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Original Article

Detection of 10 sweeteners in various foods by liquid chromatography/tandem mass spectrometry

Chui-Shiang Chang [a], Tai Sheng Yeh [b,*]

[a] Public Health Bureau, Pingtung County Government, Pingtung, Taiwan, ROC
[b] Department of Food Science and Nutrition, Meiho University, Neipu Township, Pingtung, Taiwan, ROC

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ABSTRACT

The analytical method for sweeteners in various food matrixes is very important for food quality control and regulation enforcement. A simple and rapid method for the simultaneous determination of 10 sweeteners [acesulfame potassium (ACS-K), aspartame (ASP), cyclamate (CYC), dulcin (DUL), glycyrrhizic acid (GA), neotame (NEO), neohesperidin dihydrochalcone (NHDC), saccharin (SAC), sucralose (SCL), and stevioside (STV)] in various foods by liquid chromatography/tandem mass chromatography (LC–MS/MS) was developed. The chromatographic separation was performed on a Phenomenex Luna Phenyl-Hexyl (5 μm, 4.6 mm × 150 mm) column with gradient elution of 10 mM ammonium acetate in water and 10 mM ammonium acetate in methanol. The recoveries of the 10 sweeteners were between 75% and 120%, and the coefficients of variation were less than 20%. The limits of quantification were 0.5 μg/kg for NHDC and SCL. For the other sweeteners, the limits of quantification were 0.1 μg/kg. Compared to the traditional high-performance liquid chromatography method, the LC–MS/MS method could provide better sensitivity, higher throughput, enhanced specificity, and more sweeteners analyzed in a single run. The samples included 27 beverages (16 alcoholic and 11 nonalcoholic beverages) and 15 pickled foods (1 pickled pepper, 3 candies, and 11 candied fruits). Two remanufactured wines were found to contain 7.2, 8.5 μg/g SAC and 126.5, 123 μg/g CYC, respectively. ACS-K, ASP, SCL, and NEO were detected in five beverages and drinks. The pickled peppers and candied fruits were found to contain SAC, GA, CYC, ASP, STV, NEO, and ACS-K. The wine with sweeteners detected was remanufactured wine, not naturally fermented wine. Therefore, the ingredient label for the sweeteners of remanufactured wine should be regulated by the proper authority for inspection of sweeteners.

* *Corresponding author*. Department of Food Science and Nutrition, Meiho University, 23 Pingguang Road, Neipu Township, Pingtung County 91202, Taiwan, ROC.
E-mail address: x00010091@meiho.edu.tw (T.S. Yeh).

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1. Introduction

Sweeteners are functional food additives that impart sweetness in food [1]. Sweeteners can be divided into two categories, natural and synthetic sweeteners. Synthetic sweeteners cannot be metabolized in the human body and provide no or little calories; therefore, they are also named nonnutritive sweeteners. Owing to the inherent low calories, nonnutritive sweeteners are beneficial for obesity, hypertension, diabetes, and dental caries control [2]. Demand for low-calorie foods other than soft drinks causes the increasing use of nonnutritive sweeteners; as a result, nonnutritive sweeteners have become ubiquitous in many foods. Increased consumption of nonnutritive sweeteners in recent years has become a global trend [3,4]. Occasionally, very high sweetener contents in food could happen. From the alert information of the Rapid Alert System for Food and Feed, the highest aspartame (ASP) concentration recorded in food was 6.98 g/kg [5] and the highest cyclamate (CYC) concentration recorded in food was 12.455 g/kg [6]. Because overconsumption of synthetic sweeteners can be harmful to health [7–9], regulatory monitoring of intense sweetener contents in food is necessary to protect consumers.

For the risk characterization of nonnutritive sweeteners, the Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives (JECFA) had established an acceptable daily intake (ADI) of 15 mg/kg body weight for acesulfame potassium (ACS-K), 40 mg/kg body weight for ASP, 11 mg/kg body weight for sodium CYC, 5 mg/kg body weight for saccharin (SAC), 2 mg/kg body weight for neotame (NEO), 4 mg/kg body weight for steviol glycoside, and 15 mg/kg body weight for sucralose (SCL). The JECFA has given dulcin (DUL) an ADI of "not to be used", which means that no DUL should be used in food. The JECFA did not establish an ADI for glycyrrhizic acid (GA), but the committee indicated that consumption of 100 mg/day would be unlikely to cause adverse effects in the majority of adults [10]. The Scientific Committee on Food in the European Union (EU) had established an ADI of 5 mg/kg body weight for neohesperidin dihydrochalcone (NHDC) [11].

Many countries around the world had different maximum usable dose regulations for synthetic sweeteners [11–13]. The regulatory status of the 10 sweeteners used in the present study in different countries is listed in Table 1. The EU had permitted seven artificial sweeteners—ACS-K, ASP, cyclamic acid and its salts, NHDC, NEO, SAC and its salts, and SCL—as food additives. In the recent Commission Regulation (EU) No. 1131/2011, the steviol glycosides level in food was regulated in the EU. The sweeteners ACS-K, advantame, alitame, ASP, CYC, NEO, SAC, steviol glycosides, SCL, and thaumatin were approved for use in Australia and New Zealand [14]. The US Food and Drug Administration (FDA) had only permitted five artificial sweeteners—ACS-K, ASP, NEO, SAC, and SCL—as food additives, and the use of CYC and NHDC were not permitted as food additives in the United States [15]. Even though purified steviol glycosides with rebaudioside A and STV as the principal components was considered GRAS (generally recognized as safe), crude extracts from Stevia plant were not permitted as food additives by the US FDA. There are five sweeteners—ACS-K, ASP, SAC, disodium glycyrrhizinate, and SCL—permitted for use in Japan [16]. Meanwhile, China has permitted ACS-K, alitame, ASP, CYC, GA, NEO, SAC, steviol glycosides, and SCL as food additives [17]. The sweeteners DUL and NHDC were not permitted in Taiwan, but ACS-K, ASP, CYC, GA, NEO, SAC, STV, and SCL were permitted as food additives.

There are several feasible analytical techniques for the analysis of sweeteners [18–24]. Many of the previous methods could analyze only one sweetener or simple sweetener mixtures. The synergistic use of sweeteners for cost reduction and taste quality improvement is often used, and the maximum permissible amount in food varies significantly [7,8,11,25,26]. An analytical method for the simultaneous determination of sweeteners in various food matrices is very important for food quality control and regulation enforcement.

Wasik et al [21] developed a high-performance liquid chromatography–evaporative light scattering detection (HPLC–ELSD) method for detecting six authorized

Table 1 – Regulatory status for the 10 sweeteners in the present study in different countries.

Compound	EU	US FDA	Japan	FSANZ	China	Taiwan
ACS-K	Yes[a]	Yes	Yes	Yes	Yes	Yes
ASP	Yes	Yes	Yes	Yes	Yes	Yes
CYC	Yes	No	No	Yes	Yes	Yes
DUL	No[b]	No	No	No	No	No
GA	No	No	Yes	No	Yes	Yes
NEO	Yes	Yes	No	Yes	Yes	Yes
NHDC	Yes	No	No	No	No	No
SAC	Yes	Yes	Yes	Yes	Yes	Yes
STV	Yes	No	No	Yes	Yes	Yes
SCL	Yes	Yes	Yes	Yes	Yes	Yes

ACS-K = acesulfame potassium; ASP = aspartame; CYC = cyclamate; DUL = dulcin; EU = European Union; FSANZ = Food Standards Australia New Zealand; GA = glycyrrhizic acid; NEO = neotame; NHDC = neohesperidin dihydrochalcone; SAC = saccharin; SCL = sucralose; STV = stevioside; US FDA = US Food and Drug Administration.

[a] Yes: permitted food additive.

[b] No: nonpermitted food additive.