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Perception of bitterness, sweetness and liking of different genotypes of lettuce

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ABSTRACT

Lettuce is an important leafy vegetable, consumed across the world, containing bitter sesquiterpenoid lactone (SL) compounds that may negatively affect consumer acceptance and consumption. We assessed liking of samples with differing absolute abundance and different ratios of bitter:sweet compounds by analysing recombinant inbred lines (RILs) from an interspecific lettuce mapping population derived from a cross between a wild (*L. serriola* acc. UC96US23) and domesticated lettuce (*L. sativa*, cv. Salinas). We found that the ratio of bitter:sweet compounds was a key determinant of bitterness perception and liking. We were able to demonstrate that SLs, such as 8-deoxylactucin-15-sulphate, contribute most strongly to bitterness perception, whilst 15-*p*-hydroxylphenylacetyllactucin-8-sulphate does not contribute to bitter taste. Glucose was the sugar most highly correlated with sweetness perception. There is a genetic basis to the biochemical composition of lettuce. This information will be useful in lettuce breeding programmes in order to produce leaves with more favourable taste profiles.

1. Introduction

Sesquiterpene lactones are anti-feedants and phytoalexins produced by lettuce (*Lactuca sativa* L.). Selective breeding against the bitter taste imparted by them has reduced presence of these compounds in domesticated lettuce cultivars dramatically (Wink, 1988). Many modern varieties do still contain perceivable quantities of sesquiterpene lactones and this is particularly relevant, with a move away from iceberg-type head-lettuce to bagged lettuces, which contain fewer high yielding, sweet cultivars and more red-leaved varieties, which typically contain much higher concentrations of the bitter compounds (Price, DuPont, Shepherd, Chan, & Fenwick, 1990). The perceived bitterness is enough to reduce palatability and consumption in a westernised diet, where fruit and vegetables are already under-consumed (Casagrande, Wang, Anderson, & Gary, 2007; Rogers & Pryer, 2012). It is widely believed that this bitterness can be counteracted by sweetness (Bartoshuk, 1975; Keast & Breslin, 2003); an improvement in flavour is therefore likely to be a consequence of manipulating both factors. Although sensory perception of individual sugars (Pangborn, 1963) and SLs (Price et al., 1990; Seo, Yang, Kays, Lee, & Park, 2009; Sessa, Bennett, Lewis, Mansfield, & Beale, 2000) has been previously assessed and sensory perception is well established in the case of sweet compounds, assessment of SL bitterness is sometimes contradictory and has not been considered with regard to tastant mixture suppression. Here we assess the interaction between sweet and bitter components within the natural food matrix of lettuce and additionally compare perception data to consumer liking.

Lettuce is a suitable crop in which to pursue flavour improvement as it is widely eaten across Europe and North America. Lettuce also contains a range of beneficial secondary plant metabolites, including, phenolics, ascorbate, α-tocopherol, lignans, as well as SLs (García-Macías et al., 2007; Oh, Trick, & Rajashekar, 2009); consequently, improving the flavour should increase consumer intake. Phytochemicals present in lettuce have been suggested as having a range of biological functions, from analgesic, anti-inflammatory, anti-tumor, and gastroprotective effects of the sesquiterpenoids (Giordano et al., 1990; Guzman et al., 2005; Sayyah, Hadidi, & Kamalinejad, 2004), to a cognitive effect of phenylpropanoid flavonoids (García-Macías et al., 2007; Spencer, Vauzour, & Rendeiro, 2009). Additionally lettuce, particularly the romaine type, is a source of iron and potassium and a good source

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of dietary fibre, folate and manganese, vitamins A, B1, B6, C, K, and omega-3 fatty acids (Belitz, Grosch, & Schieberle, 2009). Bitterness in lettuce is not thought to be linked to the beneficial biological effects of the same molecules, owing to distinct functional groups in the compounds (Behrens et al., 2009; Brockhoff, Behrens, Massarotti, Appendino, & Meyerhof, 2007; Chadwick, Trewin, Gawthrop, & Wagstaff, 2013) and so it is feasible to balance the reduction of those most bitter SLs while maintaining or increasing those with greatest biological function.

Sweet and bitter tastes are sensed through the binding of the tastants to G-protein coupled receptors located within papillae on the tongue. Sugars bind to type 1 receptors (T1R) (Meyers & Brewer, 2008) and bitter molecules to type 2 receptors (T2R) (Meyerhof et al., 2010). Whereas there are just two T1R receptors involved in sweet perception (T1R2/T1R3), there are 25 T2Rs responsible for binding a broad range of bitter molecules. Whereas some T2Rs are generalists and bind to a wide range of structurally diverse molecules, others are specialists, binding to a narrow range of compounds (Meyerhof et al., 2010). SLs have been found to activate the T2R46, a generalist receptor (Brockhoff et al., 2007). Within the population, it is common to categorise individuals as "bitter sensitive" or "bitter blind", and 25% of the population are "bitter blind"; however, this categorisation is due to polymorphisms of the Tas2R38 gene (Mennella, Pepino, Duke, & Reed, 2010). The receptor T2R38 is a specialist receptor, binding to thiouracil groups (as found in Brassica vegetables) and not to SLs. We therefore propose that "bitter blindness" resulting from Tas2R38 will not effect consumer perception of bitterness in lettuce.

We hypothesise that consumers are able to accurately detect sweetness and bitterness in lettuce as imparted by the compounds of interest. We also propose that taste interaction between sweetness and bitterness, as well as the absolute concentrations of the compounds, will have a significant effect on taste perception and liking. Additionally, it is broadly believed that consumers prefer foods which they perceive as sweet. To most consumers, a major factor in purchasing habits is liking for taste (Enneking, Neumann, & Henneberg, 2007) and so ultimately this will be the chief factor in delivering a positive change in consumer habits.

2. Materials and methods

2.1. Plant material and growth conditions

F_9 recombinant inbred lines (RILs) were supplied by the Michelmore lab (Genome Center, UC Davis, USA) and 102 RILs plus their parents, *L. sativa* cv. Salinas and the wild *L. serriola* UC96US23, were propagated by A.L. Tozer. For these studies, plants were grown under glasshouse conditions at The University of Reading and watered once or twice daily in accordance with the weather. The glasshouse temperature ranged from 17 to 30 °C. Seedlings were transferred from seed trays to 3½″ pots with Osmocote after 3 weeks, and were given Sangral 1:1:1 liquid fertiliser weekly. Plants were harvested after 49 days, at a mature, commercially viable, stage and prior to floral transition.

The 102 RILs were analysed by HPLC-MS (see section below) to assess SL abundance and sugar assays to assess the concentrations of sucrose, fructose and glucose (see section below) in order to determine which lines would be most informative. Eight RILs were selected, based on whether that line had high or low concentrations of sugar and SLs. The sample size was kept small to avoid fatigue in the consumer panel.

2.2. Consumer analysis and sample preparation

Lettuce samples were harvested daily on the morning of the tests and were used within an hour of preparation, being kept refrigerated and moist until they were needed in order to reduce respiration and sample wilting. Leaf samples were cut into strips, 5 cm by 1 cm, avoiding the midrib as this can contain more variable levels of SLs (Sessa et al., 2000). Samples were labelled with arbitrary three digit codes in Petri-dishes and three strips were provided per consumer. All consumer work took place in sensory booths at the University of Reading, with neutral odour, artificial daylight and controlled temperature. Forty-three consumers took part in the study, consisting of eight men and 35 women. Ages ranged from 17 to 68 with 6 over the age of 40 (mean = 29.8 years, median = 25 years). This skew in participant age was due to primary recruitment taking place on the university campus. Participants were recruited after ethical approval of the study (University of Reading Research Ethics Committee, study number 08/13) via email notification and poster advertisement and volunteers were screened by questionnaire for any dietary restrictions, allergies or health conditions that may have affected their ability to participate in the consumer study.

Consumer response was recorded using Compusense 5 software (Compusense Ontario, Canada). The study was divided into three sections. First, participants were asked to familiarise themselves with a labelled magnitude scale, rating their most bitter, sweet, salty and sour experiences on the scale. This was used to normalise their scores against other participants, to allow for high and low scale users. The main study involved rating lettuce samples presented to them, one at a time, in a balanced design for liking on a 9 point hedonic category scale (anchored from dislike extremely to like extremely), and then for perception of sweetness and bitterness, using labelled magnitude scales (where semantic descriptors from weak to strongest imaginable are positioned on a logarithmic scale, and scored 0–1.97). Participants were asked to taste each sample three times, once for liking, then sweetness and again for bitterness. Finally perception of aftertaste intensity was rated on a 5 point category scale (anchored from no after taste to very strong) after a 10 s wait period. Participants were also asked to give any additional comments on the samples. Once the assessment of one lettuce line was completed, participants were given the next sample after a 30 s rest period. Participants were given water and plain water crackers (Carr's, United Biscuits, UK) to cleanse their palate during this rest period. See supplementary data for a transcript of the questions exactly as posed. After the test, participants were given an exit questionnaire asking for further information on age, gender, frequency with which they consume lettuce, and also the regularity of their consumption of bitter foods in their diet, based on a list of 12 common bitter foods (white cabbage, green cabbage, red cabbage, cauliflower, kale, brussels sprouts, watercress, rocket, radish, coffee, tonic water, and broccoli). Finally they were phenotypically tested for bitter blindness, using PTC (phenylthiocarbamide) strips. Bitter blindness occurs in around 25% of people as the result of an inactive hTAS2R38 receptor and, while it is not directly responsible for detection of SLs, it is a widely accepted indicator of bitter taste acuity.

2.3. Chemical analysis

Sesquiterpene lactones and some polyphenols in the main population of 102 RILs were analysed by HPLC and identities confirmed by HPLC-MS, based on details published in Sessa et al. (2000); mass data for each compound were as follows; lactucin *m/z* 277; lactucopicrin *m/z* 411; 8-deoxylactucin *m/z* 332; 15-p-hydroxyphenylacetylactucin-8-oxalate *m/z* 490; lactucin oxalate *m/z* 348; lactucopicrin oxalate *m/z* 482. Full spectra are presented in Supplementary Fig. 1. Plant samples from each individual genotype were replicated in quadruplicate and analysed individually for determination of SLs. These were extracted as follows: 0.5 g of frozen homogenised leafy plant material was added to 2 ml of 70%