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Determination of theanine in tea and instant tea in solid form using high-performance liquid chromatography

Détermination de la théanine dans le thé et le thé instantané sous forme solide en utilisant la chromatographie en phase liquide à haute performance

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: www.iso.org/iso/foreword.html.

The committee responsible for this document is ISO/TC 34, *Food products*, Subcommittee SC 8, *Tea*.

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Introduction

Theanine (N-ethyl-γ-L-glutamine) is a non-protein derived (free) amino acid. In the plant world, it is a unique amino acid found in *Camellia* genus and the mushroom *Boletus badius* and *Ilex guayusa*. The most common and the most prominent occurrence is in *Camellia sinensis* (L.) O. Kuntze species.

Theanine constitutes *circa* 0,1 % to 2 % w/w of the dry weight in tea leaves (*Camellia sinensis*) and is approximately 50 % of the total free amino acids. Separation of L- and D-theanine by HPLC is not achieved by this method; however, L-theanine is the major form (more than 95 %) in all teas[2][3]. Theanine has been associated with the typical tea umami taste and is also being studied to evaluate potential beneficial effects to health.

The umami taste characteristic has been associated with theanine which is also being evaluated for its potential benefits to health. This is also creating interest in theanine as an important novel food and dietary supplement ingredient.

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Determination of theanine in tea and instant tea in solid form using high-performance liquid chromatography

1 Scope

This document specifies a high-performance liquid chromatographic (HPLC) method for the determination of theanine content in tea (*Camellia sinensis*). It is applicable to both tea and instant tea samples. Separation of L- and D-theanine is not possible using this method; however, the L-enantiomer is the major form in tea.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 1572, *Tea — Preparation of ground sample of known dry matter content*

ISO 1573, *Tea — Determination of loss in mass at 103 °C*

ISO 1839, *Tea — Sampling*

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

ISO 7513, *Instant tea in solid form — Determination of moisture content (loss in mass at 103 °C)*

ISO 7516, *Instant tea in solid form — Sampling*

3 Terms and definitions

No terms and definitions are listed in this document.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— IEC Electropedia: available at http://www.electropedia.org/

— ISO Online browsing platform: available at http://www.iso.org/obp

4 Principle

A reversed-phase (RP)-C18 column is used which is capable of separating more polar compounds using water as an eluent and UV detection at 210 nm. Theanine is separated from the other amino acids; however, the potential co-elution of theanine with methionine is not relevant due to the very small concentration of methionine in tea. It is recommended that polyamide column extract clean-up is used to remove interfering polyphenols for column protection when separation of theanine from other constituents in the extract sample is difficult. Alternatively, the column should be washed after each tea sample using acetonitrile.

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