**METHOD SPECIFICATION**

**Faculty of Biosciences, NMBU**

**Method name: Amino acid analysis (oxidized samples)**

BIOVIT No.: Msp1050

**1. Analysis method / Principle / Main instrument**

The method determines the total content (peptide-bound and free) amino acids in feed and faeces and is suitable for the amino acids cyst (e) in, methionine, aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine and arginine.

Tryptophan needs basic hydrolysis and therefore has its own method (see Msp 1051).

The procedure depends on which amino acids are to be studied. If Cysteine ​​and methionine are needed, they must be oxidized to cysteic acid and methionine sulfone before hydrolysis. The samples are further hydrolyzed with HCl for 24 hours. The amino acids are separated by ion exchange chromatography and determined by a reaction with ninhydrin and photometric detection at 570 nm (440 nm for proline).

**Main instrument:** 30+ Amino Acid Analyzer (Biochrom Ltd, Cambridge, England)

**2. Reference and any modifications**

Commission Regulation (EC) No 152/2009. 27 Jan 2009. Laying down the methods of sampling and analysis for the official control of feed. Annex III, P, Official Journal of the European Union L54 / 1 from 26/02/2009

* Determination of Amino Acids (except tryptophan) - page 19-24 (PART F)

**3. Requirements for grinding and storage**

Samples must be grinded to 0.5 mm. Moist samples must either be air-dried (at a temperature not exceeding 50 ° C) or lyophilized before painting.

Samples with a high fat content, e.g. fish feed with more than 40 % fat, are extracted with petroleum ether (bp. 40-60 ° C) before grinding.

The weighted analytical sample should contain approx. 10 mg nitrogen.

**4. Contact persons**

**Lab manager:** Hanne Kolsrud Hustoft

**Responsible for analysis:** Elin Follaug Johnsen

**5. Special remarks**

If cysteine and methionine is of no interest, the oxidation step can be skipped to save time.

It is possible to use the method to look at other amino acids than those mentioned in the reference method (1). Examples: taurine, hydroxyproline and GABA (needs another standard).

Tyrosine will degrade a bit during oxidation. This is corrected for in the spreadsheet, however if the levels of tyrosine are abnormally low, there may have been more degradation than usual.

It is important that the samples do not stand for oxidation longer than described in the method!

The method used to analyze amino acids causes a deamination of the two amino acids Asparagine and Glutamine, and they are turned into Aspartic acid and Glutamic acid.

So:

glutamic acid = glutamic acid + glutamine

aspartic acid = aspartic acid + asparagine

When publishing this may be clarified by writing: Glx and Asx

(Glx= Glu + Gln) (Asx = Asn + Asp)

Or write: “Glu + Gln” or “Asn + Asp

**6. Other literature**

* Manual for Biochrom 30+ amino acid analyzer
* Manual for Chromeleon software