Online Version ISSN: 1314-412X
Volume 3, Number 4
December 2011

AGRICULTURAL SCIENCE AND TECHNOLOGY

2011

An International Journal Published by Faculty of Agriculture,
Trakia University, Stara Zagora, Bulgaria

Scope and policy of the journal
Agricultural Science and Technology /AST/ – an International Scientific Journal of Agricultural and Technology Sciences is published in English in one volume of 4 issues per year, as a printed journal and in electronic form. The policy of the journal is to publish original papers, reviews and short communications covering the aspects of agriculture related with life sciences and modern technologies. It will offer opportunities to address the global needs relating to food and environment, health, exploit the technology to provide innovative products and sustainable development. Papers will be considered in aspects of both fundamental and applied science in the areas of Genetics and Breeding, Nutrition and Physiology, Production Systems, Agriculture and Environment and Product Quality and Safety. Other categories closely related to the above topics could be considered by the editors. The detailed information of the journal is available at the website. Proceedings of scientific meetings and conference reports will be considered for special issues.

Submission of Manuscripts
All manuscript written in English should be submitted as MS-Word file attachments via e-mail to ascitech@uni-sz.bg. Manuscripts must be prepared strictly in accordance with the detailed instructions for authors at the website http://www.uni-sz.bg/ascitech/index.html and the instructions on the last page of the journal. For each manuscript the signatures of all authors are needed confirming their consent to publish it and to nominate on author for correspondence. They have to be presented by a submission letter signed by all authors. The form of the submission letter is available upon from request from the Technical Assistance or could be downloaded from the website of the journal. All manuscripts are subject to editorial review and the editors reserve the right to improve style and return the paper for rewriting to the authors, if necessary. The editorial board reserves rights to reject manuscripts based on priorities and space availability in the journal.

Subscriptions
Agricultural Science and Technology is published four times a year. The subscription price for institutions is 80 € and for personal subscription 30 € which include electronic access and delivery. Subscription run for full calendar year. Orders, which must be accompanied by payment may be sent direct to the publisher:

Trakia University
Faculty of Agriculture, Bank account:
UniCredit Bulbank,
Sofia BIC: UNCRBGSF

IBAN: BG29UNCR76303100117681
With UniCredit Bulbank Stara Zagora

Internet Access
This journal is included in the Trakia University Journals online Service which can be found at www.uni-sz.bg.

Address of Editorial office:
Agricultural Science and Technology
Faculty of Agriculture, Trakia University
Student's campus, 6000 Stara Zagora
Bulgaria
Telephone.: +359 42 699330
+359 42 699446
http://www.uni-sz.bg/ascitech/index.html

Technical Assistance:
Nely Tzvetanova
Telephone.: +359 42 699446
E-mail: ascitech@uni-sz.bg

Online Version ISSN: 1314-412X

Volume 3, Number 4
December 2011

AGRICULTURAL SCIENCE AND TECHNOLOGY

2011

An International Journal Published by Faculty of Agriculture,
Trakia University, Stara Zagora, Bulgaria

AGRICULTURAL SCIENCE AND TECHNOLOGY, VOL. 3, No 4, pp 390 - 394, 2011

Near Infrared Spectroscopy for monitoring changes during yellow cheese ripening

S. Atanassova[1]*, N. Naydenova[2], T. Kolev[3], T. Iliev[2], G. Mihaylova[2]

[1]Department of Biochemistry, Microbiology and Physics, Faculty of Agriculture, Trakia University, 6000 Stara Zagora, Bulgaria
[2]Department of Animal Science, Faculty of Agriculture, Trakia University, 6000 Stara Zagora, Bulgaria
[3]Milk factory "Milk World 2003" Ltd, 6000 Stara Zagora, Bulgaria

Abstract. . *The aim of the study has investigation of the feasibility of Near infrared(NIR) spectroscopy in a diffusion reflection mode as a tool for the monitoring a chemical changes during ripening of Bulgarian yellow cheese from cow milk and classification of cheese according to ripening stage. The cheeses were made according to Bulgarian national standard for Bulgarian yellow cheese from cow milk. Samples for spectroscopic and chemical analysis were taken after 1, 5, 10, 15, 20, 25, 30, 35, 40 and 45 days of ripening. Total protein, water-soluble protein, titratable acidity, dry matter, and sodium chloride content were determined for all samples. NIR measurements were performed by NIRQuest 512 spectrometer (Ocean Optics, Inc.) in the region 900-1700 nm using reflection fiber-optics probe. Partial least square regression with internal cross-validation was used for calibration models development for determination of tested parameters. Soft Independent Modeling of Class Analogy (SIMCA) was implemented to create models of the cheese samples according to maturity stages based on their NIR spectra. Differences in spectral data of cheese samples during ripening were found. The biggest variation in spectral data were observed at 1215, 1350, 1644 nm, region from 1415 to 1470 and 1682-1700 nm. Determination of water-soluble protein, dry matter content and titratable acidity with coefficient of multiple correlation bigger than 0.95 and ratio of standard error of calibration and standard deviation of data set bigger than 3 was excellent. Very good determination was found for maturity rate. Prediction accuracy was good for total protein content and poor for NaCl content of cheese. SIMCA model for discrimination of cheese samples according to ripening stage was developed. We can conclude from obtained results that NIR has a potential for assessing cheese ripening related to changes in the cheese matrix during maturity.*

Keywords: Bulgarian yellow cheese, ripening, near-infrared spectroscopy.

Abbreviations: NIRS – near-infrared spectroscopy, PLS – partial least squares, PC – principal component, SIMCA – Soft Independent Modeling of Class Analogy, R– multiple correlation coefficient, SEC – standard error of calibration, SECV – standard error of cross validation, BNS – Bulgarian national standard.

Introduction

Cheese undergoes a complex series of chemical, bacterial and enzymatic reactions during maturation or ripening phase, which are responsible for transformation of fresh curd into a distinctive aged cheese. During ripening, degradation of lactose, proteins and fat are carried out and information regarding the evolution of lipolysis, glycolysis, and proteolysis may help the cheese-maker to better understand the biochemical kinetics of ripening to improve the ripening process. Determination of the degree of ripening and various chemical parameters is an important part of cheese quality evaluation. Classical methods for cheese chemical analysis are time-consuming and expensive. Considerable interest for development of instrumental techniques for objective, faster and less expensive assessments of cheese quality existed. Ideally, such techniques should also be non-invasive and non-destructive.

Near infrared (NIR) spectroscopy has been used as a method to predict the quality of different foods and agricultural products due to the speed of analysis, minimal sample preparation and low cost. The NIR spectrum of foods can give a global signature of composition which, with the application of chemometric techniques can be used to elucidate particular compositional characteristics in the food matrix. NIR has shown good prediction abilities for determination of fat, moisture, protein and inorganic salts in cheese (Adamopoulos et al., 2001; Blaquez et al., 2004, Curda and Kukackova, 2004). Cattaneo et al. (2005) used FT-IR and FT-NIR for analysis of the Italian fresh cheese Crescenza. The authors found a successful discrimination between cheeses according to their storage time using the PCA and concluded that NIR could be a suitable technique for the evaluation of the shelf-life of Crescenza cheese.

The potential of NIR to predict maturity and sensory attributes of 24 Cheddar cheeses produced using five renneting enzymes and stored at 4 C for up to 9 months has been investigated by Downey et al. (2005). The authors showed that NIR spectroscopy has demonstrated the ability to predict cheese maturity and several sensory attributes with sufficient accuracy to be industrially useful and recommended the use of NIR reflectance spectroscopy for routine quality assessment of processed cheese.

The aim of the study has investigation of the feasibility of NIR spectroscopy in a diffusion reflection mode as a tool for the monitoring a chemical changes during ripening of Bulgarian yellow cheese from cow milk and classification of cheese according to ripening stage.

Material and methods

Cheese manufacturing, sampling and physico-chemical analysis

The cheeses were made using a classical semi-hard cheese making scheme, according to Bulgarian national standard for Bulgarian yellow cheese from cow milk BNS 14-2010. Samples for

* e-mail: atanassova@uni-sz.bg

spectroscopic and chemical analysis were taken after 1, 5,10, 15, 20, 25, 30, 35, 40 and 45 days of ripening. Two or three subsamples were taken at every sampling day. Total protein, water-soluble protein, titratable acidity, dry matter, and sodium chloride (NaCl) content were determined for all samples according to the Bulgarian national standard (BNS), ISO standards and International Dairy Federation standards (IDF). The content of total protein was determined by Kjeldal method according to the BNS EN ISO 8968-1:2002 standard; the water-soluble protein by BNS EN ISO 8968-5: 2002 standard; titratable acidity by Thorner method (BNS 1111:1980) and expressed in Thorner degrees (°Th); dry matter by oven drying to constant weight at 102°C ± 2°C (BNS 1109:1989); NaCl content by BNS 8274:1982; and maturity rate as relation of water-soluble protein and total protein content (IDF 20-5:2001), respectively.

Near-infrared analysis

NIR measurements were performed by NIRQuest 512 spectrometer (Ocean Optics, Inc.) in the region 900-1700 nm using reflection fiber-optics probe without destruction or any kind of treatment of the samples. Two or three measurements at different part of the samples were made to minimise any possible effects of structural variation in the samples.

A commercial program Pirouette Version 2.0 (Infometrics, Inc., Woodinville, WA, USA) was used for performing of spectral data processing. Partial least square regression (PLS) with internal cross-validation was used for calibration models development for determination of tested parameters. The statistics used to select the best equations were multiple correlation coefficients R and the standard error of cross-validation SECV. Moreover, the mathematical treatment of spectral data – smoothing or transformation as first or second derivative using Savitsky-Golay procedure were tested in the development of calibration models. Soft Independent Modeling of Class Analogy (SIMCA) was implemented to create models of the cheese samples according to maturity stages based on their NIR spectra.

Results and discussion

Chemical analysis

The results for the range, mean values and standard deviation of Titrable acidity, Dry Matter, Total Protein, Water-soluble protein, and NaCl content of cheese samples, determined by classical chemical analyses, and calculated Maturity rate are presented in Table 1. The biggest changes during ripening were observed in titratable acidity, dry matter and water-soluble protein content in cheese samples.

Table 1. Range, mean values and standard deviation (SD) of tested parameters in cheese samples

Parameter	Min	Max	Average	SD
Dry Matter, %	56.59	62.14	58.47	2.01
Total Protein, %	23.43	26.14	24.40	0.91
Water-soluble protein, %	2.26	5.08	4.52	0.87
NaCl, %	2.45	2.69	2.59	0.13
Titratable acidity	96.8	156.6	136.5	22.26
Maturity rate	9.38	21.68	18.17	3.47

Cheese spectra

Second derivative absorbance spectra of measured samples are presented in figure 1. The second derivative technique is often used to process NIR data. It helps to separate overlapping absorption bands, remove baseline shifts and increase apparent spectral resolution. Differences in spectral data of cheese samples during ripening were found. The biggest variation in spectral data were observed at 1215, 1350, 1644 nm, region from 1415 to 1470 and 1682-1700 nm. The absorption peak at 1215 and 1350 nm could be related to the second overtone of C–H stretch vibration. The absorption in the 1415–1470 nm region might be assigned to first overtone of O–H stretch and N–H in amino and amide groups. The bands at 1644 and 1682 nm correspond to the first overtone of the C–H stretch of CH_2 groups. Analysis of cheese spectra showed that main differences in cheese chemical composition during ripening, which were connected with changes in dry matter and protein fraction, influenced significantly cheese spectra in the investigated region.

Quantitative determinations of tested parameters

The results of the quantitative determination of Titratable acidity, Dry Matter, Total Protein, Water-soluble protein, Maturity rate and NaCl content of cheese samples by PLS regression are presented in Table 2. The accuracy of each calibration equation was evaluated based on R – multiple correlation coefficient between values of cheese chemical parameter and NIR spectra, SEC-standard error of calibration, SECV – standard errors of cross validation and value of RPD- the ratio of standard deviation of data set SD to the SEC. The RPD evaluated the prediction errors in light of the standard deviation of the reference data and thus enables comparison between models for constituents with different variation ranges. The RPD values showed levels of prediction accuracy as follows: RPD between 1.5 and 2.0 indicates poor predictions; RPD between 2.0 and 2.5 indicates good prediction; RPD between 2.5 and 3.0 indicates very good prediction; and RPD >3 indicates excellent prediction. According to these criteria, determination of water-soluble protein and dry matter content and titratable acidity with R>0.95 and RPD>3 was excellent. Very good determination was found for maturity rate. Prediction accuracy was good for total protein content and poor for NaCl content of cheese. Unsuccessful prediction of NaCl could be explained by the fact that NaCl had not specific absorbance bands in the NIR region and determination of

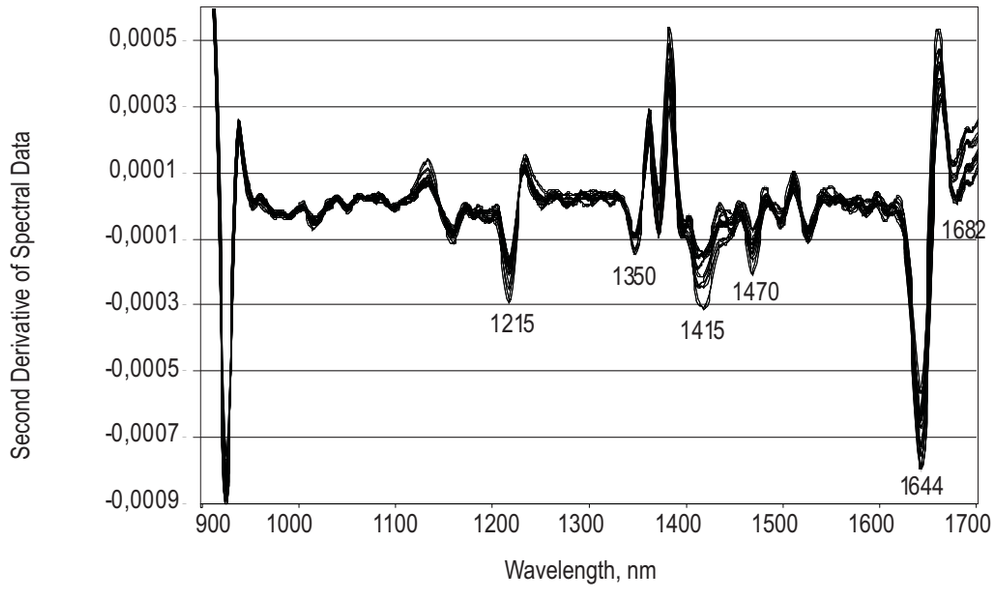

Figure 1. Second derivative absorbance spectra of measured cheese samples.

Table 2. Statistical data of the calibration equations for NIRS prediction of tested parameters' content in examined samples

Parameter	Data Transformation	PLS factors	SECV	SEC	R	RPD
Dry Matter, %	1D	7	1.40	0.65	0.95	3.11
Total Protein, %	Smooth	8	0.59	0.45	0.88	2.04
Water-soluble protein, %	1D	7	0.38	0.16	0.98	5.50
NaCl, %	Smooth	7	0.10	0.08	0.81	1.45
Titratable acidity	1D	6	9.43	4.67	0.98	4.37
Maturity rate	1D	9	1.29	0.41	0.99	2.69

SEC – standard error of calibration, SECV – standard error of cross-validation, R – coefficient of multiple correlation, RPD – ratio between SD and SEC.

NaCl was indirect. Another explanation of the poor prediction of NaCl content would be the very small range of concentration in measured samples. Figure 2-4 graphically illustrate the relationships between actual and averaged NIR spectroscopy predicted values for each days of sampling for dry matter, water-soluble protein and maturity rate of tested cheese samples. These results were in agreement with previous finding reporting that NIRS could be a suitable technique for the prediction of chemical composition of cheeses. Karoui et al. (2006) reported RPD values bigger than 2.5 for determination of total protein, non-protein nitrogen and water-soluble protein content in Emmental cheese by NIR spectroscopy. The reported accuracy of determination of water-soluble nitrogen was better than those for total protein content. Unsuccessful prediction of NaCl content was reported.

Differences in prediction accuracy of total protein and water-soluble protein content could be explained with changes in milk protein fractions during ripening. Proteolysis is one of the principal biochemical events during the ripening of a cheese. Formation of large (water-insoluble) and intermediate-sized (water-soluble) peptides is initial result of caseins hydrolysis. In the next step of ripening peptides are degraded further by enzymes from the starter and non-starter microflora of the cheese. The final products of proteolysis are free amino acids. Therefore total protein is very rough characteristics of protein content in cheese, which do not take into account changes in protein fraction of cheese during ripening.

Concentration of water-soluble proteins in cheese at any stage of ripening and maturity rate could be used as criteria for cheese maturity. Excellent NIR spectroscopy prediction accuracy of these parameters in investigated cheese, as well as titratable acidity and dry matter content, suggested that NIR has a potential as a rapid screening tool for assessing cheese ripening related to changes in the cheese matrix during ripening.

SIMCA classification

SIMCA models for discrimination of cheese samples according to ripening stage were developed. Samples were divided into 3 classes – samples taken after 1, 5, 10 and 15 day after manufacturing formed class 1, samples taken 20, 25 and 30 days formed class 2, and samples taken 35, 40 and 45 formed class 3, respectively. Correct classification of all samples was obtained with models, based on smoothed raw data and 7 PC factors in the model for class1, 8 PC in the model for class 2 and 9 PC factors in the model for class3. Graphical presentation of obtained result was presented in figure 5. The interclass distance represents the distance between the classes in SIMCA models. Greater the distance between two classes the greater is the difference in composition of samples

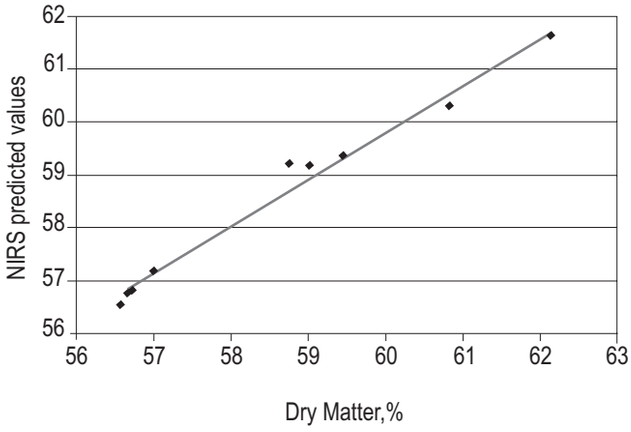

Figure 2. Relation between actual and NIRS predicted values of dry matter content in tested cheese samples.

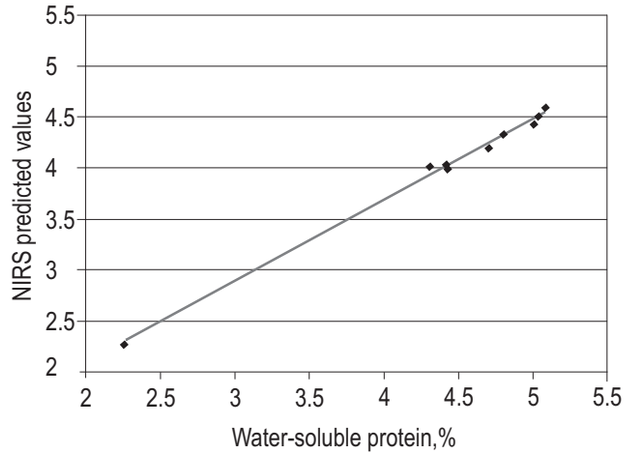

Figure 3. Relation between actual and NIRS predicted values of water-soluble protein in tested cheese samples.

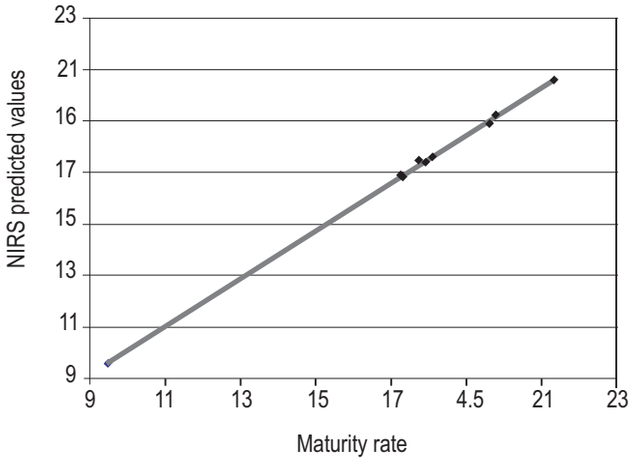

Figure 4. Relation between actual and NIRS predicted values of maturity rate of tested cheese samples.

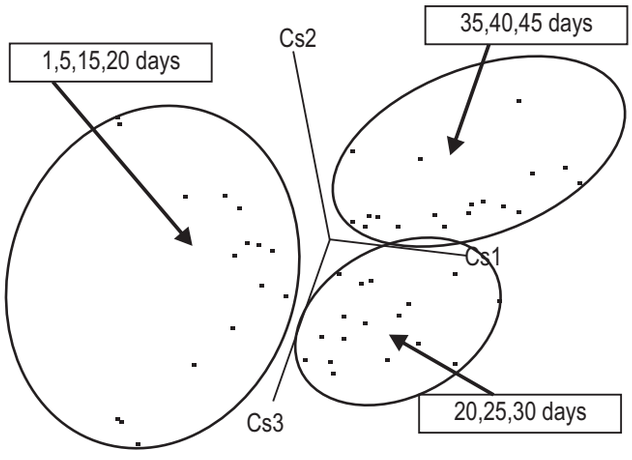

Figure 5. SIMCA classification plot for discrimination of cheese samples according to ripening time.

belonging to those clusters. As a rule of thumb, a distance of over 3 indicates that the samples are well separated. Parameter "Interclass distance" from SIMCA procedure between class 1 and class 2 was 1.49, between class 2 and class 3 was 1.74, and between class1 and class 3 - 3.47, respectively. The interclass distance bigger than 3 was obtained between models for samples from the beginning and the end of ripening which showed good separated classes.

Conclusion

Differences in near-infrared spectral data of cheese samples during ripening were found. The biggest variation in spectral data were observed at 1215, 1350, 1644nm, region from 1415 to 1470 and 1682-1700nm.

The results of quantitative determination indicated the suitability of near-infrared spectroscopy for the determination of dry matter, total protein and water-soluble protein content, titratable acidity and maturity rate of Bulgarian yellow cheese. SIMCA models for discrimination of cheese samples according to ripening stage were developed. Correct classification of all samples was obtained with models, based on smoothed raw data. NIR has a potential as a rapid screening tool for assessing cheese ripening related to changes in the cheese matrix during maturity. The method is rapid and non-destructive, no sample preparation is necessary. The application of NIR spectroscopy can improve the dairy production economy through the optimised laboratory efficiency, the increased product quality and the tighter production control.

Acknowledgements

Financial support for the study was provided by the National Scientific Fond of Bulgarian Ministry of Youth, Education and Science, project DO 02-143/2008.

References

Adamopoulos G, Goula AM and Petropakis HJ, 2001. Quality

control during processing of Feta cheese-NIR Application. Journal of Food Composition and Analysis, 14, 431–440.
Blaquez C, Downey G, O'Donnell C, O'Callaghan D and Howard V, 2004. Prediction of moisture, fat and inorganic salts in processed cheese by near infrared reflectance spectroscopy and multivariate data analysis. Journal of Near Infrared Spectroscopy, 12, 149–157.
BNS 1111:1980. Milk and milk products. Determination of acidity.
BNS 8274:1982. Milk and milk products. Methods for determination of NaCl.
BNS. Milk and milk products. Methods for determination of moisture and dry matter.
BNS EN ISO 8968-1:2002. Milk - Determination of nitrogen content - Part 1: Kjeldahl method (ISO 8968-1:2001).
BNS EN ISO 8968-5:2002. Milk - Determination of nitrogen content - Part 5: Determination of protein-nitrogen content (ISO 8968-5:2001).
Cattaneo T, Giardina C, Sinelli N, Riva M and Giangiacomo R, 2005. Application of FT-NIR and FT-IR spectroscopy to study the shelf-life of Crescenza cheese. International Dairy Journal 15, 693–700.
Čurda L and Kukacková O, 2004. NIR spectroscopy: A useful tool for rapid monitoring of processed cheeses manufacture. Journal of Food Engineering, 61, 557-560.
Downey G, Sheehan E, Delahunty C, O'Callaghan D, Guinee T and Howard V, 2005. Prediction of maturity and sensory attributes of Cheddar cheese using near infrared spectroscopy. International Dairy Journal, 15, 701–709.
IDF 20-5:2001 Milk -- Determination of nitrogen content -- Part 5: Determination of protein-nitrogen content
Karoui R, Mouazen AM, Dufour É, Pillonel L, Schaller E, De Baerdemaeker J and Bosset JO, 2006. Chemical characterization of European Emmental cheeses by near infrared spectroscopy using chemometric tools. International Dairy Journal, 16, 1211–1217.

CONTENTS **1 / 2**

CONTENTS **2 / 2**

Instruction for authors

Preparation of papers

Papers shall be submitted at the editorial office typed on standard typing pages (A4, 30 lines per page, 62 characters per line). The editors recommend up to 15 pages for full research paper (including abstract references, tables, figures and other appendices)

The manuscript should be structured as follows: Title, Names of authors and affiliation address, Abstract, List of keywords, Introduction, Material and methods, Results, Discussion, Conclusion, Acknowledgements (if any), References, Tables, Figures.

The title needs to be as concise and informative about the nature of research. It should be written with small letter /bold, 14/ without any abbreviations.

Names and affiliation of authors

The names of the authors should be presented from the initials of first names followed by the family names. The complete address and name of the institution should be stated next. The affiliation of authors are designated by different signs. For the author who is going to be corresponding by the editorial board and readers, an E-mail address and telephone number should be presented as footnote on the first page. Corresponding author is indicated with *.

Abstract should be not more than 350 words. It should be clearly stated what new findings have been made in the course of research. Abbreviations and references to authors are inadmissible in the summary. It should be understandable without having read the paper and should be in one paragraph.

Keywords: Up to maximum of 5 keywords should be selected not repeating the title but giving the essence of study.

The introduction must answer the following questions: What is known and what is new on the studied issue? What necessitated the research problem, described in the paper? What is your hypothesis and goal ?

Material and methods: The objects of research, organization of experiments, chemical analyses, statistical and other methods and conditions applied for the experiments should be described in detail. A criterion of sufficient information is to be possible for others to repeat the experiment in order to verify results.

Results are presented in understandable tables and figures, accompanied by the statistical parameters needed for the evaluation. Data from tables and figures should not be repeated in the text.

Tables should be as simple and as few as possible. Each table should have its own explanatory title and to be typed on a separate page. They should be outside the main body of the text and an indication should be given where it should be inserted.

Figures should be sharp with good contrast and rendition. Graphic materials should be preferred. Photographs to be appropriate for printing. Illustrations are supplied in colour as an exception after special agreement with the editorial board and possible payment of extra costs. The figures are to be each in a single file and their location should be given within the text.

Discussion: The objective of this section is to indicate the scientific significance of the study. By comparing the results and conclusions of other scientists the contribution of the study for expanding or modifying existing knowledge is pointed out clearly and convincingly to the reader.

Conclusion: The most important consequences for the science and practice resulting from the conducted research should be summarized in a few sentences. The conclusions shouldn't be numbered and no new paragraphs be used. Contributions are the core of conclusions.

References:

In the text, references should be cited as follows: single author: Sandberg (2002); two authors: Andersson and Georges (2004); more than two authors: Andersson et al.(2003). When several references are cited simultaneously, they should be ranked by chronological order e.g.: (Sandberg, 2002; Andersson et al., 2003; Andersson and Georges, 2004). References are arranged alphabetically by the name of the first author. If an author is cited more than once, first his individual publications are given ranked by year, then come publications with one co-author, two co-authors, etc. The names of authors, article and journal titles in the Cyrillic or alphabet different from Latin, should be transliterated into Latin and article titles should be translated into English. The original language of articles and books translated into English is indicated in parenthesis after the bibliographic reference (Bulgarian = Bg, Russian = Ru, Serbian = Sr, if in the Cyrillic, Mongolian = Mo, Greek = Gr, Georgian = Geor., Japanese = Ja, Chinese = Ch, Arabic = Ar, etc.)

The following order in the reference list is recommended:

Journal articles: Author(s) surname and initials, year. Title. Full title of the journal, volume, pages. Example:

Simm G, Lewis RM, Grundy B and Dingwall WS, 2002. Responses to selection for lean growth in sheep. Animal Science, 74, 39-50

Books: Author(s) surname and initials, year. Title. Edition, name of publisher, place of publication. Example:

Oldenbroek JK, 1999. Genebanks and the conservation of farm animal genetic resources, Second edition. DLO Institute for Animal Science and Health, Netherlands.

Book chapter or conference proceedings: Author(s) surname and initials, year. Title. In: Title of the book or of the proceedings followed by the editor(s), volume, pages. Name of publisher, place of publication. Example:

Mauff G, Pulverer G, Operkuch W, Hummel K and Hidden C, 1995. C3-variants and diverse phenotypes of unconverted and converted C3. In: Provides of the Biological Fluids (ed. H. Peters), vol. 22, 143-165, Pergamon Press. Oxford, UK.

Todorov N and Mitev J, 1995. Effect of level of feeding during dry period, and body condition score on reproductive performance in dairy cows, IX th International Conference on Production Diseases in Farm Animals, Sept.11 – 14, Berlin, Germany, p. 302 (Abstr.).

Thesis:

Penkov D, 2008. Estimation of metabolic energy and true digestibility of amino acids of some feeds in experiments with muscus duck (Carina moshata, L). Thesis for DSc. Agrarian University, Plovdiv, 314 pp.

The Editorial Board of the Journal is not responsible for incorrect quotes of reference sources and the relevant violations of copyrights.

AGRICULTURAL SCIENCE AND TECHNOLOGY

Volume 3, Number 4
December 2011

Journal web site:
www.uni-sz.bg/ascitech/index.html

Publisher:
www.alfamarket.biz