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Bitterness and cucurbitacin levels in New Zealand-grown zucchini cultivars

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Copy 10 of 10

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Contents

1 Executive summary

Early in 2002 bitter-tasting zucchini caused unpleasant gastric pain in some New Zealand consumers. The findings of a literature study carried out in the months that followed on the causes of this phenomenon led to further research in the 2002-03 season.

Because the synthesis of cucurbitacin, the product responsible for the bitter taste, is under strong genetic control, a range of zucchini cultivars was assessed to determine those responsible for producing bitter fruit. A trial containing one plot each of 18 plants from each of 24 cultivars of zucchini sourced from seven seed companies was established in Pukekohe. At the end of the season, in early March, 300 fruit from 238 plants were harvested from this trial for assessment. Every fruit was tasted individually (tasted raw) and scored for bitterness level. Fruit varied in bitterness with some exhibiting moderate to strong levels.

Ten cultivars and fruit with the highest bitterness levels along with two with the lowest levels were examined by extraction and analysis using an HPLC method and compared with standards of cucurbitacin-E and cucurbitacin-E-glucoside. These compounds had been reported in the literature as being responsible for bitter flavours in zucchini. None of the samples contained cucurbitacins that matched the standards used.

In some fruit there was, however, evidence of small quantities of compounds that eluted in the correct position for cucurbitacin-E-glucoside. Although the spectral characteristics of these compounds did not match the authentic standard, their identity and connection with the bitter tastes observed is of real interest, but outside the scope of this project.

The absence of compounds matching the cucurbitacin standards used does not imply that these cultivars will not produce plants with bitter fruit or fruit containing detectable cucurbitacins under other circumstances. We know that bitter fruit have been generated in previous seasons but we do not know whether the compounds causing this bitterness were the same as the standards used.

Further research is required to determine the identity of the bitter compounds tasted and the conditions under which they are produced.

2 Introduction

In the summer of 2001-02 several reports were received from the New Zealand public that bitter-tasting zucchini were being produced by plants throughout the country. Some of these fruit were eaten and caused unpleasant gastric pain and distress. Vegfed commissioned a literature search to determine the reasons for this phenomenon and this was published in *Crop & Food Research Confidential Report No. 598* entitled "Cucurbitacins in bitter zucchini," J A Heyes, April 2002.

Following this report, which found that cucurbitacin synthesis is under strong genetic control (Herrington 1983) and appears to be associated with off-type plants, a trial was grown in Pukekohe in the 2002-03 season. This trial included one plot each of 18 plants from each of 24 cultivars sourced from seven seed companies. The trial was grown on a commercial grower's property in Ostrich Farm Road, approximately 8 km from the Pukekohe Research Station. We found no published evidence suggesting that cucurbitacin synthesis in zucchini fruit is affected by seasonal factors such as weather or pest outbreaks. However, bitter fruit and stomach upsets were reported during February so we decided to take our samples at the end of February/beginning of March when the plants were near the end of their productive period and might be under some stress.

3 Method

3.1 Harvest and sensory procedure

Crop & Food Research was approached in mid February and asked to analyse the zucchini variety trial to determine if there were any toxins or bitter tasting compounds like those present last year.

1. The trial was harvested on 11 March 2003 by Crop & Food Research staff.
2. Fruit were labelled by plant number and a maximum of four fruit were harvested per plant, depending on fruiting vigour of the individual plants on the day of harvest.
3. Fruit were chilled and couriered overnight to Lincoln.
4. Each fruit was tasted individually in its raw state and assessed on a scale from 0 to 5.
   - 0 = sweet and pleasant tasting
   - 1 = no bitterness, bland
   - 2 = slight bitterness

- 3 = moderate bitterness
- 4 = very bitter and unpleasant
- 5 = unpalatable and extremely bitter

Any fruit showing moderate (3) or stronger (>3) bitterness was frozen for separate analysis, and three slices were taken from all other fruit to produce a bulked frozen sample for each cultivar.

After the HPLC tests had been completed, the frozen samples were tasted again alongside a 1 ppm and a 10 ppm solution of cucurbitacin-E glycoside. This was to compare the bitter taste of the glycoside with that of the bitter tasting fruit.

3.2 *HPLC analysis*

Ten of the 24 cultivars were tested by HPLC using a modification of the method outlined by Hutt & Herrington (1985). The 10 were selected based on the sensory results to give eight cultivars with and two cultivars without bitter tasting fruit. The toxic material in zucchini is reported to be the cucurbitacin E glycoside (Hutt & Herrington 1985). Cucurbitacin standards were obtained from Professor Dick Robinson (now retired), a cucurbit breeder from Cornell University, USA. These standards were left with him by Dr Piotr Gorski, the principle biochemist working with him on the cucurbitacin project. Prof. Robinson's opinion was that it would be possible to bulk squash fruit for analysis and still detect one bitter fruit among many normal fruit since the cucurbitacin content is so much higher in bitter fruit. An additional standard sample of cucurbitacin E (a triterpenoid) was purchased from Extrasynthese of Genay, France.

3.2.1 *Preparation of cucurbitacin extracts*

Approximately 5 g (exact weight recorded) of randomly selected tissue was weighed into a 50 ml 'Falcon' centrifuge tube. Chloroform (20 ml) was added and the sample homogenised using the Ultra-Turrax T25 homogeniser for 1 minute at 9500 rpm. Anhydrous sodium sulfate (~2.5 g) was added to dry the sample. The homogenate was then transferred into a filter funnel fitted with a Whatman #4 paper filter and the filtrate collected into a 50 ml round-bottomed flask. The solid collected in the filter paper was washed with additional chloroform (3 x 5 ml) and the washings combined with the original filtrate. The combined filtrate was evaporated to dryness (at 20°C) using a rotary evaporator. The homogeniser was thoroughly cleaned with deionised water, then methanol and finally chloroform prior to processing the next sample.

The sample was prepared for HPLC analysis by reconstituting the residue in 1 ml of HPLC grade methanol.

3.2.2 *HPLC analyses*

A Waters liquid chromatograph consisting of a model 626 pump and controller, model 717-plus autosampler and a model 996 photo diode array ultraviolet-visible detector was used. The detector output was stored, integrated and manipulated using a personal computer running Waters

'Empower' software. Samples were separated with a 250 x 4.6 mm Beckman Ultrasphere C18 (5 micron) column fitted with a 7.5 x 4.6 mm Beckman Ultrasphere C18 (5 micron) guard column thermostatted to 25°C using a Waters column heater. The mobile phase was degassed HPLC-grade methanol:water (70:30 v/v). Solvent flow rate was 1.0 ml/minute. Injection volume for both cucurbitacin standards and zucchini extracts was 5 μl.

Identification of cucurbitacins was based on HPLC retention time. Detector response at 235nm (λ_{max}) was linear over the concentration range 0-2.0 mg/ml. Standard samples exhibited less than 2% variability in individual calculated concentrations between triplicate injections of the same sample.

4 Results

4.1 Sensory

Table 1 gives the sensory scores for bitterness averaged over all fruit from that cultivar. Fruit were harvested from all plants bearing fruit on the day of harvest. Up to four fruit were harvested from some plants.

Fruit from the same plant did not have similar bitterness levels. The level of bitterness was low in most fruit. However some fruit did exhibit strong bitterness.

On Friday 23 May (more than two months after harvest) dilute (1 ppm and 10 ppm) solutions of the cucurbitacin-E glycoside standard were tasted alongside thawed slices of cultivars 15, 17, 20, 21, 22 and 23. These cultivars were selected for tasting again because of the HPLC results reported below. Slices from cultivar/plant 17/4, 20/6, 20/7, and 21/1 were all very bitter, but slices from 22, 21/2, 15 and 23 were not as bitter. The sensation of bitterness in the dilute solutions was very similar but not as strong as that experienced in the thawed fruit.

*Table 1: Sensory scores for bitterness for fruit tasted (those marked with a * were tested by HPLC analysis).*

Cultivar number	No. of plants harvested	No. of fruit tasted	Mean bitterness score	No. of fruit more than slightly bitter
1	12	14	1.07	0
2	8	12	1.08	0
3	12	13	0.92	0
4	8	9	1.78	1
5	15	18	1.44	1
6	12	16	0.93	0*
7	7	9	1.44	1*
8	11	13	1.31	0
9	7	8	1.25	0

Cultivar number	No. of plants harvested	No. of fruit tasted	Mean bitterness score	No. of fruit more than slightly bitter
10	9	10	1.1	0
11	9	12	1.25	1
12	14	17	2.00	5*
13	9	21	1.19	0
14	11	16	0.81	0*
15	12	13	1.85	4*
16	12	14	1.07	0
17	4	10	1.6	2*
18	10	11	0.82	0
19	10	11	1.00	0
20	8	11	2.45	4*
21	14	16	1.69	3*
22	6	7	2.71	6*
23	10	12	1.33	1*
24	8	9	0.89	0

4.2 *HPLC cucurbitacin tests*

Using the quantitation method outlined in the method above, **none** of the samples contain the cucurbitacins used as standards. However, in a few of the samples (cultivars 15, 20, 21 and 22) there were very small peaks that eluted in the correct position for cucurbitacin-E-glucoside but their spectral characteristics were incorrect when compared with the authentic standard. There are many different compounds in the cucurbitacin family and the sugars to which they attach as glycosides also vary. The calculated concentrations for these samples (assuming the identity was correct) would have been around 4-6 ppm which would have made them very bitter, which does match up with some of the tasting results.

5 *Conclusions*

This analysis was based on one harvest only. Fruit were harvested from every plant that had fruit of a suitable size, and every fruit harvested was tasted individually to determine the incidence of bitter fruit. This was to show any plant-to-plant differences within a cultivar. Other researchers have noted this difference in bitterness between zucchini plants. Some fruit certainly appeared to be more bitter than others, and were definitely unpalatable. Where individual fruit were more bitter than other fruit of the same cultivar, these fruit were analysed separately by HPLC (e.g. cultivars 15, 17, 20 and 23 had fruit exhibiting bitterness levels between moderately bitter and very

bitter, and cultivars 17 and 20 had especially bitter fruit). Some cultivars had similar bitterness levels in all fruit (e.g. cultivars 22 where 6 of the 7 fruit were moderately bitter) and so fruit from these cultivars were bulked for analysis.

It was interesting that cultivars 15, 20, 21 and 22 all had small HPLC peaks in the correct position. Three of these cultivars were yellow-skinned and one (15) was green-skinned. Although the spectral characteristics of these compounds did not match the authentic standard, the identity of these compounds and their connection with the bitter tastes observed is of real interest but outside the scope of this project. The compounds causing the strongest bitter tastes were not identified in our tests. It is possible that these could be cucurbitacins or glycosides other than the standards used.

Although cucurbitacin-E was not found in any cultivar tested, we did detect high levels of bitterness in some fruit. Although we did not find this specific compound in any fruit, we cannot draw the conclusion that other similar compounds are not implicated or that such compounds would never develop in these cultivars under different conditions. The propensity for these toxic compounds to be produced appears to be a combination of both genetic inheritance and cultural conditions. Even though the cultural conditions did not induce the specific compounds at the trial site for the one harvest analysed, we cannot draw the conclusion that they may not be induced under different conditions.

Neither can we be sure that the bitter compounds tasted were cucurbitacins. Fruit varied in their bitterness, but taste panellists used words such as astringent, hot, grassy and bitter aftertaste, to describe the sensation produced. When a dilute (10 ppm) solution of the cucurbitacin E glycoside standard was tasted the sensation of bitterness was very similar but not as strong as that experienced from the fruit.

6 Recommendations

The propensity for any of the cultivars tested to produce bitter fruit under different conditions cannot be ruled out. Conclusions based on the results of one harvest at one site would be premature.

The bitter compounds tasted were not chemically identified as a result of this research.

It is recommended that the identity of the bitter compounds is further investigated and that trials are conducted to determine whether cultivars produce bitter fruit under different conditions.

It is also recommended that any bitter fruit reported by consumers are obtained for analysis.

7 References

Gorski, P.M.; Jaworski, A.; Shannon, S.; Robinson, R.W. 1985: Rapid TLC and HPLC test for cucurbitacins. *Cucurbit Genetics Cooperative, USA, No. 8:* 69-70.

Herrington, M.E. 1983: Intense bitterness in commercial zucchini. *Cucurbit Genetics Cooperative, USA, No. 6:* 75-76.

Heyes, J.A. 2002: Cucurbitacins in bitter zucchini. *Crop & Food Research Confidential Report No. 598.* Palmerston North, New Zealand Institute for Crop & Food Research Limited.

Hutt, T.F.; Herrington, M.E. 1985: The determination of bitter principles in zucchinis. *Journal of the Science of Food and Agriculture 36:* 1107-1112.

Appendix

Cultivar ID

Cultivar number	Cultivar name	Seed company	Cultivar number	Cultivar name	Seed company
1	Blackjack	SPS	13	Gold Rush	Yates
2	Black Beauty	Kings Seeds	14	Regal Supreme	Hendersons
3	Shimmer	S&G	15	ZU357	S&G
4	42688	Webling & Stewart	16	41688	Webling & Stewart
5	Panther	SPS	17	Gold Rush	S&G
6	Blackjack	Yates	18	Gold Coast	S&G
7	Costata Romanesco	Kings Seeds	19	Commander	Yates
8	Arlesa	S&G	20	Zephyr	Kings Seeds
9	Eclipse 6114	Asian Seeds	21	Butterfingers	SPS
10	Congo	SPS	22	Solar Flare	Kings Seeds
11	Ambassador	Kings Seeds	23	Black Belt	Hendersons
12	Regal Black	SPS	24	Eclipse 611T	Asian Seeds