant lower moisture content and in a significant increase in both redness (a*) and yellowness (b*) when calf rennet is used.

The lactic microflora isolated from traditional, raw milk Italian cheeses: a tool to define cheese authenticity?
La microflora lattica isolata da formaggi italiani tradizionali al latte crudo: uno strumento per definire l'autenticità dei formaggi?

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Many Italian cheeses are characterized by the presence of a wide and heterogeneous lactic microflora. Lactic acid bacteria (LAB) present in raw milk, natural starters, and the cheese environment reach the cheese curd and, therefore, confer the "microbial printing" of the original ecosystem. In such cheeses, the original raw milk microbiota is found at high, and often dominant, levels also in the ripened product. This complex LAB community can be further differentially selected and modulated according to the technological parameters and the intrinsic biological characteristics of the individual microbial components. With the aim of identifying potential microbial markers of cheese authenticity, in the present work the LAB composition of 12 artisan cheese typologies made with raw milk without starter (or using natural starters) was carried out. A wide microbial diversity accounting on thirty-four LAB species was observed. *Lactobacillus paracasei, Lactobacillus plantarum, Lactococcus lactis, Enterococcus faecium*, and *Enterococcus faecalis* were the prevailing species. RAPD-PCR carried out on about a thousand isolates evidenced a wide strain heterogeneity, which seemed related to the various cheese production areas or cheese types. In some cases, species and strain grouping and distribution suggested that technological factors may have caused a selection of some dominant biotypes within different cheeses. On the contrary, some *L. paracasei, Lc. lactis* e *Lb. delbrueckii* subsp. *lactis* strains tended to be typically associated to a given product, suggesting their use as "microbial markers of cheese authenticity". Further work will be needed to verify spatial (geographic) and overtime persistence of proposed biomarkers.

Diversity of Caciocavallo cheese produced in different regions of Southern Italy
Diversità del Caciocavallo prodotto in diverse regioni dell'Italia meridionale

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Caciocavallo Silano is a PDO cheese, historically produced in Southern Italy. The traditionally-made Caciocavallo cheese is produced by coagulating the raw milk added of calf rennet and natural whey starter. The curd is then subjected to a spinning process in hot water, followed by moulding into the traditional shape. The Geographical area of production includes provinces located in different regions and, among these, the type of production can significantly vary.
In this work, 8 traditionally home-made Caciocavallo cheeses, withdrawn at the 6th month of ripening in artisanal dairy plants located in 4 different regions of the PDO area (Calabria, Campania, Molise and Puglia), were assayed in order to highlight differences/similarities of the microbial composition by using a culture-indipendent method (PCR-DGGE). Moreover, physical-chemical analyses were performed to point out pH, Acidity, Humidity, as well as protein and amino acidic composition of the samples.
Analysis by DGGE of nucleic acids extracted directly from the samples evidenced composite profiles, with several bands placed in different migration positions. In particular, some bands with the same position resulted present in all samples, whereas other ones were distinctive of cheeses from different regions or from the same region but withdrawn in different plants. As for physical-chemical parameters, also in this case a strong variability was evidenced. In particular, the evaluation of total free amino acids, highlighted the presence of samples with a marked proteolytic activity, in comparison with other samples in which proteolysis resulted very low. Humidity and protein composition resulted also variable among assayed samples, whereas pH and acidity slightly differed.

The flavour profile of Mozzarella cheese: natural vs. defined starter cultures.
Profilo aromatico della Mozzarella: confronto fra colture naturali e selezionate.

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Quality branded (PDO) soft "pasta filata" cheese like "Mozzarella di bufala Campana" are of both scientific and economic interest because of the growing sale trend and because of problems related to protection of their quality traits.
The use of the natural whey starter cultures is required by the standard of identity of the PDO cheese. Although it is one of the main factors affecting the tipicality of the product, it is also responsible for the variability of its sensory quality. Commercial defined starter cultures used for other varieties of soft Mozzarella cheese may significantly improve the hygienic quality of the product and reduce its variability but they also result in a flattening of important sensory properties with a significant reduction of consumer acceptance.
In the framework of a research project whose main objectives where the standardization of the quality, the increase of the shelf-life of water-buffalo Mozzarella cheese, whilst protecting its typical flavour characteristics, we developed a multiple strain starter containing *Lactobacillus delbrueckii* ssp. *bulgaricus*, *Streptococcus thermophilus* and *Lb. rhamnosus*. The use of the MSS allowed to obtain an excellent product reproducibility from both the rheological and sensory standpoint. Proteolysis and aroma profile of Mozzarella cheese produced using the MSS and of control produced using the traditional technology was compared. Primary proteolysis was evaluated by UREA-PAGE while gas-chromatography was used to evaluate volatile organic compounds and organic acids. The control and the MSS Mozzarella cheese did not show any significant difference in the proteolytic profile, while minor differences where found in organic acids. Acetic acid content was 4 times lower in the control, in which lactic acid was slightly higher compared to the MSS cheese. Both formic and citric acid content were similar in the two cheeses.
We conclude that the MSS can be used to control the variability of water-buffalo Mozzarella cheese without negatively affecting its typical flavour.

ELISA for monitoring the freshness of water buffalo curd
Uso di tecniche ELISA per monitorare la freschezza del latte di bufala

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Caseins are initially broken down into large, well characterized fragments. This initial step, called primary proteolysis, is catalysed by endogenous milk proteases, such as plasmin, cathepsin D, and possibly somatic cell proteinases, and by the coagulant enzymes (chymosin and pepsin) to a variable extent in the curd production. Proteolysis in milk occurs mainly by plasmin and continues its action in the curd where the plasmin is concentrated. Plasmin preferentially hydrolyzes β-casein to γ-caseins. The monitoring of quantitative formation of γ-CN by indirect ELISA allowed us to evaluate curd freshness. To perform this analytical procedure we produced anti-peptide antibodies against major cleavage sites of plasmin in β-CN sequence. The peptide sequences corresponded to β-CN f(20-39). In this case antibodies recognized only intact β-CN and its diminution indicated the proteolysis degree in the fresh and stored curd. The results showed different β-CN between seven fresh and seven stored curds. The most stored curd showed a β-CN content up to ten times lower than fresh one. The development of this methodology can allow to disposing of a powerful tool to guarantee the quality of dairy products to consumers and to defend protected dairy production.

Biochemical characterization of "Laticauda" ovine hard cheese to value and to safeguard a traditional dairy product of "Sannita" bio-territory
Caratterizzazione biochimica del pecorino da Lauticauda per la valorizzazione e la salvaguardia di un prodotto tradizionale del bioterritorio Sannita

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The research concerned the characterization of 'Laticauda' ovine hard cheese obtained from milk of autochthon genotype Laticauda sheep. In this note we reported results of the lipidic and proteic profiles obtained by analyzing cheese with different initial weight (eight cheese moulds: four of about 4kg and four of about 8kg) at different ripening times (3, 6, 9, 12 months). During the ripening, numerous changes occurred into cheeses to ascribe to milk endogenous, rennet and bacterial flora enzymes. The aim of this work was to value 'Laticauda' cheese through the identification of protein and lipid components to define nutritional, healthy and extranutritional properties and to obtain an useful tool for traceability of this dairy product. The components of the lipid and protein fraction were identified by gas chromatography, two-dimensional gel elctrophoresis and mass spectrometry MALDI-TOF, respectively.
These preliminary results showed:
(a) in relation to ripening time:
- an increase of C4÷C6 and C8÷C12 fatty acids and an overall decrease of long chain fatty acids as function of mould cheese weight;
- a profile of CLA content increasing in the cheese mould of 4kg and decreasing in the cheese mould of 8kg;
(b) in relation to only mould cheese weight:
- a higher degree of proteolysis in cheese mould of 4kg with respect to that of 8kg.
Overall, the determination of the CLA content and the proteomic analysis showed a faster chemical kinetics in ovine cheese of 4kg with respect to that of 8kg.

Hygienic, microbial and physico-chemical characteristics of Fagagna, a semi-hard cheese of Friuli Venezia Giulia region
Caratteristiche igieniche, microbiologiche e fisico-chimiche del Fagagna, un formaggio semi-duro del Friuli Venezia Giulia

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The aim was the study of the microbial ecology and the physico-chemical characteristics (pH, proteolysis index, biogenic amines, and volatiles compounds) of Fagagna cheese from artisanal factory. Fagagna cheese is produced with cow milk, with a natural starter culture and ripened over two months. It presents a good flavour and odour and it is considered safe. Three different lots of production were studied. The microbial characteristics were evaluated in fresh milk, after adding starter, after curd cooking, at the end of brining, after 15, 30, 60 days of ripening. The volatiles compounds and the biogenic amines were evaluated at the beginning of ripening and at 30 and 60 days. The ripening index was also investigated. Different strains of typical lactic acid starter (SLAB), and various non-starter lactic acid bacteria (NSLAB) were isolated, identified and characterized by traditional and molecular (PCR-DGGE) methods. Lactic acids bacteria are the dominant microbial population in all the phases of cheese production. They increased till the 28th day, reaching 9 log CFU/g , then they decreased during the ripening. After adding the starter culture the value of LAB was 7 log CFU/ml. Coagulase negative cocci population also increased but their value was at 8.3 CFU/g level. *Enterobacteriaceae*, *E. coli* and *St. aureus*, were present in low concentration, less than 2-3 log CFU/ml, they never grew and decreased after curd cooking at 44-46°C and during ripening. Finally yeast population grew within 15 days of production and then its value was at level of 4 log CFU/g till the end of ripening. They grew in the curd and cheese. On the cheese surface moulds and yeasts were isolated but they were not observed. No *Salmonella* spp. and *Listeria monocytogenes* were isolated. The identification of isolated bacteria demonstrated the existence of a difference between the traditional method based on biochemical tests and molecular methods. By traditional method 15 strains were identified, whereas

by molecular 7 strains. The method based on PCR-DGGE and sequencing of the obtained amplicons gave real and precise identification. The traditional one can give subjective and inaccurate interpretations. *Lactobacillus delbrueckii* and S*treptococcus thermophilus* were the typical SLAB, whereas the various NSLAB were represented by *L. rhamnosus*, *L. fermentum*, S*. macedonicus, Enterococcus faecalis* and *Leuconostoc pseudomesenteroides*. *L. delbrueckii* and *S. thermophilus* predominated in the wild starter culture used in order to produce Fagagna cheese. The production of the wild starter cultures is often uncontrollable and it frequently varied, for this reason it consists in a mix of homofermentative end heterofermentative lactic acid bacteria. This bring to a widely presence of little holes in the Fagagna cheese, that the consumers consider a good property and not a defect. The proteolitic index was 8 at 30 days and 14 at 60 days. These values are similar to those of Montasio cheese, a typical cheese of Friuli Venezia Giulia region. The biogenic amines were tested at 30 and 60 days of ripening. At 30 days 2-phenilethilamine, cadaverine were detected, and at 60 days also istamine. Triptamine, putrescine, tiramine, spermidine and spermine were never identified. Moreover the total amine concentration was less than 50 ppm at 30 days and less than 100 ppm at 60 days. However both SLAB and NSLAB strains were able to produce "in vitro" biogenic amines. So considering the absence of pathogenic bacteria and the low amount of total biogenic amines, the Fagagna cheese could be considered safe for the consumers. The aromatic compounds were also investigated, they came from the sugar fermentation activities of bacteria and from the oxidative activities of the curd compounds. In particular they were represented by acetic acid, ethanol and esters. Thirty-four compounds were identified and shared in 8 classes: carboxylic acids, ketons, alcohols, esters, aldheydes, hydrocarbons, sulphite compounds and lactons.
Considering its hygienic, microbial and physico-chemical characteristics, Fagagna cheese must be safe.

Development of new starter cultures for traditional cheeses produced in the mountain areas of the Veneto Region
Sviluppo di sistemi di colture starter per formaggi tradizionali prodotti nelle regioni montagnose del Veneto

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Natural starter cultures are complex association of species and strains of lactic acid bacteria which can affect both the cheese-making procedure and the ripening profile. Although natural starters are an important source of strains with desirable technological properties, fluctuations in their composition may result in variable performance. On the other hand, the replacement of natural cultures with defined starters composed of a limited number of strains may lead to cheeses with poor flavour and aroma. The aim of this work was to develop new starter cultures derived from natural milk cultures traditionally used for the manufacturing of cheeses in the mountain areas of the Veneto region. Thirty-one natural milk cultures collected in different seasons were studied for their microbiological characteristics and for their acidification ability. Furthermore, their biodiversity at species and strain level was evaluated by molecular methods. Most of the cultures were characterized by the dominant presence of *S. thermophilus*, whilst thermophilic lactobacilli and enterococci were not detected, or were present at very low concentrations. Yeasts, coliforms and coagulase positive staphylococci were not found in most of the examined samples; however, some cultures were contaminated by Gram-negative spoilage bacteria. Two milk cultures characterized by good microbiological and technological properties (absence of spoilage microorganisms, high concentration and biodiversity of *S. thermophilus*, proper acidification ability) were reproduced and freeze-dried. Evaluation of the culture composition in different steps of production by RAPD-PCR showed a different composition; however, after growth of the freeze-dried cultures in milk the typical composition of the natural culture was re-established. Results suggest that the freeze dried natural milk cultures could be a valid alternative to the natural liquid cultures (which are prepared daily), or to the defined commercial starters.

Employment of selected autochthonous starters for quality improvement and preservation of typical features of PDO cheeses
Uso di starter autoctoni selezionati per il miglioramento della qualità e per il mantenimento delle caratteristiche tipiche dei formaggi DOP

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Fiore Sardo, Pecorino Sardo and Pecorino Romano are the three Sardinian Protected Denomination of Origin (PDO) cheeses manufactured from ewe's milk. The respective PDO protocols allow the inoculation of milk with autochthonous lactic acid bacteria. In this way the typical features of the cheese are connected to the area of manufacturing also through the use of the specific microflora which evolved and adapted to that environment.
In this study we evaluated the influence of selected (experimental) autochthonous starter cultures on some microbiological and physicochemical parameters of the three PDO cheeses manufactured according to the respective PDO specifications. Control cheeses consisted of Fiore Sardo and Pecorino Sardo manufactured without the addition of any starter culture and Pecorino Romano manufactured with the addition of scotta-fermento. The selected microbial starters used in the experimental trials were isolated from sheep milk and from the different PDO cheeses and were extensively characterized for their cheese-making attributes. This was done in an attempt to maintain the typical features of cheeses and properly handle the entire cheese-making process.
Microbiological analyses showed that the number of lactic microorganisms significantly increased in all the experimental cheeses, vs controls, particularly in the fermentative phase. A parallel reduction of spoilage microorganisms was also observed in these cheeses, Fiore Sardo in particular, compared to controls. Experimental cheeses also showed a significantly higher amount of free amino acids and free fatty acids. Overall, the experimental cheeses manufactured with the selected autochthonous microorganisms, and according to the respective PDO specifications, showed improved nutritional, sensory and healthy attributes.

Use of the microfluidic "Lab-on-a-chip" technique as alternative method for the study of milk proteins.
Uso di tecniche a microfluidi "Lab-on-a-chip" come metodi alternativi per lo studio delle proteine del latte

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Aim of our work was to use a new analytical approach, based on the "Lab-on-a-chip" version of the size-based micro-capillary electrophoresis, to study the protein profile of raw cow, donkey, goat and sheep milk. Recently, this system has been taken into consideration as an ideal tool for the analysis of food proteins, providing information on their size and concentration in a single assay. Advantages of using such system include automated separation, good reproducibility and digitized data output. Furthermore, the miniaturization of analysis ensures much shorter times of run and avoids use and handling of great quantities of hazardous wastes.
Experimentation and Results. Just 3 µl of each sample were sufficient for the analysis, that were mixed with a SDS solution, β-mercapthoethanol and a reducing buffer, to have a final volume of 90 µl. 6 µl of each mixture were charged in the well of a micro-chip, previously filled with 48 µl of gel-staining and 12 µl of gel-destaining solutions. After only 3 minutes between running and analysis of data for each sample, the micro-system permitted us to distinguish and quantify (in terms of ng/µl) almost all their major proteins (α-lactalbumin, β-lactoglobulin, α_{s1}-casein, α_{s2}-casein, β-casein and κ casein) present in.
Significance and impact of the study. The results indicated that the microfluidic-chip might be a rapid alternative or complementary technology for the separation and quantification of proteins in milk protein based systems.

Characterization and safety evaluation of enterococci isolated from Manchego cheeses
Caratterizzazione e valutazione della sicurezza di enterococchi isolati dal formaggio Manchego

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Enterococci are common constituents of some dairy products as cheese, in which they would seem to contribute to the development of some sensory properties. However, in recent years, enterococci have emerged as serious pathogens in the environment of hospitals, where vancomycin-resistant strains are in increase, and their presence in foods has provoked concern. In this study, 140 enterococci, isolated from Manchego cheeses made from raw ewe's milk at two dairies, were first identified by using specific-PCR and API galleries and later screened for production of biogenic amines (Bover-Cid et al., 1999) and antibiotic resistance.

Isolates were identified as belonging to the species *Enterococcus faecalis* and *E. faecium*. Genomic typification using randomly amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) displayed 27 different genotypes defined at a minimum similarity level of 82%, the value determined in the reproducibility study. Thirteen major clusters grouped two or more isolates and the remaining showed singular RAPD-PCR patterns. Most of major clusters contained isolates from both dairies.

None of the strains decarboxylated histidine, lysine and ornithine, but the majority produced tyramine from tyrosine. Profiles of antibiotic resistance, using ATB ENTEROC 5 (Biomerieux, France), were variable being most of them not inhibited by rifampicin and quinupristin-dalfopristin.

cis-trans-retinol isomerization as a process marker in "pasta filata" cheeses
Isomerizzazione del *cis-trans*-retinolo come marker di processo nei formaggi a "pasta filata"

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Product markers are molecules that could be used for the traceability of raw materials and technological processes. In dairy foods several process markers have been identified, such as furosine, lactulose, whey proteins and enzymes.
Vitamin A, that in milk is present as esterified forms of retinol, for several years has been considered heat resistant and stable during storage. However, by using proper analytical techniques it has been possible to show that retinol could undergo to cis-isomerization in dairy products, under light exposition and acid conditions (pH<4.5), during storage and after growth of different microorganisms. Therefore the isomerization index, i.e the ratio between 13-*cis* and *trans* retinol, has been proposed as process and product index in dairy products.
In the light of the microbial influence on retinol isomerization and to better investigate the role of this parameter as a process index, in this work the retinol isomerization has been analysed during the technological process of different "pasta filata" cheeses. The found isomerization index has been related to the microbial levels during the production process.

Methods for the evaluation of innovative drying techniques of "pasta filata" cheeses
Metodi per la valutazione di tecniche innovative per l'essiccamento di formaggi a "pasta filata"

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The drying process of cheese is one of the longest and most expensive step of the technological process. The traditional drying processes of local cheese industries occur in chambers without controlled moisture and temperature levels, thus obtaining products with a variable composition and characterized by a specific microbial microflora proliferation. For these reasons the cheese industry aims at finding innovative drying processes able to have controlled temperature and humidity parameters and to obtain short time ripened products, of known microbial quality and a better "shelf life". With the aim to standardize plants and methods for drying "pasta filata" cheeses, with respect to the traditionality and the safety of the finished products, "pasta filata" cheese samples (caciocavallo and scamorza) have been dried at different times by using an innovative drying plant, easily applicable to small local industries.
Different methods, such as image analysis and dynamometric measures, were applied on the finished products in order to evaluate the efficacy of the innovative drying technique used.

Free amino acids in "pasta filata" cheeses made from milk of different origin
Aminoacidi liberi in formaggi a "pasta filata" prodotti con latte da diverse fonti

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Traditional foods are moving towards new proposals of characterization in order to meet customer satisfaction.
If "pasta filata" cheeses made from buffalo milk are a consolidated reality, the same products made from goat/cow and sheep/cow milks are difficult to obtain due to the different milk composition which influences the chemical-physical properties and the yield.
In this work "pasta filata" cheeses ("caciocavallo") have been realized from different milks, mixed at different concentration, respecting the traditionality of the finished products. On the different realized products the quali-quantitative composition of free amino acids has been determined in order to evaluate the possibility to use this parameter as a distinctive characteristic, due mainly to the original milk composition and also to the specific microbial microflora, as reported by other authors. Moreover the evolution of free amino acids during ripening has been investigated in order to verify if free amino acids could be used as an useful index to estimate the ripening period also in products made from milk of different origin.

Bacterial population of Pecorino Crotonese cheese and its influence in flavour formation
Le comunità batteriche del Pecorino Crotonese cheese e la loro influenza sulla formazione del flavour

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The diversity and dynamics of the dominant bacterial population throughout manufacture and ripening of two artisanal Pecorino Crotonese cheeses, provided from different farms, were investigated by the combination of culture-dependent and –independent approaches. Three hundred and thirty-three strains were isolated from selective culture media, clustered by using Restriction Fragment Length Polymorphism and identified by 16S rRNA gene sequencing. Results indicate that a decrease in biodiversity throughout the ripening was observed, revealing the presence of *Lactococcus lactis* and *Streptococcus thermophilus* species in the curd and in aged cheese samples and the occurrence of several lactobacilli throughout cheese ripening, with the dominance of *Lactobacillus rhamnosus* species. Bacterial dynamics determined by Denaturant Gradient Gel Electrophoresis provide a more precise description of the distribution of bacteria highlighting differences in the bacterial community between cheese samples, and to detect *Lactobacillus plantarum*, *Lactobacillus buchneri* and *Leuconostoc mesenteroides* species, which were not isolated. Moreover, the concentration of flavour compounds produced throughout cheese ripening was investigated and related to lactic acid bacteria presence. Fifty-seven compounds were identified in the volatile fraction of Pecorino Crotonese cheeses by Gas Chromatography-Mass Spectrometry. Esters, alcohols, and free-fatty acids were the most abundant compounds, while aldehydes and hydrocarbons were present at low levels.

A preliminary evaluation of physico-chemical and microbiological characteristics of "Pecorino di Farindola" cheese
Una valutazione preliminare delle caratteristiche fisico-chimiche e microbiologiche del "Pecorino di Farindola"

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Pecorino di Farindola is a traditional cheese made in Abruzzo region. It is produced from raw ewe's milk and the coagulation is obtained by pig rennet paste. Since literature data are lacking on the characterization of this particular type of cheese, the aim of this work was to approach an investigation on its physico-chemical and microbiological properties. The research was carried out on 10 different cheese samples produced in 5 factories distributed in the provinces of Pescara and Teramo (Abruzzo region) and taken after ripening for 90 days. By considering the higher and lower value for each characteristic among all the samples, a high variability was observed, e.g. lactococci and lactobacilli ranged between 10^5-10^7 cfu/g; coliforms and *Enterobacteriaceae* were detected in the range 10-10^4 cfu/g. Nevertheless, all the samples were characterized by the absence of pathogens at the time of evaluation. The pH ranged between 5.0 and 5.7, the protein content from 33 to 44%, and fat from 40 to 57%. By evaluating the content of molecules with supposed functional properties, it was found that *cis*-9, *trans*-11 conjugated linoleic acid (CLA) occurred in all samples examined, at concentration between 0.92 and 1.27%. Even if few samples were considered here, a high variability among cheeses was observed, probably related to different production protocol. Further work on Pecorino di Farindola cheese will consider a complete characterization of the cheese during ripening with a special focus on NSLAB population and its technological and functional properties.

Promising analitical approaches for cheese characterization
Approcci analitici possibili per la caratterizzazione dei formaggi

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Foodstuff characterisation is actually one of the most crucial issue for nutrition, food quality and consumer protection. This is true for cheese, especially in the italian agricultural and food economy. Due to the complexity and analysis cost, usually the informations related to declared origin and authenticity of cheese products are incomplete. To obtain a complete foodstuff description elemental analysis, lipid, protein and aminoacid composition, volatile components, colour and texture profile are needed.
For this work were used FID-GC for cholesterol and lipid profile, ionic HPLC with amperometric detector for total and free aminoacid composition; volatile components were detected with electronic nose for comparative determination, while SPME coupled with GC-MS was used for qualitative determinations. A colorimeter with L*a*b* color space was used for color determination. Cheese texture profile (springiness, cohesion force and energy, gumminess, chewiness) was determined with a dynamometer. All of these determinations confirm that cheese characterisation is really a complex issue.
NIR spectrometry, that is normally used for moisture, lipid, protein and ash determination, was applied to obtain more informations on lipid profile, aging and lipolytic index. A well defined NIR calibration allows analysis as qualitative/quantitative screening but also a more precise determination of type of cheese, permitting to discriminate it among similar cheeses, in short time and cheap mode.

Characterization of coagulase-negative cocci involved in the ripening of Manteca cheese
Caratterizzazione dei cocchi coagulasi-negativi coinvolti nella maturazione della Manteca

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Manteca cheese is a traditional product of some regions of southern Italy, such as Calabria, Campania, Puglia and Basilicata. It is made from the whey obtained during the production of Caciocavallo Podolico, a cow's milk- 'pasta filata'-ripened cheese. The aims of this work were to identify and characterize for some technological properties the coagulase-negative cocci (CNC) present throughout the ripening process. This study was carried out on samples (whey, ricotta and butter) of Manteca cheese from two dairies in Basilicata and Campania regions. Generally, the initial counts of CNC (10^7 cfu/g) decreased during ripening, and theirs numbers at the end of ripening (about 150 days) were relatively low (10^3 cfu/g). A total of 202 CNC was isolated and identified by phenotypic and molecular methods. The predominant species were *Macrococcus caseolyticus* (98 strains), *Kytococcus sedentarius* (26), *Kocuria kristinae* (19), *Micrococcus luteus* (19), while *Staphylococcus cohnii* subsp. *cohnii* (16), *Staphylococcus caprae* (10) and *Staphylococcus hominis* (5) were identified with a lower frequency. Additional phenotypic characters of technological interest such as growth at 15°C and 45°C, tributyrin, gelatine and Tween 80 hydrolysis, and the ability to produce free amino acids (FAA) from casein were determined. No strains were able to hydrolyse tributyrin and Tween 80 but all the isolates were able to grow at 15°C and several strains (81%) at 45°C. Eighty-two *M. caseolyticus* strains hydrolysed skim-milk and only four hydrolysed gelatine. Most of the strains possessed caseinolytic activity. Also, CNC were tested for susceptibility to 20 antibiotics: 50% were susceptible to less than 2 antibiotics, while two *M. caseolyticus* strains showed to be resistant to more than five antibiotics.

Application of Artificial neural networks for the traceability of mountain cheeses
Applicazione delle Rete Neurali Artificiali per la rintracciabilità delle produzioni casearie di alpeggio

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Artificial Neural Networks (ANN) are particular mathematical structures that process information by means of a parallel approach and are used in many scientific fields to obtain results when mathematical models do not exist or these models, if any, are so complex that makes them unusable. The aim of this study was to evaluate the application of ANNs to trace mountain cheeses. In particular ANNs were applied to the terpenic compounds, evaluated by SPME-GC/MS, of cheeses from different alpine pastures. Using specific software different architectures (MLP, GRNN, LN) of ANNs were defined to highlight the differences among samples and select those with the greatest capacity of sample re-classification. Also a variable selection among those identified by SPME-GC was performed to optimize the ANN structure. The obtained results showed that it is possible through terpenic component, despite its variability, and a "dynamic" system for calculating as the ANNs, discriminate against products and build an exportable model useful for control of origin.

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