New Zealand Institute for Crop & Food Research Limited
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CROP & FOOD RESEARCH
Mana Kai Rangahau




Crop & Food Research Confidential Report No. 598

# Cucurbitacins in bitter zucchini

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April 2002

A report prepared for
**Vegfed (The NZ Vegetable and Potato Federation Inc.)**

Copy 8 of 8

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# Contents

# 1 Executive summary

In the summer of 2001/02 there were media reports of fourteen people seeking medical attention after eating bitter zucchini in Auckland and Christchurch. Bitter zucchini have previously been associated with public health scares in Australia and the US in the early 1980s.

This report was requested to cover the following specific aspects:

1. Cucurbitacins' action/toxicity level/published levels in vegetables, including an outline exposure assessment made on the basis of published data.
2. Levels of cucurbitacins detectable by taste v. toxicity levels.
3. Control of cucurbitacin synthesis: weather, pest pressure.
4. Species and strain differences in levels of cucurbitacins in vegetables.
5. Any special links to New Zealand: are there any strains of cucurbits that are high in cucurbitacins and particularly grown in New Zealand?

In summary, the mode of action of cucurbitacins is manifold, with principal effects on the gastro-intestinal, circulatory and central nervous systems. The threshold for toxicity for the most common cucurbitacin in zucchini fruit appears to be around 2-20 mg; a lethal dose for mice and rats is around 1-40 mg/kg body weight. Bitter zucchini can contain 600-7000 ppm cucurbitacins. Cucurbitacins are detectable by taste in zucchinis at 2 ppm, which has led to reassurances that people are likely to reject bitter zucchini before they are harmed. Unfortunately, bitterness is not detected rapidly in the mouth and the toxic dose is so low that the accidental swallowing of 3 g (i.e. the first mouthful) of a very bitter fruit leads to the consumption of a toxic dose.

I have searched for, but not found, published evidence of cucurbitacin synthesis in zucchini fruit being controlled by weather or pest pressure. Rather, it is stated to be under strong genetic control and appears to be associated with off-type plants. This is in marked contrast to cucumbers; and to reports in the media. It is imperative research is done to verify this point, as it has marked implications for preventing further outbreaks.

# 2 Cucurbitacins' action/toxicity level/published levels in vegetables, including an outline exposure assessment made on the basis of published data

Bitterness in foodstuffs is often associated with the presence of toxic compounds and it has been argued that people's aversion to bitterness is a primeval survival trait (Rouseff 1990). Cucurbitacins are oxygenated tetracyclic triterpenes (Guha & Sen 1975). There are at least 24 different cucurbitacins, differing in their substituents (details in Miro 1995); many have glycosylated forms as well as the aglycone. The most common type in the fruit of zucchini is cucurbitacin E-glycoside.

Vegetables containing cucurbitacins have been used since ancient times as purgatives, vermifuges, narcotics and anti-malarials (Merck Index). Miro (1995) details the pharmacological effects of cucurbitacins. Briefly, these include:

- gastrointestinal effects (they stimulate stomach secretions and have purgative properties);
- cytotoxic and anti-tumour action (although their anticancer properties may not be greatly below their toxic concentrations);
- hepatoprotective and hepatocurative activities (protective or curative properties against liver diseases such as hepatitis and cirrhosis);
- anti-inflammatory activity;
- interference with ovulation in mice;
- lowering blood pressure by increasing capillary permeability;
- central nervous system effects (possibly including narcotic or hallucinogenic effects);
- antimicrobial activity (both bactericidal and fungicidal);
- and antihelminthic activity.

In addition to these pharmacological effects, there are reported effects on plants, where they exhibit anti-gibberellin activity; and important effects on insects, which are referred to below.

## 2.1 Toxicity

Various publications quote $LD_{50}$ values for cucurbitacins: 1-7 mg/kg of cucurbitacins A, B or C in rats and mice (Ferguson et al. 1983); 40 mg/kg of cucurbitacin E-glycoside in mice (EPA 1999); 1 mg/kg for cucurbitacin D administered intravenously, with death caused by respiratory-cardiac failure (Miro 1995).

Stock poisoning by cucurbitacins occurs in southern Africa, particularly during drought when there are limited alternative food supplies (Ferguson et al. 1983).

Toxicity in humans can be deduced from the concentrations at which clinical symptoms have been observed. In an Australian outbreak in the early 1980s Herrington (1983) recorded that ingestion of 3 g of bitter zucchini (i.e. the first mouthful) was sufficient to cause nausea followed by collapse, stomach cramps for a further three days, and diarrhoea which persisted for longer. Vomiting was rare. The taste was apparent and penetrated the whole meal; but just chewing the affected fruit or swallowing small amounts was sufficient to produce diarrhoea and sometimes stomach cramps. Ferguson et al. (1983) wrote of the same incident and said that when five people consumed approximately 700 g each of zucchini within a six-hour period, two people collapsed and the others suffered severe cramps and diarrhoea; but not all of this zucchini was bitter. In an outbreak reported from California and Alabama, also in the early 1980s, symptoms of severe cramps, persistent diarrhoea and collapse followed within 1-2 h of eating 3 g of bitter zucchini (Fenwick et al. 1990). These figures, combined with the concentration data below, suggest that serious symptoms can be induced in adult humans by the ingestion of 2-20 mg of cucurbitacin E.



# 3 *Levels of cucurbitacins detectable by taste v. toxicity levels*

Cucurbitacins are among the most bitter substances known (Fenwick et al. 1990). Zucchini flesh containing 2 ppm cucurbitacins was recognisably bitter in taste tests (Hutt & Herrington 1985). Individual extracted cucurbitacins in water can be detected at 1-10 ppb in taste tests (cucurbitacins B and E respectively, Fenwick et al. 1990).

This fact has encouraged several observers to make comments such as 'Most herbivores, including man, will not feed on plants containing cucurbitacins, which taste bitter in dilutions as low as 1 ppb' (Rhodes et al. 1980). Against this reassuring thought is the reality of repeated stock and human poisoning. It seems that the high concentrations, which can accumulate in zucchini fruit, mean a single mouthful can be toxic. This problem is exacerbated by the facts that bitterness is detected at the rear of mouth and the intensity of bitterness is slow to register; it can be strongest as an after taste (Fenwick et al. 1990). Cucurbitacins are also lipid soluble, so it may be significant that the bitter zucchini which affected a Christchurch couple in March 2001 were stir-fried (New Zealand Herald, Auckland, 2 March 2002). Possibly the bitterness had spread through the meal and was masked by other flavours? Six other people in Christchurch were affected at one dinner party in 2002 (The Press, 2 March 2002).

The media frequently referred to a smell 'like cat's urine' associated with bitter zucchini (New Zealand Herald, 2 March 2002; The Press, 2 March 2002; Produce Week, 12 March 2002) but I have not found this reported in

scientific papers. The only references I have found are the web-based information sheet from Prof Ian Shaw, where he states 'the smell is pungent and has been described as like cat urine' (http://www.vegetables.co.nz/about/7_press.cfm); and a personal communication from Winna Harvey (Crop & Food Research) who reported that a grower who noticed zucchini with a bitter, stinging taste reported that they also had a 'funny smell'.

# 4 Control of cucurbitacin synthesis: weather, pest pressure

The production of cucurbitacins appears to be under strong genetic control. Several studies have implicated a single dominant gene responsible for bitter fruit (including Herrington 1983). In zucchini (*Cucurbita pepo*) the 'bitter fruit' gene was independent from a gene for 'seedling bitterness' (Dane et al. 1989; Rehm 1968). The progeny of a bitter-fruited *Cucurbita pepo* 'custard squash' also indicated fruit bitterness was conferred by a single dominant gene, as a 3:1 ratio of bitter fruit was obtained in the F2 generation (Herrington 1983). The same was reported for cucumbers (*Cucumis sativus*) (Andeweg & de Bruyn 1959). In the same paper, melon (*Cucumis melo*) seedling bitterness is reported as being under the control of a single dominant gene.

There was a major effort in Holland in the 1950s to breed out bitterness from cucumbers. Researchers screened a huge range of varieties looking for seedlings that were not bitter, hoping these would never produce fruit that were bitter. None of the 87 varieties tested consistently produced non-bitter seedlings. One plant (out of 15 000 tested) of the American variety, Improved Long Green, had no bitterness in any plant part and was used in a breeding programme (Andeweg & de Bruyn 1959).

Zucchini fruit are generally regarded as being of very low bitterness, but their leaves contain higher levels of cucurbitacins than closely related squash, e.g. 'Early Golden Bush Scallop'. The latter are resistant to cucumber beetles and the former highly susceptible, as these insects respond to cucurbitacins as a feeding stimulant (Wiseman et al. 1961; Sharma & Hall 1971).

This strong genetic control is also reported for watermelons, where it was found that the extreme bitterness character can introgress into commercial populations from naturalized plants growing in field margins or as volunteers from earlier crops (Herrington et al. 1986). Herrington suggests (pers. comm.) that the same may happen for zucchini, and recommends that care is taken whenever off-type bushes are found (more trailing stems), or off-type fruit (more squat, bulbous, warty). In his experience, bitterness is much more common in these off-type plants. Occasional plants producing bitter fruit are easier to envisage in open-pollinating varieties; but if they are found in F1 hybrids, which make up an increasing proportion of the seed companies' range, it would suggest that the parent lines are not pure or the hybrid seed is contaminated. If home gardeners save seed from hybrid varieties, again this

could account for the occurrence of bitter progeny. It certainly means that where zucchini bitterness is reported it is imperative to identify the source of the plants; if they were supplied by a commercial seed company, they may need to check the integrity of the breeding programme from which they source their seed.

In cucumbers there is considerable published evidence for an additional environmental effect on fruit bitterness. There was increased bitterness when cucumbers were grown in raised beds or fertilised with municipal sludge (Fenwick et al. 1990). Bitterness in cv. Kagafutokyuri was stronger in younger fruit and in more vigorous plants, with more bitter fruit on the first lateral than secondary lateral (Kano et al. 1997). Bitterness was enhanced by N fertilisation (Kano et al. 2001; Kano et al. 1999). Charles Voigt (vegetable extension specialist, University of Urbana, Illinois; pers. comm.) says cucumber bitterness increases under water and heat stress and perhaps under conditions of poor pollination.

The evidence for an environmental effect on zucchini fruit bitterness is nowhere near as strong. The cucurbitacin content in **leaves** of zucchini cv. Black increased when they were mechanically injured (Tallamy 1985). Charles Voigt (pers. comm.) states that viral diseases can produce bitterness in zucchini **fruit** as part of their symptomology; but this should be accompanied by marked fruit deformation. The Alabama Co-operative Extension System website http://www.aces.edu/department/ipm/avgcukes.htm is categorical: cucumber bitterness is under environmental control but 'in squash the bitter flavour is because of genetic factors that are not influenced by environmental stress. Squash bitterness is much rarer than cucumber bitterness and has only been reported in zucchini and yellow straightneck varieties'. It seems likely that the statement on the Vegfed website that the gene for cucurbitacin production in zucchini 'appears to be switched on by particular weather conditions' (http://www.vegetables.co.nz/about/7_press.cfm) is an extrapolation from research in cucumbers.

The evidence for really bitter-fruited zucchini carrying a dominant gene for fruit bitterness seems persuasive to me. Rather than looking for environmental or insect-related causes, attention should be focussed on the varieties of zucchini and source of seed which caused the problems observed this year.

Some recent developments may also be relevant. Cucurbitacins are such powerful feeding stimulants for some species of beetle that they can be used as an additive to conventional pesticides to make the pesticides vastly more effective. Florida Food Products is producing 'Invite', and MicroFlo Co. produces 'Slam' and 'Adios'. These are low quantities of insecticide (e.g. carbaryl) laced with cucurbitacin which makes the insects deliberately consume the insecticide. The amounts of cucurbitacins in these products is claimed to be very small: less than 3.4 g cucurbitacin will be applied per acre per season; and at this level it would certainly not be hazardous to humans. Diabroticid beetles (such as the corn root borer) can detect amounts of cucurbitacins as small as 0.1 ng (Halaweish & Tallamy 1998).

But the problem is that the usefulness of cucurbitacins as feeding stimulants for use in these 'safe' pesticides has led to a drive to develop high-yielding cucurbits with high cucurbitacin content. Breeding work reported by Rhodes

et al. (1980) describes the deliberate crossing of *Cucurbita pepo* with the wild species *Cucurbita texana*. Cucurbitacin contents in the hybrid fruit (predominantly cucurbitacin E and cucurbitacin E-glycoside) reached 480 ppm in the tissue. This breeding programme is still continuing (references in Halaweish & Tallamy 1998). It is purely speculation on my part, but it seems possible that introgession from wild *Cucurbita* spp. or cultivated high-cucurbitacin species may be responsible for the continuing problem of fruit bitterness in commercial zucchini crops.



# 5 *Species and strain differences in levels of cucurbitacins in vegetables*

## 5.1 *Zucchini (*Cucurbita pepo*; summer squash, courgette)*

Commercial zucchini must, by definition, be low in bitterness, since it is detectable at 2 ppm in a taste panel (Hutt & Herrington 1985). Fruit cucurbitacin contents in wild *Cucurbita pepo* were measured by Sharma & Hall (1973) and Metcalf et al. (1982) at 20-40 ppm. Occasional plants, which produce extremely bitter fruit, have appeared periodically. Bitter zucchini containing around 600 ppm cucurbitacins were found when there was an outbreak of poisoning in Queensland (Hutt & Herrington 1985). Fruit samples sent from these plants for analysis in the US contained up to 1120 ppm (Ferguson et al. 1983). Bitter zucchini containing 3000-7000 ppm cucurbitacin E were found during the California and Alabama outbreak; canned zucchini had 930 ppm cucurbitacin E, on a whole-can basis (Fenwick et al. 1990). If 2-20 mg cucurbitacin is required to cause medical symptoms, this could be achieved by eating less than 1 g of the most concentrated samples.

In the Australian outbreak, 16 of 20 farms identified as the source of bitter fruit grew only cv. 'Blackjack'. This is a vigorous, widely-grown variety, still popular with the home gardener in New Zealand and with commercial growers for rapid production. Another implicated variety in Queensland was 'Castle Verde' (Herrington 1983). However, Herrington (1983) reported that plants producing bitter fruit in a Blackjack crop were off-type. The seed from which they grew may been collected from volunteers in the seed production field or from a weedy contaminant.

I have no information about the varieties responsible for the California/Alabama outbreak. Steps to identify the source of the fruit causing trouble this summer have not been reported. Winna Harvey (pers. comm.) said one grower reported bitter fruit in the 2000/01 season from a crop of glasshouse-grown Watkins' 'Black Beauty'. This is an open-pollinated older variety; a source at Webling and Stewart advised me that they supply only hybrid varieties now.

## 5.2 *Squash (winter squash,* Cucurbita maxima*; some* Cucurbita pepo *(e.g. spaghetti squash),* Cucurbita moschata *(e.g. butternut))*

All three of these species are generally regarded as non-bitter with less than 20 ppm cucurbitacins in the fruit (Metcalf et al. 1982). A golden variety of acorn squash (*Cucurbita pepo*) was removed from the market because it was too often bitter (Charles Voigt, pers. comm.).

## 5.3 *Cucumber* (Cucumis sativus)

Bitter fruit may contain over 1000 ppm cucurbitacin (Rehm et al. 1957). One cucumber variety, Reusrath's Bitterfreie, was reported as always producing non-bitter fruit (Andeweg & de Bruyn 1959).

## 5.4 *Melons* (Cucumis melo*: musk melons, honeydew,* Citrullus lanatus*: watermelon*)

Some watermelons contain significant quantities of cucurbitacins in the fruit. Concentrations of 50-90 ppm were measured (Sharma & Hall 1973). A bitter mutant of the Hawkesbury watermelon contains abundant quantities of cucurbitacin E-glycoside, which makes it ideal as an industrial source: juice containing 300-800 ppm was readily extracted from the fruit for use as a feeding attractant in pesticides (Matsuo et al. 1999).

## 5.5 *Wild cucurbits*

In a survey of 18 of the 24 known Cucurbita species, Cucurbita andreana had the highest fruit cucurbitacin concentration, at 2780 ppm cucurbitacin B plus 420 ppm cucurbitacin D (Metcalf et al. 1982). Cucurbita texana (used as a breeding parent in the commercial production of cucurbitacins) had 750 ppm cucurbitacin E glycoside and 367 ppm cucurbitacin I in the same study.

# 6 *Any special links to New Zealand: are there any strains of cucurbits that are high in cucurbitacins and particularly grown in New Zealand?*

All established varieties of zucchini in commercial production are low in cucurbitacins, by definition: consistent bitterness would rule out a variety for consumption. One yellow acorn squash has been withdrawn from sale because of the number of complaints associated with it (Charles Voigt, pers. comm.). Where new varieties are introduced to the market, these should perhaps receive closer scrutiny.

The varieties available commercially in New Zealand include:

**SPS seeds (Winter 2001 newsletter)** supply Congo, Panther and Sunline zucchini. Mention is made in the newsletter of new varieties under trial: the powdery-mildew-tolerant 'Vaquero' and multi-virus tolerant zucchini.

**Webling and Stewart** supply Texas (main seller) and Blackjack, which is very vigorous. It is favoured by growers for rapid fruit production early or late in the season, but is also grown mid-season.

**Yates NZ (catalogue)** lists Blackjack, Commander and Goldrush. Watkins Seeds, now owned by Yates NZ, sold Black Beauty in 2001.

**LeFroy Valley** supplies Congo.

**Kings Seeds (catalogue, 2001/03)** lists Cocozelle, Black Beauty, Costata Romanesco, Gold Rush, Solar Flare and Zephyr.

I do not know if any attempts have been made to trace the origin of the bitter fruit responsible for this season's outbreaks, or even if it has been verified that the fruit came from commercial growers; it seems necessary to establish this, but it is beyond the scope of this contract.

Several of the varieties named above have been associated previously with occasional bitter fruit, but this may simply be because of their widespread popularity (e.g. Blackjack). If there is indeed no particular environmental contribution to zucchini bitterness, I would recommend Vegfed, seed companies and the Public Health Group of the Ministry of Health collaborate to pinpoint the source of zucchini responsible for toxicity.

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