

Determination of ketone bodies and citric acid in cow's milk for diagnostic purposes  
summary of PhD thesis

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## I. INTRODUCTION

The energy deficiency in high-yielding dairy cows can cause subclinical or clinical ketosis, in which the concentration of ketone bodies (acetone, acetoacetate and  $\beta$ -hydroxybutyrate) increases in the different body fluids. The subclinical ketosis is associated with decreased body conditions and milk yield, impaired reproductive performance and increased risk of clinical ketosis. The early detection of elevated levels of ketone bodies during the early, subclinical stage of energy deficiency is highly recommended as on this basis appropriate corrections in the herd management can be introduced to reduce the development of clinical ketosis - consequently the profit-loss.

The aim of this study was:

- method development, optimisation and validation for reference and routine laboratory analysis of ketone bodies and citrate,
  - as a preliminary study clarifying for diagnostic reason that connection between elevated ketone body formation and citrate concentration in milk exists or not.
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## II. METHODS AND METHOD DEVELOPMENTS

### II.1. Determination of acetone

- As a reference methods a new gas chromatographic (GC) and a headspace gas chromatographic (HS-GC) methods were developed and validated for determination of acetone in cow's milk.
- A rapid, automated flow injection (FIA) method was optimised and validated for routine laboratory analysis of acetone in milk samples. The optimisation procedure was carried out by experimental design.

### II.2. Determination of total ketone bodies

- For determinations of oxidized, reduced and total ketone bodies from raw milk samples the HS-GC method including appropriate sample preparations was applied. The sample preparation is based on chemical oxidation of the ketone bodies to acetone in three consequent steps.

### II.3. Determination of citrate

- A sensitive and specific fluorimetric method for determination of citrate in raw milk samples [Hori et al., 1974] was applied with some modifications in sample preparation.

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### III. NEW SCIENTIFIC RESULTS

#### III.1. Method development

- The results of investigation of analytical parameters of GC and HS-GC methods proved their suitability for accurate, precise, sensitive and reproducible measurement of acetone in milk samples.
- Based on time and cost consumption and application in large sample numbers the new gas chromatographic methods were compared. The author suggests using HS-GC method as reference method of acetone determination of raw milk samples.
- The optimised FIA method was tested in 102 raw milk samples. Based on the results of the comparison FIA and HS-GC determinations it was established
- that the rapid and cheap flow injection analysis could be a very good alternative to gas chromatography for determination of acetone in milk.
- The HS-GC total ketone body analysis can be used as a reference method in milk samples.

#### III.2. Ketone bodies and citrate concentrations in cow's raw milk

- Under real conditions of milk sampling, transport and storage, milk samples of 119 dairy cows were investigated. Based on the results of ketone bodies and citrate determinations the author established the follows:
- during the traditional sample handling the sample's acetoacetate spontaneously decarboxylised to acetone;
- in the metabolically most critical first 6 weeks of lactation the  $\beta$ -hydroxybutyrate and the acetone concentration showed an opposite change: the acetone content decreased, while  $\beta$ -hydroxybutyrate increased in milk;
- significant positive correlation and linear connection between acetone and  $\beta$ -hydroxybutyrate levels was found in the samples with acetone concentration  $>0.4$  mmol/l;
- the author found parallel drop of citrate and acetone during the lactation, which can be clearly explained on the basis of biochemistry of tricarmonic acid cycle and ketogenesis;
- significant negative correlation between citrate and  $\beta$ -hydroxybutyrate and acetone was also found in the samples with acetone concentration  $>0.4$  mmol/l.

Focusing on these results related to the data in the literature found, a routine laboratory analysis of milk citrate could be a useful tool in early detection of energy deficiency in high-producing dairy cows.

To prove this hypothesis, it is necessary

- to develop a routine laboratory method (i.e. enzymatic FIA or Fourier transformation infrared spectroscopy) for determination of citrate in milk;
  - to investigate more fresh milk samples from cows with well known health-status, precisely described and controlled conditions of sample origin, sampling, transport and storage.
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#### IV. PUBLICATION LIST

##### Papers:

1. Tömösközi, S., Baticz, O. and Lásztity, R.: Determination of total cholesterol content in foods by flow injection analysis with immobilized cholesterol oxidase enzyme reactor, *Nahrung/Food*, 46 (1), 2002.
2. Baticz, O., Vida, L. and Tömösközi, S.: Determination of acetone in cow's raw milk by flow injection and gas chromatographic methods, *Acta Alimentaria*, Vol. 30 (3), 297-311, 2001.
3. Tömösközi, S., Lásztity, R., Haraszi, R. and Baticz, O.: Isolation and study of the functional properties of pea proteins, *Nahrung/Food*, 45 (6), 399-401, 2001.
4. Baticz, O., Tömösközi, S., Vida, L. and Gaál, T.: Relationship between concentration of citric acid and ketone bodies in raw cow's milk, *Acta Vet. Med.* 2002. – accepted, in press
5. Lásztity, R., Khalil, M.M., Haraszi, R., Baticz, O. and Tömösközi, S.: Isolation, functional properties and potential use of protein preparations from lupin, *Nahrung/Food* 45 (6), 396-398, 2001.
6. Tömösközi, S., Baticz, O. and Örsi, F.: Élelmiszerek koleszterin-tartalmának meghatározása és a mérések automatizálásának lehetőségei, *Élelmezési Ipar*, LII. 6., 1998.
7. Tömösközi, S., Veress, T., Baticz, O., Simándi, B. and Örsi, F.: Szuperkritikus fluid extrakció, mint mintaelőkészítési módszer élelmiszerek koleszterintartalmának kivonására, *Olaj, szappan, kozmetika*, 49. évf. Különszám, 109-111, 2000.
8. Baticz, O., Kemény, S., Vida, L. and Tömösközi, S.: Kísérlettervezés alkalmazása acetontartalom FIA-eljárással történő meghatározásának optimalizálására, *Élelmiszervizsgálati Közlemények*, XLVII. kötet, 3-4. füzet, 113-126, 2001.
9. Baticz, O., Tömösközi, S., Vida, L. and Gaál, T.: Concentrations of citrate and ketone bodies in cow's raw milk and their relationships under real conditions, *J. of Dairy Sci.*, 2002 – in press

##### Oral presentations:

1. Tömösközi, S., Baticz, O., Haraszi, R. and Kemény, S. : Analitikai vizsgálatok minőségbiztosítása, *Hungalimentaria '99. konferencia*, Budapest 1999.
2. Tömösközi, S., Baticz, O., Csete, Zs., Gundel, J., Katona, F. and Prokai, S.: Takarmányozási problémák feltárása és a ketózis kialakulásának előrejelzése a nyerstej oxidált ketontest-tartalmának automatizált FIA technikával történő meghatározásával, 11. Magyar Buiatrikus Kongresszus, Siófok, 1999.
3. Tömösközi, S., Baticz, O. and Vida, L.: Acetontartalom meghatározása nyerstejben áramló injektációs analízissel, 296. tudományos kollokvium (KÉKI), Budapest, 1999.
4. Baticz, O., Tömösközi, S., Kemény, S. and Vida, L.: Kísérlettervezés alkalmazása acetontartalom FIA-eljárással, tejből történő meghatározásának optimalizálására, XIII. Élelmiszer Minőségellenőrzési Tudományos Konferencia, Székesfehérvár, 2000.
5. Baticz, O., Nógrádi, S. and Tömösközi, S.: Az áramló injektációs analitika környezetvédelmi alkalmazása, XV. Országos Környezetvédelmi Konferencia, Siófok, 2001.
6. Baticz, O., Tömösközi, S., Vida, L. and Gaál, T.: A nyers tehéntejben mérhető ketonanyag- és citromsav-koncentrációk összefüggései, MTA akadémiai beszámoló, Budapest, 2002.

##### Posters:

1. Tömösközi, S., Veress, T., Baticz, O., Simándi, B. and Örsi, F.: Szuperkritikus fluid extrakció, mint mintaelőkészítési módszer élelmiszerek koleszterintartalmának kivonására,

SFE konferencia, Budapest, 1999.

2. Baticz, O., Vida, L. and Tömösközi, S.: Relationship among quantities of different ketone bodies in raw milk, Reactions in Food IV. konferencia, Prága, 2000