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INNOVATIVE METHOD FOR QUALITY AND SAFETY CONTROL OF ALCOHOLIC BEVERAGES

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Summary. *A gas chromatography flame ionization detector (GC-FID) method based on the use of ethanol as an internal standard was developed and compared with the official Chinese method for the determination of methanol in various alcoholic beverages. Both official and developed methods were approbated for the analysis of wide range of alcoholic beverage samples with ethanol concentrations from 6.5 to 70.0 % by volume.*

One of the most important indicators of the quality and safety of alcoholic products is the quantitative content of volatile compounds. Methyl alcohol is one of the most controlled volatile compounds in alcoholic beverages and raw materials for their production at the legislative level in the world.

In accordance with the Chinese official methods for the analysis of alcoholic beverages (GB/T 15038-2006, GB/T 11858-2008, GB 5009.266-2016, etc.) the determination of methanol in alcoholic beverages is carried out by GC-FID. Quantitative calculation of the mass concentration of the methanol is carried out according to the method of an internal standard (IS). The most abundant internal standard substances are *n*-butanol, 4-methylpentan-2-ol and 2-methylbutan-2-ol. 4-methylpentan-2-ol is also used as an IS in the European official methods for the analysis of alcoholic beverages (Commission Regulation (EC) No 2870/2000 of 19 December 2000 laying down Community reference methods for the analysis of spirits drinks, OIV-MA-AS312-03A, etc.).

Unfortunately, the official internal standard method has disadvantages, associated with preparation of standard solutions and manual procedure for the quantitative addition of an internal standard substance into the test sample, that affect the accuracy of the final analysis results.

The authors developed modified internal standard method, based on the use of ethanol as an internal standard. The method avoids the aforementioned disadvantages of the traditional internal standard method. There is no need to add any internal standard to the analysed sample, since ethyl alcohol is an essential compound of any alcoholic beverage. The developed method is applicable to the analysis of any liquid products containing ethanol, which can be analysed using GC systems. The concentration of ethanol in any ethanol product, expressed as the mass of ethanol per liter of absolute alcohol, is the density of ethanol, so the concentration of the internal standard used in the developed method is always known. For this reason, the method allows to obtain the concentration of methanol directly from gas chromatographic measurements in legally required (GB/T 11858-2008) dimension of mass per litre of absolute alcohol (ethanol) units (g/hL of absolute alcohol (AA) or mg/L AA).

Both official and developed methods were approbated for the analysis of wide range of alcoholic beverage samples. As samples (with corresponding ethanol volume concentration in %) were studied: rum (40 %), whiskey (40 %), bourbon (43 %), grain spirit (40 %), brandy (40 %), grappa (40 %), calvados (40 %), gin (47 %), vodka (40 %), slivovice (45 %), tsikoudia (38 %), sake (14.5 %), tequila (38 %), vermouth (15 %), nalewka (18 %), mulled wine (8.5 %), rectified spirit (70 %), cocktail (27.5 %), sambuca (38 %), egg (17 %), herbal (35 %), limon (25 %), cherry (16 %), raspberry (16.5 %) and sloe gin (35 %) liqueurs). 4-methylpentan-2-ol was used as IS for the official method. The comparison of the obtained results was perfomed at a 0.05 significance level, employing the statistical Student's test (*t*-Test: Paired Two Sample for Means) and ANOVA

(Single factor) for both the official and developed methods. As a null hypothesis, the similarity between the concentration for the official and developed methods was taken.

The relative difference between the results, obtained for both official and developed method, was calculated according to the following formula

$$\Delta = \left(C(D) - C(O)\right) / C(O) \cdot 100\ \%, \quad (1)$$

where *C*(*D*) and *C*(*O)* are the concentrations of an analyte in the studied sample of alcoholic beverage, obtained by the developed and official methods, correspondingly, mg/L AA.

The obtained results of analysis are presented in the tab. 1.

Table 1 – The results of determination of methanol concentration and its standard deviation (*SD*) in alcoholic beverages by both official and developed methods

Table 1

Result	Rum	Whiskey	Bourbon	Grain spirit	Brandy
C(*O*)±*SD*, mg/L AA	22.2±0.5	132±2	88.4±1.2	110±1.6	297±2
C(*D*) ±*SD*, mg/L AA	22.3±0.6	130±1	88.9±0.5	111±0.7	297±1
Δ, %	0.7	–0.9	0.6	0.9	–0.2
Result	Grappa	Calvados	Gin	Vodka	Slivovice
C(*O*)±*SD*, mg/L AA	414±5	910±5	4.16±0.09	21.8±0.2	10546±97
C(*D*) ±*SD*, mg/L AA	412±2	913±2	4.19±0.16	21.7±0.2	10603±18
Δ, %	–0.6	0.3	0.8	–0.7	0.5
Result	Tsikoudia	Sake	Tequila	Vermouth	Nalewka
C(*O*)±*SD*, mg/L AA	755±50	18.2±1.3	1456±35	17.5±0.1	168±5
C(*D*) ±*SD*, mg/L AA	761±20	18.1±1.4	1460±10	17.6±0.2	169±4
Δ, %	0.8	–1.0	0.3	0.6	0.9
Result	Mulled wine	Rectified spirit	Cocktail	Sambuka	Egg liqueur
C(*O*)±*SD*, mg/L AA	25.3±3.0	6.05±0.39	77.3±0.7	2.32±0.04	9.75±0.28
C(*D*) ±*SD*, mg/L AA	25.1±2.7	6.03±0.40	76.3±1.5	2.34±0.05	9.81±0.14
Δ, %	–0.6	–0.4	–1.2	0.8	0.7
Result	Liqueurs				
	Herbal	Limon	Cherry	Raspberry	Sloe gin
C(*O*)±*SD*, mg/L AA	19.5±0.1	29.1±0.9	9.77±1.34	127±5	20.5±0.7
C(*D*) ±*SD*, mg/L AA	19.6±0.1	29.4±1.0	9.82±1.27	126±4	20.7±0.4
Δ, %	0.4	0.8	0.5	–1.1	0.5

The comparison of the results obtained for both official and developed showed that the relative difference between the values of concentrations is less than ±1.5 %. Both statistical tests (Student's test and ANOVA) confirmed that the difference between the means, obtained for both methods for all the studied samples is statistically insignificant at a 0.05 significance level.

The obtained results show, that the developed method can also be validated for a number of volatile congeners in alcoholic products and ethanol containing products in general. The obtained results can be an occasion for launching the interlaboratory study of the developed modified internal standard GC-FID method in order to improve official methods of analysis and make them simpler, faster, easier, cheaper, more reliable and robust.

Authors have prepared improved version of the National standards of People's Republic of China GB/T 15038 [1] and GB/T 11858 [2] based on the use of ethanol as an internal standard substance. The yellow text highlights the text to be deleted. Green highlights text that needs to be inserted into the documents. The simplicity and accessibility of the implementation of the method in the daily practice of testing laboratories is impressive. Video presentation of the method is presented at open access resources [3].

References

1. Improved document National standard of People's Republic of China GB/T 15038-2006 Analytical methods of wine and fruit wine [Electronic resource]. – Mode of access: https://elab.bsu.by/download.php?id=309. – Date of access: 24.10.2022.

2. Improved document National standard of People's Republic of China GB/T 11858-2008 National Food Safety Standards Vodka [Electronic resource]. – Mode of access: https://elab.bsu.by/download.php?id=307. – Date of access: 24.10.2022.

3. Video presentation of the method in Chinese [Electronic resource]. – Mode of access: https://www.youtube.com/watch?v=uKut-YQaVxg. – Date of access: 24.10.2022.

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МИКРОБИОТА ЖЕЛУДОЧНО-КИШЕЧНОГО ТРАКТА И ЦИРКАДНЫЕ РИТМЫ ЧЕЛОВЕКА

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Summary. *The paper shows the relationship "microbiota-gut-brain" and the importance of studying the prognostic significance of the nature of the metabolic profile of the intestinal microflora against the background of stress from circadian rhythm disorders. It is recommended to include up-to-date data on the biochemical and physiological features of the intestinal microbiota and its effect on the human body in curricula when teaching biological disciplines such as "Microbiology" and "Normal Physiology".*

На сегодняшний день в научной среде сформировались определенные взгляды на функционирование микрофлоры и ее значение для организма хозяина. Доказано наличие взаимодействия между микробиотой кишечника и эндокринной, нейро-иммунной, иммунной системой, которое тем не менее недостаточно изучено. Перспективным направлением исследований является изучение оси микробиота-кишечник-мозг и различных расстройств (нарушений циркадного ритма, режима сна). Модификация кишечной микробиоты с помощью пробиотиков, пребиотиков и постбиотиков открывает новые подходы к изменению функций мозга и лечению стрессовых расстройств с учетом новых взаимосвязей «микробиота-кишечник-мозг». В связи с чем, изучение прогностической значимости характера метаболитного профиля кишечной микрофлоры (состава короткоцепочечных жирных кислот) на фоне стресса, влияние на данный профиль микробиома введения биологически активных добавок может послужить научным обоснованием для разработки отечественных биологически активных добавок для коррекции микробиоты кишечника в условиях воздействия стрессовых факторов на организм.

Кишечные бактерии все чаще признаются критически важным органом в организме человека, необходимым для развития и поддержания целостности и барьера кишечника, а также оптимального сбора энергии. Они играют важную роль в контроле метаболизма и иммунитета хозяина [1–2]. Таким образом, неудивительно, что микробиота кишечника и ее метаболиты также потенциально могут влиять на циркадные ритмы хозяина [3]. Метаболизм человека адаптирован к циркадному ритму продолжительностью около 24 часов, который синхронизирован с земным 24 – часовым циклом свет/темнота. Этот ритм управляется мозгом в гипоталамусе, который, в свою очередь, синхронизирует остальные части тела. Этот механизм молекулярных часов играет важную роль в регуляции ритмической экспрессии генов, контролируемых часами, которые, в свою очередь, регулируют синтез, хранение и расход энергии [4]. Цикл свет/темнота – это наиболее мощный внешний, или экологический сигнал для человеческого организма [4]. Круглосуточный образ жизни, состоящий из сменной работы, ранних утренних подъемов, позднего отхода ко сну, смены часовых поясов, может привести к нарушению циркадных ритмов, поскольку наши внутренние часы могут не