Bitterness of the non-nutritive sweetener Acesulfame Potassium varies with polymorphisms in *TAS2R9, TAS2R31* and *TAS2R38*.

Alissa L. Allen[1], John E. McGeary[2], and John E. Hayes[1]

1 Department of Food Science, College of Agricultural Sciences, The Pennsylvania State University, University Park, PA.

2 Providence Veterans Affairs Medical Center, Providence, RI

Background

• Sweetness is innately liked by humans (reviewed by (Steiner, Glaser et al. 2001)), even prior to birth (Snoo 1937). Many highly liked foods contain high endogenous amounts of natural sugars, or have sugars or other sweeteners added during processing. However, due to health risks such as cardiovascular disease, diabetes and obesity (Hill and Prentice 1995; Howard and Wylie-Rosett 2002), there has been a demand for reduced added-sugar in foods. To retain desired levels of sweetness while reducing calories, bulk carbohydrates are often replaced with non-nutritive sweeteners.

• Acesulfame potassium (AceK) was approved by the US Food and Drug Administration for use in dry foods in 1994; approval as a general-purpose sweetener followed in 2002. However, in addition to eliciting sweet sensations, many non-nutritive sweeteners also have objectionable side tastes, such as bitterness, that are experienced by some individuals but not others. Thus, better understanding of the mechanisms underlying this variability may facilitate improved product formulation, with the potential to substantially impact health and wellness.

• Numerous studies have demonstrated that the perception of bitter taste in humans is moderated by genetic variation in TAS2R genes (Duffy, Davidson et al. 2004; Pronin, Xu et al. 2007; Reed, Zhu et al. 2010; Hayes, Wallace et al. 2011; Roudnitzky, Bufe et al. 2011). Kuhn and colleagues demonstrated receptors encoded by *TAS2R31* (formerly called *TAS2R44*) and *TAS2R43* are activated by saccharin and AceK *in vitro* (Kuhn, Bufe et al. 2004). Roudnitzky and colleagues (2011) were able to demonstrate *in vitro* that the Arg35Trp (R35W) polymorphism in *TAS2R31* was causal. However, they also found that even in the presence of the high functioning Trp35 allele, other *TAS2R31* mutations abolished the ability of T2R31 to respond to AceK and saccharin. A third study showed that a polymorphism at position 187 in *TAS2R9* was associated with the ability to be activated in the presence of a bitter drug (ofloxacin) (Dotson, Zhang et al. 2008).

• Here, we explore relationships between functional SNPs in *TAS2R9, TAS2R31* and *TAS2R38* with AceK bitterness in addition to PROP bitterness.

Methods

Participants – Reportedly healthy, non-smoking adults (n = 97; 24 men; aged 18-45 years) were recruited from the Penn State community. Procedures were IRB approved, informed consent was obtained, and participants were paid for their time.

Stimuli – PROP phenotype was determined using a standard PROP concentration series (ie, Hayes et al 2008). In a separate portion of the first session, subjects also rated the intensity of 25 mM AceK. Stimuli were presented as 10mL aliquots in plastic medicine cups at room temperature. Subjects were instructed to take the entire sample into his or her mouth, swish for 3 seconds, and then spit prior to rating. Subjects rinsed with 20C water prior and between every trial.

Measuring Intensity – Prior to rating samples, subjects were oriented to a generalized Labeled Magnitude Scale (gLMS) with a list of 15 imagined or remembered sensations that includes oral and nonoral items. Instructions encouraged subjects to make ratings in a generalized context by indicating the top of the scale should reflect the 'strongest sensation of any kind'. Data were collected via Compusense *five* (Guelph ONT)

Genetic Analysis – DNA was collected from saliva, using Oragene collection kits according to manufacturer instructions (Genoteck. Inc; Ontario, Canada). SNPs in *TAS2R9, TAS2R31* and *TAS2R38* were determined using Sequenome MassARRAy software.

Aggregate Genetic Bitterness Score – Using one SNP each from *TAS2R9, TAS2R31* and TAS2R38, we constructed an Aggregate Genetic Bitterness Score (AGBS) based on the total number of functional alleles each participant carried.

Results

Finding 1: Variation in the *TAS2R9* Val187Ala allele predicts AceK bitterness

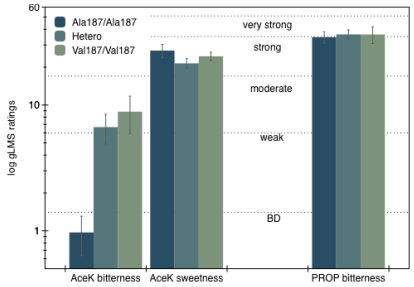

Figure 1: Effect of *TAS2R9* polymorphisms on the bitterness and sweetness of AceK and bitterness of PROP. Adjectives refer to semantic labels on the general Labeled Magnitude Scale (gLMS). BD refers to 'barely detectable'.

Finding 2 : Variation in the *TAS2R31* Val240Ile allele predicts AceK bitterness

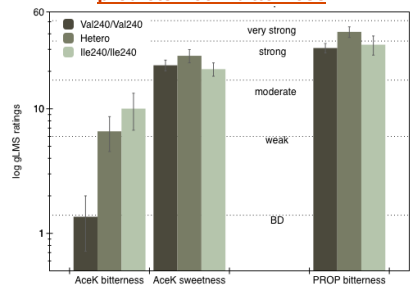

Figure 2: Same as Figure 1, but for *TAS2R31*.

Finding 3 : Variation in the *TAS2R38* Ala49Pro allele predicts AceK bitterness and PROP bitterness

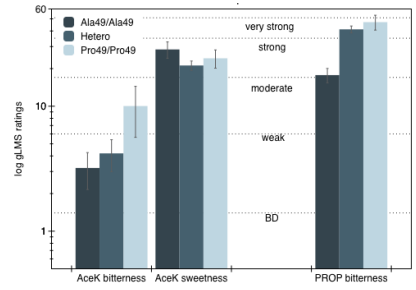

Figure 3: Same as Figure 1, but for *TAS2R38*.

Finding 4: Aggregate Genetic Bitterness Score predicts the bitterness of AceK

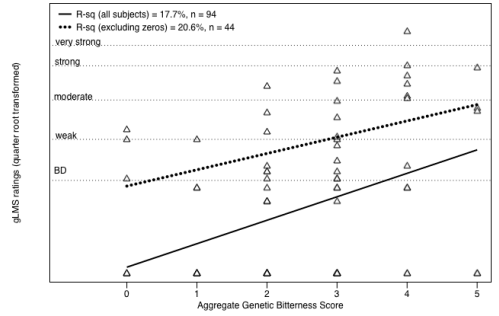

Figure 4: Correlation between Perceived Bitterness of AceK and the Aggregate Genetic Bitterness Score (AGBS). The solid line is for all 97 participants; the dotted line is the fit after non-responders have been removed.

Discussion and Application

• Using suprathreshold psychophysics in humans, we explained variation in the perceived intensity of AceK bitterness using a candidate SNP approach across multiple TAS2R genes. These data suggest more than one receptor is responsible for the perception of AceK bitterness.

• Tag SNPs believed to be in complete linkage disequilibrium with the putatively causal SNP in *TAS2R31* predicted variation in AceK bitterness. In addition, a polymorphism Ala187Val in *TAS2R9* has not been previously reported as being functional *in vivo* for AceK was shown to predict bitterness. Lastly, *TAS2R38* haplotype was found to be a predictor of AceK bitterness, with the PA_ homozygotes perceiving more bitterness than the AV_ homozygotes. Conversely, a putatively functional SNP in *TAS2R4* did not predict bitterness.

• Polymorphisms in three bitter receptor genes on different chromosomes all appeared to contribute to the suprathreshold bitterness of AceK, and a simple Aggregate Genetic Bitterness Score (AGBS) was able to explain 18% of the variance in perceived bitterness. Upon the removal of non-raters of AceK bitterness the AGBS explained 20.6%.

• Present data suggest additional polymorphisms may contribute to the perception of AceK bitterness. More research is needed to determine if other receptors confer additional response, and whether rare polymorphisms in the genes studied here attenuate responses to AceK.

• Given the diversity and complexity of the TAS2R bitter taste system, using a single bitter compound to screen panelists is not sufficient when screening panelists for their ability to detect bitterness. Selecting panelists using the sample of interest will ensure the ability to perceive the bitterness.

• A high number of participants that reported no bitterness from AceK. This observation reemphasizes the need for product developers and sensory specialists to consider individual differences in perception that may be partially or completely hidden by overall group means. Genotyping or phenotyping panelists may improve selection for participation in sensory studies, and begin to inform work on genetically driven product segmentation.

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