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## Glycyrrhizinic acid in licorice products on the Danish market

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### ABSTRACT

Glycyrrhizinic acid (GA) is a natural compound found in licorice root and its extract, and is used in a large number of food products. It has been documented that excess consumption of licorice can cause adverse health effects, due to their content of GA. Mandatory labelling rules are therefore in place in the EU to inform consumers of the possible adverse health effects. In this study, 219 samples of confectionery, ice cream, and tea with licorice were sampled in Denmark and analyzed for their GA content. The content of GA was measured with an UPLC-UV method. The results showed that 10% of the samples were inadequately labelled. The samples in each category reaching the highest GA content were confectionery made of pure licorice extract at 23,154 mg/kg, ice cream at 1690 mg/kg, and brewed tea at 1203 mg/L. An intake of 4.3 g of confectionery, 59 g of ice cream, or 83 ml of tea, would surpass the provisional limit of 100 mg GA/day for regular ingestion. In addition to this health risk scenario from high content products, a larger consumption of licorice products with a lower GA content could also pose a health risk. We suggest campaigns to increase public awareness of the potential adverse health effects of excessive licorice consumption. In addition, it was investigated if 18 $\beta$ -glycyrrhetic acid (GE) was liberated during the processing steps of licorice production. GE is the pharmacologically active aglucone of GA. 18 $\beta$ -Glycyrrhetic acid was not identified in any product.

### 1. Introduction

Licorice is an important commodity, primarily originating from root extracts from the plant species of *Glycyrrhiza glabra*. This species is widely distributed in Europe, Caucasus, Central Asia, Mongolia, and China (Hayashi et al., 2003; Mamedov & Egamberdieva, 2019). The popularity of *G. glabra* is linked to the content of the sweet triterpenoid saponin glycyrrhizinic acid (GA), which is estimated to be 170 times sweeter than sucrose (Mizutani et al., 1994). Upon ingestion, GA is hydrolyzed to the aglucon 18 $\beta$ -glycyrrhetic acid (GE), which is the pharmacologically active compound (Vampa, Benvenuti, & Rossi, 1992). *Glycyrrhiza glabra* has been used for millennia as a medicinal remedy (Davis & Morris, 1991). More recent studies claim numerous positive pharmacological effects of licorice (Hasan, Ara, Mondal, & Kabir, 2021; Mamedov & Egamberdieva, 2019; Pastorino, Cornara, Soares, Rodrigues, & Oliveira, 2018; Sidhu et al., 2020) even in the combatting of COVID-19 and similar infections (Gomaa & Abdel-Wadood, 2021; Gowda, Patrick, Joshi, Kumawat, & Sen, 2021). So, should we consider the use of licorice extract in chewing tobacco,

confectionery, herbal remedies, beer, and tea as a health promoting benefit? In any case, studies have clearly demonstrated adverse health effects after excessive licorice consumption (Deutch, Grimm, Wehland, Infanger, & Krüger, 2019), thus consumption as food should be limited to safe intake levels. Symptoms including hypertension, hypokalemia, and edema are well documented in review articles (Isbrucker & Burdock, 2006; Luís, Domingues, & Pereira, 2018; Nazari, Rameshrad, & Hosseinzadeh, 2017; Omar et al., 2012) as well as case reports including cardiac arrest (Albermann, Musshoff, Hagemeyer, & Madea, 2010; Allcock & Cowdery, 2015; Bengel, Shah, Yamaguchi, & Josef, 2020; Celik et al., 2012; Chehri, Holmager, Stender, & Friis-Hansen, 2021; Edelman, Butala, Avery, Lundquist, & Dighe, 2020; Kwon, Oh, & Choi, 2020; McHugh, Nagabathula, & Kyithar, 2021; Murphy, Agger, & Rainey, 2009; Støving et al., 2011; Varma & Ross, 2017). In Europe, these health concerns were addressed officially by the Scientific Committee on Food (SCF) who expressed the opinion that “regular ingestion should not exceed 100 mg glycyrrhizin per day from all sources, this figure is regarded as provisional until more extensive data becomes available” (SCF, 1992). A more recent opinion from SCF concluded that the amount

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of new toxicological data is too limited for an ADI to be established, and that the 100 mg GA/day provides sufficient protection to the majority of the population, however, this might not apply for certain sensitive subgroups (SCF, 2003). Størmer, Reistad, and Alexander (1993) find that 100 mg GA/day is the lowest-observed-adverse-effect level and suggests a safety factor of 10, placing a safe dose for most individuals at 10 mg GA/day (Størmer et al., 1993), while van Gelderen, Bijlsma, van Dokkum, and Savelkoul (2000) on the basis of results from a human volunteer study, proposed a safe intake to be 0.2 mg/day/kg body-weight, which equals 14 mg GA/day for an adult of 70 kg (van Gelderen et al., 2000). Others have looked at individual groups, and one report suggests that pregnant women are especially vulnerable (Steffensen et al., 2018, p. 19). To estimate consumers' total GA intake, it is pertinent to have quantitative information concerning the content of GA from various food commodities. Studies examining GA in licorice products have been performed earlier on confectionery (Maas, 2000; Sabbioni, Ferranti, Bugamelli, Forti, & Raggi, 2006; Størmer et al., 1993), tea (Maas, 2000), dietary supplements (Li, Nikolic, & van Breemen, 2016), herbal medicines (Bi, Tian, & Row, 2010), and weiyanning granule (Zhou, Zhang, Duan, & Mei, 2015) but studies are limited as is the number of samples analyzed.

The United States of America has implemented rules in the US Code of Federal Regulations (sec. 184.1408) specifying the maximum use levels of glycyrrhizinic acid in different food categories ranging from 0.1% in alcoholic beverages to 16.0% in hard candy (FDA, 2020). The European Commission has implemented EU regulation 1334/2008 (EU Commission, 2008) that specifies maximum levels of aroma compounds herein GA and ammoniated GA in several food products. In addition, EU regulation 1169/2011 (EU Commission, 2011) lays down rules for the labelling of licorice products. A labelled warning must be included in the following two cases: "Confectionery containing glycyrrhizinic acid or its ammonium salt due to the addition of the substance(s) as such or the liquorice plant *Glycyrrhiza glabra* at concentrations of 4 g/kg or above" and "Beverages containing glycyrrhizinic acid or its ammonium salt due to the addition of the substance(s) as such or the liquorice plant *Glycyrrhiza glabra* at concentrations of 50 mg/L or above, or of 300 mg/L or above in the case of beverages containing more than 1.2% by volume of alcohol". These must be labelled with "contains liquorice – people suffering from hypertension should avoid excessive consumption" and shall be added immediately after the list of ingredients, and "In the absence of a list of ingredients, the statement shall accompany the name of the food". To comply with EU regulation and gain insight into the GA content in food products, annual official control projects were conducted from 2019 to 2022. Glycyrrhizinic acid was investigated in confectionery, ice cream, and brewed tea with licorice as part of the product name or present in the list of ingredients. The measured content of GA, in combination with the presence or absence of the labelled warning, determined compliance or non-compliance. Ice cream was not explicitly mentioned in the EU regulation 1169/2011 (EU Commission, 2011), but the Danish Veterinary and Food Administration (DVFA) chose to include ice cream in the category of confectionery.

Several articles report quantitative methods for determination of GA in licorice. These methods include capillary zone electrophoresis (Sabbioni et al., 2005), HPLC-UV (Bi et al., 2010; Giah, Jahadi, & Khosravi-Darani, 2021), HPLC-PAD (Sabbioni et al., 2006), and more advanced methods such as UPLC-qTOF MS/MS (Gupta, Verma, Anand, & Khajuria, 2013). This paper describes a simple and fast UPLC-UV method that measured the content of GA and its aglucone GE in confectionery, ice cream, and brewed tea. The objective of this study was to determine how well the mandatory EU regulatory labelled warning applied and how much licorice intake would exceed the provisional limit of 100 mg GA/day. In addition to this objective, the content of GA in various foods containing licorice is important for estimating consumer exposure, which is essential for assessing the health implications that licorice products pose to the public. 18 $\beta$ -Glycyrrhetic acid (GE) was included in the measurement to investigate its possible release

during processing.

## 2. Materials and methods

### 2.1. Chemicals and standards

Acetonitrile (Optigrade, Gradient Grade) was purchased from Pro-mochem, (Germany), ammonium acetate (Emsure) was purchased from Merck (Germany), glycyrrhizinic acid ammonium salt, and 18 $\beta$ -glycyrrhetic acid were purchased from Sigma Aldrich (Denmark).

### 2.2. Food samples

A total of 219 food samples with licorice in its name or in the ingredient list were collected annually from 2019 to 2022 as part of a governmental inspection program at the DVFA. The number and category of yearly samples are presented in Table 1 and involved confectionery (n = 145), ice cream (n = 15), and tea (n = 59) from local stores, supermarket storage facilities, and directly from importers. Confectionery included dragée, drops, hard and soft candy, licorice coated with chocolate, and pure licorice (licorice extract). Ice cream included single serving bars/popsicles as well as larger tubs. In addition to pure licorice root tea, tea included black tea, herbal tea, as well as mixtures with a varying content of licorice root and aroma. Tea was either packed in teabags or sold as loose-leaf tea. Sample collection was performed accredited and equally spread throughout Denmark. Upon arrival at the laboratory, confectionery samples were frozen at  $-80$  °C and subsequently crushed and homogenized in a Combi Cutter (cc34, Nilma, Parma, Italy). Ice cream samples were received at  $-18$  °C and were also homogenized in the Combi Cutter. Crushed and homogenized samples were stored at  $-18$  °C until further analysis. Teabag and loose-leaf tea samples were stored at room temperature prior to analysis. Analysis was performed no later than 30 days after sample arrival.

### 2.3. Validation samples and design

#### 2.3.1. Precision

Four validation samples (VS) were used for the precision studies and labelled VS1 (confectionery), VS2 (confectionery), VS3 (spiked confectionery), and VS4 (brewed Earl Grey tea). VS1 and VS2 had a natural GA content of approximately 2850 mg/kg and 730 mg/kg, respectively, VS3 and VS4 had no measurable content of GA and were spiked to 500 mg/kg and 35 mg/kg, respectively. Six replicates of each sample were subjected to analysis on three individual days, and quantified with comparison to dilution curves prepared each day.

#### 2.3.2. Limit of detection (LOD) and limit of quantification (LOQ)

A de-glycyrrhizinated licorice supplement (VS5) and a brewed chamomile tea (VS6) with no natural GA content were spiked with GA to 5.0 mg/kg and 2.0 mg/L, respectively, which corresponded to the smallest possible chromatographic signals that were easily discriminated from the background. On one day, ten subsamples of spiked VS5 and VS6 were freshly prepared and analyzed under repeatability conditions. LOD was calculated as the mean content of GA + 3  $\times$  the standard deviation (SD<sub>r</sub>) and LOQ was calculated as the mean content of

**Table 1**  
Number of samples and product category sampled from year 2019–2022.

Year	Sample category			Total
	Confectionery	Ice cream	Tea	
2019	79	0	11	90
2020	33	0	11	44
2021	33	15	17	65
2022	0	0	20	20
Total	145	15	59	219

GA + 6 × SD<sub>r</sub> (Wenzl, Haedrich, Schaechtele, Robouch, & Stroka, 2016).

### 2.3.3. Recovery and accuracy

Results from the spiked VS3 and VS4 used in the precision study were also used to establish GA recovery. For measuring accuracy, certified reference material or collaborative trials are preferred, however, no such material or trials were internationally identified. One randomly chosen licorice sample was therefore analyzed at the DVFA and was also sent to a commercial laboratory for a quantitative LC-MS analysis of GA. Results from the two laboratories were disclosed after completion and a comparison performed.

### 2.3.4. Linearity

Dilution curves consisted of six dilutions of GA in water with concentrations of 0.0, 20.0, 40.0, 60.0, 80.0, and 100.0 mg/L (corresponding to a GA range from 0.0 to 5000 mg/kg when 0.5 g of sample was used). Linear regression was performed on six dilutions on each dilution level to investigate linearity.

### 2.4. Teabag homogeneity

In the case of tea sample No. 199, homogeneity of extracted GA from different teabags was investigated. Ten teabags from the collected sample No. 199 (batch 1) and ten teabags from the same brand but a different batch were purchased in a shop (batch 2).

### 2.5. Analytical procedure

Tea was brewed as instructed on the tea package. Depending on the instruction, 200–250 ml of 80–100 °C water was added to one teabag or a specified mass (in case of loose-leaf samples) and incubated for 3–7 min prior to filtration. Similar to the other food products, GA was extracted from the filtered tea brew. If no brewing instruction was provided, 2.5 g of tea leaves was added 250 ml of 100 °C water and incubated for 5 min.

An extraction and chromatographic combination was inspired by different sources (Tian, Yan, & Row, 2008; Zhou et al., 2015). In brief, 0.5 g of homogenized licorice or tea brew, or 5.0 g of ice cream was placed in a 50 ml tube and added 25 ml extraction solution consisting of 70% ammonium acetate (0.01M) in acetonitrile. The mixture was placed in an ultra sonicator (Bransonic® Ultrasonic Cleaner 5510) at 60 °C for 60 min followed by 15 min shaking at 250 rpm. One and a half ml of the mixture was transferred to centrifugation tubes and centrifuged for 10 min at 11,000 × g. The supernatant was filtered through a 0.22 µl polyvinylidene difluoride (PVDF) filter directly into HPLC vials. The sample content of GA was quantified with comparison to a dilution curve consisting of six dilutions of GA with concentrations of 0.0, 20.0, 40.0, 60.0, 80.0, and 100.0 mg/L (corresponding to 50 × higher levels in samples, mg/kg). The dilutions were prepared in the extraction solution from a stock containing 1000 mg GA per liter extraction solution. Samples with a GA content above 2500 mg/kg were diluted to reach a concentration near the middle of the dilution curve. Samples above the labelling requirement were analyzed in duplicate. All other samples were analyzed in a single determination. In each analytical series, one licorice or one brewed tea sample was spiked with 0.250 ml of the GA stock solution directly in the sample extraction mixture to conclude 25 ml in total. The result from the non-spiked sample was subtracted from the result from the spiked sample and recovery calculated. The recovery percentage was used to adjust results to 100%. In addition, this recovery percentage was included for quality assurance purposes and monitored throughout the project. The method was accredited in 2021.

### 2.6. UPLC instrumentation and chromatographic conditions

The Waters ACQUITY UPLC H-class (Milford, MA) consisted of a separation module and a PDA detector. The system was equipped with a

Waters ACQUITY UPLC HSS T3 Column 2.1 × 100 mm, 1.8 µm and a 5 mm VanGuard™ precolumn made of the same column material. The chromatographic system consisted of eluent A and B. Eluent A contained 100% acetonitrile, and eluent B contained 0.01 M ammonium acetate. Eluents were filtered through a 0.22 µm nylon filter. The chromatographic gradient consisted of the following segments: Initial eluent was 20% A followed by 30% A at 2 min (curve 8), 70% A at 4.3 min (curve 9), 100% A at 5 min (curve 1), and 20% A at 9 min (curve 1). Flow rate was 0.4 ml/min, column temperature 60 °C, and injection volume 5 µl. Chromatograms used for quantification were obtained at 252 nm. Sample vials were kept in the auto sampler at 20 °C and injected every 10th min. Glycyrrhizic acid and GE were identified in the chromatogram at a retention time around 2.6 min and 5.3 min, respectively.

## 3. Results and discussion

### 3.1. Validation

Validation parameters for GA in licorice and tea are presented in Table 2. Ice cream was a simple matrix with only the licorice fraction influencing the chromatogram. Validation parameters obtained for confectionery was therefore adopted for ice cream. As evident from the Results and Discussion, section 3.2, GE was not identified in any sample, and the inclusion of validation parameters for GE are therefore restricted to the limit of quantification (LOQ). Additional validation parameters for GE were comparable to GA.

#### 3.1.1. Precision

VS1 showed a relative standard deviation of repeatability (RSD<sub>r</sub>) of 1.6% and a relative standard deviation of reproducibility (RSD<sub>R</sub>) of 7.9%. VS2 showed an RSD<sub>r</sub> of 0.8% and an RSD<sub>R</sub> of 1.6%. VS3 showed an RSD<sub>r</sub> of 2.5% and an RSD<sub>R</sub> of 3.0%. VS4 showed an RSD<sub>r</sub> of 9.0% and an RSD<sub>R</sub> of 10.2%.

#### 3.1.2. Limit of detection (LOD) and limit of quantification (LOQ)

The VS5 showed a mean GA content of 4.86 mg/kg with a standard deviation of 0.23 mg/kg, which corresponded to an LOD of 5.5 mg/kg and an LOQ of 6.2 mg/kg (rounded up to 10 mg/kg). VS6 showed a mean GA content of 2.055 mg/kg with a standard deviation of 0.045 mg/kg, which corresponded to an LOD of 2.2 mg/kg and an LOQ 2.3 mg/kg (rounded up to 5 mg/kg). VS3 and VS6 with no measurable content of GE were spiked to 35 mg/kg and 2 mg/kg, respectively. LOQ for GE was calculated to 38.0 mg/kg in licorice and 2.7 mg/kg in brewed tea.

**Table 2**

Selected glycyrrhizic acid (GA) validation parameters including coefficient of variation (CV), recovery, and limit of quantification (LOQ).

Validation sample code	Sample type	GA content, spiked or natural	CV (%)	Recovery (%)	LOQ
VS1 <sup>a</sup>	Licorice, natural content	2850 mg/kg	7.9	–	–
VS2 <sup>a</sup>	Licorice, natural content	730 mg/kg	1.6	–	–
VS3 <sup>a</sup>	Licorice, spiked	500 mg/kg	3.0	93	–
VS4 <sup>b</sup>	Earl Grey tea, spiked	35 mg/L	10.2	92	–
VS5 <sup>b</sup>	De-glycyrrhizinized licorice supplement, spiked	5.0 mg/kg	4.7	–	10 mg/kg
VS6 <sup>b</sup>	Chamomile tea, spiked	2.0 mg/L	2.2	–	5.0 mg/L

<sup>a</sup> Results obtained under reproducibility conditions.

<sup>b</sup> Results obtained under repeatability conditions.

### 3.1.3. Recovery and accuracy

The spiked VS3 and VS4 showed a recovery of 93% and 92%, respectively. The results for measuring accuracy were disclosed and compared after completion of both analyses. The commercial laboratory reported 790 mg GA/kg ( $n = 1$ ), and the DVFA found a mean content of 789 mg GA/kg ( $n = 6$ ;  $s = 8.6$  mg/kg). Inclusion of the standard deviation in the DVFA mean content suggests that the results obtained from the two laboratories are comparable across the different methodologies.

### 3.1.4. Linearity

The dilutions prepared showed a regression line  $Y = 9894x - 5477$  with a determination coefficient ( $r^2$ )  $> 0.9998$ . The  $r^2$  in combination with a visual evaluation of a residual plot (not presented) showed a satisfactory linearity.

## 3.2. Food samples

A total of 219 food samples were analyzed in a governmental inspection program at the DVFA and compared to the EU regulation 1169/2011 (EU Commission, 2011). Table 3 shows the minimum, maximum, and mean GA content from all official control samples. Fig. 1 shows the results from 145 confectionery samples and 15 ice cream samples. Confectionery samples were subdivided into two groups. Confectionery A consisted of licorice candy excluding products of pure licorice with or without aroma. Confectionery B consisted of pure licorice with or without aroma. Confectionery A had a content between  $< \text{LOQ}$  and 4936 mg/kg, with a mean content of 1066 mg/kg. Confectionery B had a content between 14,935 mg/kg and 23,154 mg/kg, with a mean content of 17,922 mg/kg. Another study (excluding pure licorice) found a mean GA content of 1700 mg/kg ( $n = 19$ ) in licorice sold on the Dutch market (Maas, 2000), which is higher than our mean content of 1066 mg/kg for the Confectionery A samples. This difference in the mean content could be explained by actual differences between products on the Danish and Dutch market, or it could be the low number of analyzed Dutch samples that are unrepresentative of the actual content. In the ice cream category, 15 samples were analyzed and showed a content of GA between 408 and 1690 mg/kg, with a mean content of 920 mg/kg. Unfortunately, no reports of the GA content in this category were found, which disables a comparison. In the tea category, 59 samples were analyzed and showed a content of GA between  $< \text{LOQ}$  and 1203 mg/L (Fig. 2) with a mean content of 133 mg/L. If the high content (1203 mg/kg) from the only tea sample with licorice, licorice root, and aroma on the ingredient list is removed, the mean GA content is reduced to 114 mg/L with a maximum content of 534 mg/L. This is similar to the GA mean ( $n = 10$ ) content of 126 mg/L found in a study of tea samples with licorice as a plant ingredient (Maas, 2000). Six confectionery samples (all in the Confectionery B group) that exceeded the limit of 4000 mg GA/kg lacked the proper regulatory labelled warning, 4 samples were labelled

**Table 3**  
Minimum, maximum, and mean content of glycyrrhizinic acid in confectionery, ice cream, and tea.

Category	Minimum	Maximum	Mean
Confectionery total ( $n = 145$ )	$< \text{LOQ}$	23,154 mg/kg	1996 mg/kg
Confectionery A <sup>a</sup> (137)	$< \text{LOQ}$	4936 mg/kg	1043 mg/kg
Confectionery B <sup>b</sup> (8)	14,935 mg/kg	23,154 mg/kg	17,922 mg/kg
Ice cream ( $n = 15$ )	408 mg/kg	1690 mg/kg	920 mg/kg
Tea ( $n = 59$ )	$< \text{LOQ}$	1203 mg/L	133 mg/L
Tea <sup>c</sup> ( $n = 58$ )	$< \text{LOQ}$	534 mg/L	114 mg/L

<sup>a</sup> Licorice candy excluding products of pure licorice extract with or without aroma.

<sup>b</sup> Pure licorice with or without aroma.

<sup>c</sup> The result of 1203 mg/L was excluded in the calculation as it was the only tea sample with licorice, licorice root, and aroma on the ingredient list. Limit of quantification (LOQ): 10 mg/kg for confectionery and ice cream, and 5 mg/L for brewed tea.

with the warning even though it was not required, and 7 samples were collected without labelled information. In total, 10 confectionery samples were considered non-compliant. Ice cream was evaluated in accordance with the rules that applies for confectionery, and all samples correctly lacked the labelled warning. Six brewed tea samples exceeded the limit of 50 mg GA/L and lacked the proper regulatory labelled warning, 5 samples were labelled with the warning even though it was not required, and 1 sample was collected without labelled information and could not be evaluated. In total, 11 tea samples were considered non-compliant. Across the different product categories, 21 samples were non-compliant, which means that 10% of all samples ( $n = 219$ ) were non-compliant. In the 8 confectionery samples that contained GA  $< \text{LOQ}$ , licorice was part of the confectionery name but not mentioned in the ingredient list. This explains the absence of GA, but questions the legitimacy of the confectionery product name (that included licorice), as it only mentioned aroma or anise in the ingredient list.

The products with high levels of GA within each product category might easily pose a health problem as relatively small amounts will exceed the provisional limit of 100 mg GA/day (SCF, 2003). One sample labelled “pure licorice” contained 23,154 mg GA/kg, resulting in 4.3 g (corresponds to 4.6 pieces of licorice) of confectionery being enough to reach the provisional limit of 100 mg GA/day. The high content of GA in ice cream (1690 mg/kg) and in tea (1203 mg/kg) samples, results in 59 g and 83 ml (approx.  $\frac{1}{3}$  of a cup), respectively, being enough to reach the provisional limit of 100 mg/day.

One recent Danish case study of severe hypokalemia resulted from a two months intake of more than 2 L of licorice tea/day as a remedy for cough (Chehri et al., 2021). The tea brand consumed in this case study was equivalent to the brand of sample No. 199 (personal communication with Dr. Chehri), which contained 163 mg GA/L in our analysis. No information about the actual batch consumed was available, and we therefore investigated the variation of teabags and batches, and found a mean content of 159 mg GA/L ( $n = 20$ ;  $s = 6.6$ ) (Table 4). A daily intake of 2 L of tea with a content of 159 mg GA/L, would correspond to an intake of 318 mg GA, exceeding the provisional daily limit by more than three-fold.

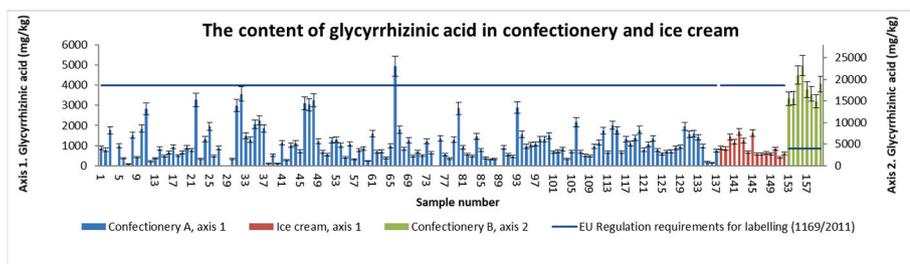
In general, if we use the mean content of GA in confectionery (1996 mg/kg) and in tea (133 mg/L), 51 g of confectionery or 770 ml of tea will reach the provisional limit of 100 mg GA/day. This is comparable to other studies that found that 60–70 g of licorice confectionery or 800 ml of tea (Maas, 2000) will reach the provisional limit of 100 mg GA/day. It should be noted that because many consumers have brand loyalties toward specific products, the GA content can easily exceed the GA mean content in each food category. Another concern is that consumers who like the licorice taste, may regularly consume a combination of various products that contain licorice. Combining intake from multiple sources will decrease the amount that can be consumed of each food type if the intake is to be kept below the provisional limit of 100 mg GA/day.

All samples were screened for GE to investigate if GA was hydrolyzed to GE in some products. The lack of samples with a measurable GE content demonstrated that this was not the case, which is in contrast to another study (Sabbioni et al., 2006).

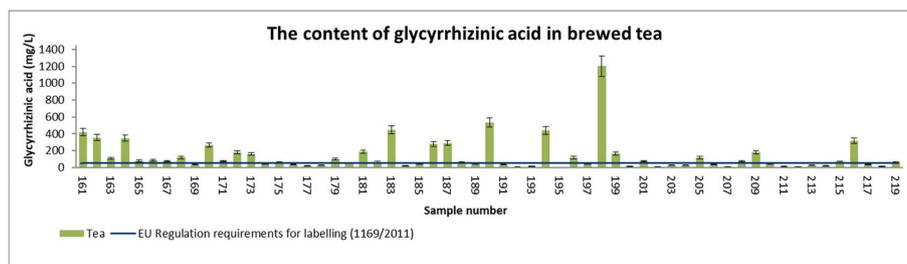
## 4. Conclusion

This is currently the most comprehensive investigation of GA in licorice products, and we hope it can contribute to future risk assessments. This study showed a large variation in the GA content, both between the individual categories of confectionery, ice cream, and brewed tea but also within each category. With the amounts of GA found in licorice products in this study, some consumers with an appetite for high GA content products, or a combination of products can easily exceed a daily intake of 100 mg GA. It should be noted that consumers might have tea brewing preferences that vary from the instruction on the product and therefore consume more or less GA than estimated in this study.

We doubt that consumers in general are well informed about the



**Fig. 1.** The content (mg/kg) of glycyrrhizic acid in confectionery and ice cream samples. The large difference in glycyrrhizic acid in confectionery products prompted a secondary axis. Axis 1 is used for Confectionery A, which are products excluding pure licorice extracts with or without aroma. Axis 2 is used for Confectionery B, which are products of pure licorice extracts with or without aroma. The highest RSD<sub>(R)</sub> (10.2%) obtained in the validation study is used for the added error bars.



**Fig. 2.** The content (mg/L) of glycyrrhizic acid in brewed tea samples. The highest RSD<sub>(R)</sub> (10.2%) obtained in the validation study is used for the added error bars.

**Table 4**

Minimum, maximum, and mean GA results from brewed tea from two batches of the same teabag brand.

Type of calculation	Batch 1 (n = 10) mg/L	Batch 2 (n = 10) mg/L	Batch 1 and 2 (n = 20) mg/L
Minimum	143	154	143
Maximum	166	172	172
Mean	156	163	159
Standard deviation	6.2	5.2	6.6

potential adverse health consequences of licorice consumptions. Even if they acknowledge the risk, they have no chance to determine the actual GA content in their preferred licorice products. Nonetheless, EU labelling requirements offer some assistance for consumers to make an informed choice, but the vague EU regulatory labelled warning “Contains licorice - people suffering from hypertension should avoid excessive consumption” is problematic. GA levels in licorice products may differ across countries, and the total number of analyzed products is still limited. We therefore encourage similar studies to be conducted in other countries, to reduce the uncertainty of GA exposure assessments among licorice consumers. Products with licorice intended for future studies could include alcoholic and non-alcoholic beverages, cakes, chewing gum, chewing tobacco, chocolate, cookies, spice (powdered pure licorice extract) intended for home cooking, and throat pastilles.

**CRedit authorship contribution statement**

**Nicolai Z. Ballin:** Conceptualization, Methodology, writing original draft, Validation, Project administration, Supervision. **Dorte Møller Larsen:** Methodology, Investigation, Validation. **Sofie Tjagvad Jensen:** Formal analysis, Validation. **Laila Brock Andersen:** Formal analysis. **Pelle Thonning Olesen:** Writing – review & editing, Conceptualization.

**Declaration of competing interest**

None.

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