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Determination of Cucurbitacins in untreated and roasted kernels of *Citrullus colocynthis*

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Introduction:

The cucucrbitacins are bitter and toxic natural substances, found mainly in plants of the family *Cucurbitacea* that includes the common pumpkins and gourds. Later cucucrbitacins were also found in plants of many different families. The chemical structure of a cucurbitacin is depicted in figure 1. There are several related cucurbitacins that are chemically only slightly different, e.g. some lack a double bond that others have, or have an acetyl-group, where other have a hydroxyl-group. The different cucurbitacins are named with a letter, e.g. cucurbitacine A or cucurbitacine E. Two forms exist of each cucurbitacin, the "free" form or *aglycone*, and a form with an attached glucose-molecule, the *glucoside*. The glucosides are much better soluble in water than the aglycones.

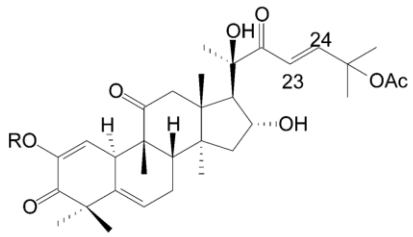

Figure 1: Structure of cucurbitacine E (R = H). Drawing taken from [1]

Colocynth *(Citrullus colocynthis*), a desert plant related to watermelon, is a nonhardy drought-resistant perennial herbaceous vine, originally from tropical Asia and Africa, now widely distributed in the Saharo-Arabian and the Mediterranean region [2].

The fruits are a powerful cathartic and were used in traditional medicine for different purposes [3].

Colocynth seeds were found in several Northern African and Near Eastern archeological sites, dating from 3800 BC to Roman times (in Libya) and the pre-pottery Neolithic levels in Israel. The seeds contain 17-19% oil. In ancient times, this oil was used for lamps. Despite the extremely bitter taste of the seeds, the oil is considered edible [4].

In particular, when being roasted, the bitterness of the seeds is considerably reduced. [5]. Since the bitter compounds are also the toxic ones, this may indicate a simultaneous reduction of the toxicity.

As far as the author is aware, there are no published investigations on the cucurbitacin content of colocynth seeds. Objective of the present study was therefore the determination of cucurbatacin concentrations in untreated kernels and in those which were roasted in a so called „Clayton Ring". The latter treatment was performed in an archaeological experiment by Carlo Bergmann who, in

2007, hypothesized that moderate roasting of poisonous colocynth kernels under an almost complete exclusion of air would reduce their toxic properties thus, turning the seeds into edible matter. [6]

Cucurbitacins found in colocynths are mainly (a)cucurbitacin E, free and as (b) glucoside, and minor amounts of (c) glucosides of the cucurbitacins I, K, L and B [7, 8]

Figure 2: Clayton Ring roasting test [5]

Materials and Methods

Extraction

Untreated colocynth seeds collected in the surroundings of Obak, Eastern Desert of the Sudan and roasted seeds from the same source material were ground in a blade grinder. The fine, sticky powder was mixed with different solvents and treated in an ultrasonic bath to extract the cucurbitacins.

Analysis

The extracts were analyzed with a liquid chromatography system coupled to a triple quadrupole mass spectrometer (Agilent Technologies 1200 series HPLC, 6410B mass spectrometer).

In the qualitative experiments masses from 400-800 were scanned to get an overview, which cucurbitacins are present in the extract.

Quantitative determination was performed with the spectrometer set to detect only the masses of the identified cucurbitacins (and some common fragments).

The calibration standard was cucurbitacin E (purity 98,4 %, Phytolab, Vestenbergsgreuth, Germany), the major cucurbitacin found in the colocynth kernels. Since there were no standards of the glucoside or the other cucurbitacins available, all substances were quantitated against the cucurbitacin E, i.e. assuming the same response. There is certainly some error associated with this approach, but the order of magnitude of the concentration should be right. The focus of the archaeological experiment [6] and subsequently of this analytical determination was on the change of concentration caused by roasting of the seeds.

Results

Qualitative Experiments

The main cucurbitacins found in untreated colocynth seeds are, as expected, cucucrbitacin E and its glucoside, in lower amounts the glucosides of the cucurbitacins B, I, L and J/K. Figure 2 shows the chromatogram of a colocynth extract. Each peak in the chromatogram shows the presence of a particular substance with the area (or height) corresponding to the concentration of this substance. The detection of cucurbitacin E and its glucosid are shown in orange and green respectively.

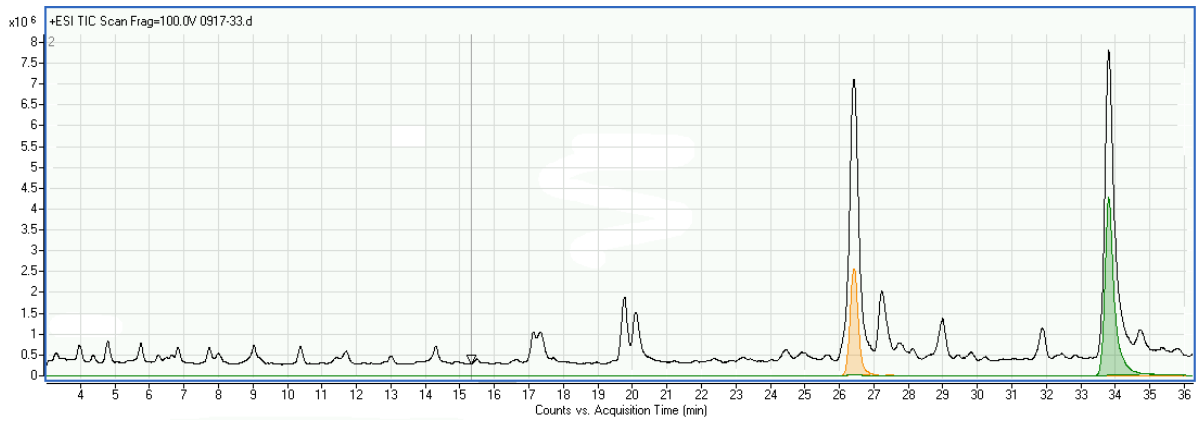

Figure 3: Qualitative chromatogram of an acetonitrile extract of colocynth seed
total ion chromatogram with overlayed extracted ion traces of m/z 736.3 (cucurbitacin E glucoside, orange) and 574.3 (cucurbitacin E, green)

Quantitative determination

The concentrations of cucurbitacins in unroasted seeds are presented in table 1. The values in the table are mg/l measured in the extracts. Since 100 mg sample were extracted with 10 ml extractant, multiplying by a factor of 100 will give the concentrations of cucurbitacins in the seeds in mg/kg (fresh weight). The relative standard deviation of 3 replicate extractions is between 10 and 20%.

The “other cucurbitacins” reported in the table are calculated as the sum of cucurbitacin J/K-glucoside, I glucoside, L glucoside, dihydrocucurbitacin E glucoside and dihydrocucurbitacin E.

Table 1: Measured cucurbitacin concentrations in extracts from untreated seeds, prepared with different extractants

extractant	cucurbitacin E	cuc. E-glucoside	other cucurbitacins
	mg/l		
50% methanol in water	**3.90**	**3.81**	0.78
100% methanol	3.68	2.02	0.38
aqueous acetate buffer pH 5	**1.93**	**4.27**	0.72

The results for the roasted seeds are shown in table 2.

Table 2: Measured cucurbitacin concentrations in extracts from roasted seeds

extractant	cucurbitacin E	cuc. E-glucoside	other cucurbitacins
	mg/l		
50% methanol	**2.94**	**0.90**	0.21
acetate buffer pH 5	**0.49**	**0.57**	0.11

Discussion

From table 1 can be seen, that methanol is extracting more agylcone from the seeds, but with some water the recovery of the glucosides is better. Some other solvent mixtures than the reported ones were also tested but not quantitatively evaluated. All in all, 50% methanol gave the best overall recoveries.

There was initially the idea to split the glucose-molecule off from the glucosid with the help on an enzyme, to be able to measure glucoside and aglycone together. Unfortunately this did not work with the tested ß-glucosidase from almonds.

The roasting of the seeds clearly reduced the amount of extractable cucurbitacins in the seeds. Especially the glucosides were reduced – in the 50 % methanolic extract by about 75 % (from 3.81 mg/l to 0.90 mg/l), in the acetate extract by about 85% (from 4.27 mg/l to 0.57 mg/l). The aglycon of cucurbitacin E in the methanolic extract was reduced by 25% (3.90 to 2.94 mg/l), in the acetate buffer extract by 75 % (1.93 to 0.74 mg/l).

Since the glucosides are significantly more bitter and more toxic than the aglycones the roasting of the colocynth seeds could have made them edible. The reported oral LD50[1] for mice are: cucurbitacin E - 340 mg/kg body weight, cucurbitacin E glucoside - 40 mg/kg body weight [9].

The acetate buffer extract that is probably nearer to the conditions in the human stomach than a methanolic extract, contained 4.27 mg/l cucurbitacin E glucoside or 427 mg per kg seeds. After

[1] LD50 = lethal dose 50; dose at which half of the test animals die

roasting only about 60 mg per kg seeds remain. Probably some 100 g (estimated 400 g) of these seeds could be eaten without intoxication[2].

It might moreover be possible, that some of the remaining cucurbitacin glucoside is hydrolyzed under the acidic conditions in the human stomach, further reducing the toxicity. This is at least suggested by a half-quantitative experiment, where the seeds were extracted with dilute hydrochloric acid.

Though the presented results are not an unequivocal proof that colocynth seeds are edible after roasting, they present some evidence that this might be the case, because the extractable content of cucurbitacin glucosides is significantly reduced by roasting.

Literature

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[2] Calculation assuming similar toxicity in mice and humans: LD50 = 40 mg/kg body weight, i.e. 2400 mg for a 60 kg adult. Assuming a safety factor of at least 100, 24 mg might be tolerable. This would be the cucururbitacin E glucoside content of 400 g roasted seeds.