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Modification of the bitterness of caffeine

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ABSTRACT

Caffeine is the world’s most consumed psychoactive chemical and as such is a valuable commodity to the food and beverage industry. Caffeine also activates the bitter taste system causing a potential problem for manufacturers wanting to develop products containing caffeine. In the present study both oral peripheral and central cognitive strategies were used in an attempt to suppress the bitterness of caffeine. Subjects (n=33) assessed the influence of sodium gluconate (100 mM), zinc lactate (5 mM), sucrose (125, 250 mM), milk (0, 2, 4% milkfat), and aromas (coffee, chocolate, mocha) on the bitterness of caffeine (1.5, 3, 4.5 mM). The oral peripheral strategies proved most effective at suppressing the bitterness of caffeine: zinc lactate (-71%, p<0.05), non-fat milk (-49%, p<0.05), and sodium gluconate (-31%). Central cognitive strategies were partially effective: 250 mM sucrose (-47%, p<0.05) and mocha aroma (-10%) decreased bitterness, while chocolate (+32%) and coffee (+17%) aromas increased perceived bitterness. Overall, zinc lactate was the most effective bitterness inhibitor, however the utility of zinc in foods is negated by its ability to inhibit sweetness.
INTRODUCTION

Caffeine is the most commonly ingested psychoactive drug in the world, naturally occurring in coffee, cocoa (chocolate), and black teas, and as an additive in soft- and energy-drinks. The stimulant effect of foods that contain caffeine, such as the coffee bush berry, has been known for hundreds of years (Flament, 2002). The stimulant effect was due to caffeine and related methyl xanthines such as theophylline and theobromine whose physiologic mode of action is antagonism of the adenosine A1 and A2 receptors in the brain (Rainnie, Grunze, McCarley, & Greene, 1994).

As the site of action of caffeine is similar to the site of action of addictive drugs, it is not surprising that caffeine, at levels in common beverages, has been proposed to be an addictive drug (Holtzman, 1990). The dose of caffeine required to modify behaviour in humans is low (~50 mg) (Nehlig, 1999), similar to the dose delivered in 500 ml common cola soft-drinks (~53-65 mg, 0.55-0.67 mM). Behavioural studies have shown that the consumption of caffeine promotes a physiologic and psychologic dependence that is reinforced with repeat consumption (Garrett & Griffiths, 1998; Hughes, Oliveto, Bickel, & Higgins, 1993; Schuh & Griffiths, 1997). The common method of repeat caffeine consumption is via caffeinated foods such as coffee, tea, cocoa, and soft drinks. Caffeine may promote liking and consumption of these foods via the development of flavor preferences; where individuals associate (unconsciously) a food/flavor with its post-ingestive consequences. The mode of action of caffeine in developing flavour preference is not immediate (Yeomans et al., 2000) as, for example, we experience with a sucrose solution (sweet and appetitive) but the positive influences occur post-consumption with increased vigilance and attention, enhanced mood and arousal as well as enhanced motor activity.
Suppression of caffeine bitterness

Caffeine may elicit bitterness depending on the concentration (Keast & Roper, 2007) and this can be a problem for food and beverage manufacturers. Solving this problem is complicated by the observation that the human bitter taste system is complex. It is subserved by approximately two dozen putative G-protein coupled receptors, the TAS2Rs (Adler et al., 2000; Chandrashekar et al., 2000), and several post-receptor transduction mechanisms (Huang et al., 1999; Kinnamon & Margolskee, 1996; Rossler, Kroner, Freitag, Noc, & Breer, 1998; Spielman, Huque, Whitney, & Brand, 1992; Wong, Gannon, & Margolskee, 1996). Moreover, caffeine may modify flavour at the peripheral level via interference with taste transduction (Peri et al., 2000; Rosenzweig, Yan, Dasso, & Spielman, 1999).

In general, there are three approaches to suppressing bitterness: physio-chemical interactions in a food or beverage matrix, oral peripheral physiological interactions with receptor cells (e.g., via receptor inhibitors), and central cognitive mixture suppression (e.g., via taste-taste and taste-aroma interactions).

Physio-chemical interactions can change flavour intensity or even generate new flavours. They occur in a simple aqueous solution: weak attractive forces, such as hydrogen or hydrophobic bonding, will result in altered structures; precipitation of the compounds will render them weaker or tasteless. The chemical composition of a food matrix will influence perceived flavour, changing from an aqueous to emulsion system (oil-in-water) decreased bitterness (Metcalf & Vicker, 2002).

Sodium and zinc salts inhibit the bitterness of certain compounds whether or not the salts elicit a taste, indicating that the inhibition is peripheral rather than based on perceptual interactions (Bartoshuk & Seibyl, 1982; Breslin & Beauchamp, 1995a; Keast & Breslin, 2002, 2005; Keast, 2003; Kroeze & Bartoshuk, 1985). As well as lipid having a physio-chemical influence on bitterness, the components of fats, fatty
Suppression of caffeine bitterness

acids, may also modify bitterness via interactions in the oral periphery (Koriyama, Wongso, Wananabe, & Abe, 2002).

Central cognitive effects can occur when different qualities of taste stimuli are mixed together and the perceived intensity of one or more of the components is diminished by the perception of the others. This is labeled mixture suppression (Pangborn, 1960) and is caused by cognitive interactions among taste qualities. As one example, mixture suppression occurs when you add sugar to coffee, both the sweetness of the sugar and bitterness of the coffee are reduced. Also, the combination of taste and aroma may influence the intensity of both the taste and aroma (Frank & Byram, 1988; Frank, Ducheny, & Mize, 1989). The primary requirement for an aroma influencing the perceived intensity of a taste is the congruency of the aroma-taste pair. For example, strawberry aroma increases perceived sweetness (we associate strawberry odour with sweetness) whereas peanut butter aroma does not increase perceived sweetness (we do not associate peanut butter aroma with sweetness).

The aim of this study was to investigate both central cognitive and oral peripheral factors that may modify the bitterness of caffeine.
MATERIALS AND METHODS

SUBJECTS

Subjects (n=33, 23±4 years old, 28 female) between the ages of 18 and 38 were University students in Melbourne, Australia. All subjects were volunteers and agreed to participate and provided informed consent on an approved Institutional Review Board form. The subjects were asked to refrain from eating, drinking or chewing gum for one hour prior to testing. Not all subjects participated in all experiments: Experiment 1, n=30; Experiment 2, n=31; Experiment 3, n=33; Experiment 4, n=32.

SUBJECT TRAINING

Subjects were initially trained in the use of the general Labeled Magnitude Scale (gLMS) following the published standard procedures (Green et al., 1996; Green, Shaffer, & Gilmore, 1993) except the top of the scale was described as the strongest imaginable sensation of any kind (Bartoshuk, 2000). The gLMS is a psychophysical tool that requires subjects to rate perceived intensity along a vertical axis lined with adjectives: barely detectable = 1.5, weak = 6, moderate = 17, strong = 35, very strong = 52, strongest imaginable = 100; the adjectives are placed semi-logarithmically, based upon experimentally determined intervals to yield data equivalent to magnitude estimation (Green et al., 1996; Green et al., 1993). The scale only shows adjectives, not numbers, to the subjects, but the experimenter calculates numerical data from the scale. The gLMS was chosen for this study as it provides ratio quality data (Bartoshuk, 2000). A computerized data-collection program (Compusense 5) was used in all sessions.
Suppression of caffeine bitterness

Subjects were trained to identify each of the five taste qualities by presenting them with exemplars. Salty taste was identified as the predominant taste quality from 150 mM NaCl, bitterness as the predominant quality from 0.50 mM quinine-HCl, sweetness as the predominant quality from 300 mM sucrose, sourness as the predominant quality from 3 mM citric acid, and umami the predominant quality from a mixture of 100 mM MSG and 50 mM IMP. In addition, astringency was identified from 0.5mM tannic acid. To help subjects understand a stimulus could elicit multiple taste quality, a mixture of 0.50 mM quinine-HCl & 3 mM citric acid (bitter and sour) and a mixture of 150 mM NaCl & 300 mM sucrose (salty and sweet) were employed as training stimuli. The gLMS was the scale used during taste quality training. All subjects were able to correctly identify taste and astringent qualities after training.

STANDARDISATION OF gLMS RATINGS WITH HEAVINESS RATINGS

The gLMS standardization methodology followed previously published methodology (Delwiche, Buletic, & Breslin, 2001) and is employed to minimise individual scale use bias. Subjects rated the heaviness of five visually identical weights (opaque, sand-filled jars at levels 52, 294, 538, 789, and 1028 g). All ratings were made on the gLMS. Subjects were asked to rate the intensity of heaviness and all judgments were made within the context of the full range of sensations experienced in life. All weights were presented twice in blocks of ascending order.

To determine a standardization factor, each subject’s average intensity for heaviness was divided by the grand mean for heaviness across weight levels and subjects. Each individual’s intensity ratings were multiplied by his or her personal standardization factor for scale-use bias. The data collected were normally distributed therefore did not require log transformation.
Suppression of caffeine bitterness

STIMULI

Caffeine, sodium gluconate, zinc lactate, and sucrose were purchased from Sigma Chemical (St. Louis, MO) and were the highest purity available. Zinc and sodium were selected as they are both known bitterness inhibitors and the concentrations were selected to ensure tastes from the salts did not overly interfere with judgements of bitterness (Keast & Breslin, 2005; Keast, Canty, & Breslin, 2004a; Keast, 2003). Milk of varying fat concentrations was prepared according to manufacturers instructions (Anchor powdered milk range). Coffee and chocolate aromas were supplied by IFF and prepared according to manufacturers instructions. Caffeine concentrations were chosen as they are in the range commonly occurring in foods and beverages and the chosen concentrations could be perceptually distinguished in preliminary testing.

All solutions were prepared with deionized (di) filtered water and were stored in glass bottles at 4°-8°C and were brought to room temperature prior to testing. Filtered di water was used as a control stimulus in experiments 1 and 2 and the rinsing agent in all experiments.

STATISTICAL ANALYSIS

Numerical results are expressed as arithmetic means ± standard error. Statistical analysis of results was determined with repeated measures analysis of variance (ANOVA) with Bonferroni correction using SPSS version 14. Statistical analyses of bitterness intensity ratings are included in results presented from experiment one, three and four, and bitterness and sweetness intensity ratings are
Suppression of caffeine bitterness

presented for experiment two. Ratings of the other qualities were collected to minimize halo dumping effects (Clark & Lawless, 1994). These ratings were generally not statistically different across conditions, low in magnitude (less than weak on the gLMS), and not relevant to the objectives of this research project. P values <0.05 were considered statistically significant. Significance levels for pairwise post-hoc tests were determined after applying Bonferroni corrections. The post-hoc significance level for experiments 1, 2 and 3 were: 0.05/18= p<0.0027; and experiment 4, 0.05/30= p<0.0016. Calculation of percent bitterness inhibition was calculated using the formula:

\[
\frac{MB}{OB} \times 100 = Y \%
\]

\[Y - (-100) = Z \%
\]

where MB = mean modified bitterness pooled across caffeine concentrations, OB = original bitterness pooled across caffeine concentrations, Y = percent original bitterness remaining, Z = percent bitterness inhibition
Suppression of caffeine bitterness

METHODS

Experiment 1: The effect of sodium and zinc ions on bitterness of caffeine

Water (di), three caffeine concentrations (1.5, 3, 4.5 mM), and 100 mM Na-gluconate (or 5 mM Zn-lactate) were tasted individually and in combination to yield a total of 8 solutions per-session per-subject (full factorial design). There were two different trays (one for each salt), and each tray was tasted on at least three separate occasions, resulting in a total of 6 sessions on 6 separate days. The testing protocol was as follows: Solutions (10 ml) were presented in 30 ml plastic medicine cups (McFarlane Medical and Scientific, Melbourne) on numbered trays. The solutions were presented in random order across subjects. Subjects rinsed with di water at least four times over a 2-minute period prior to testing. The subjects were instructed to pour the whole sample in their mouth while wearing nose-clips, hold it in their mouth for a few seconds, and rate the solution for sour, sweet, bitter, salty, umami, and astringent perceptions prior to expectorating. All subjects rinsed with di water 4 times during the interstimulus interval of 2min. The gLMS was used as the rating method.

Experiment 2: The effect sweetness on bitterness of caffeine

Water (di), three caffeine concentrations (1.5, 3, 4.5 mM), and two sucrose concentrations (125 and 250 mM) were tasted individually and in combination to yield a total of 12 solutions per-session per-subject (full factorial design). Each tray was presented on three separate occasions on three separate days. The testing protocol was the same as experiment 1.

Experiment 3: The effect of milk fat on bitterness of caffeine
Suppression of caffeine bitterness

Four caffeine concentrations (0, 1.5, 3, 4.5 mM), and three powdered bovine milks (0%, 2%, 4% milkfat) were tasted individually and in combination to yield a total of 12 solutions per-session per-subject (full factorial design). Each tray was presented on three separate occasions on three separate days. The testing protocol was the same as experiment 1.

Experiment 4: The effect of congruent aroma on bitterness of caffeine

Chocolate and coffee aromas were assessed for bitterness in aqueous solutions with subjects wearing noseclips. There was no perceivable bitterness elicited by the solutions. Three caffeine concentrations (1.5, 3, 4.5 mM) in 0% milkfat bovine milk with four aromas (blank, coffee, chocolate, mocha (½ coffee, ½ chocolate)) were tasted individually and in combination to yield a total of 12 solutions per-session per-subject (full factorial design). Each tray was presented on three separate occasions on three separate days. The testing protocol was the same as experiment 1 except subjects did not wear noseclips to allow for the influence of aroma on bitterness perception.
Suppression of caffeine bitterness

RESULTS

Experiment 1: The effect of sodium and zinc ions on bitterness of caffeine

Results from a 3 x 4 (salt v caffeine) two-way repeated measures ANOVA with bitterness as the dependent variable revealed there was a significant main effect of salt \[F(2,58) = 12, p<0.0005\] and a significant main effect of caffeine \[F(3,87) = 40.8, p<0.0005\]. Both Na-gluconate and Zn-lactate significantly inhibited bitterness (pooled across caffeine concentrations) \(p<0.05\), and there were significant differences in bitterness across all caffeine concentrations (pooled across salts) \(p<0.0005\). There was a significant interaction among the salts and caffeine \[F(6,83) = 30, p<0.0005\] indicating differences in bitterness intensity of specific combinations of caffeine and salts. Post hoc analysis with Bonferroni correction revealed that Na-gluconate did not inhibit the bitterness at any caffeine concentration \(p>0.0027\). However Zn-lactate significantly inhibited the bitterness of 3 & 4.5 mM caffeine \(p<0.0027\) (Figure 1).

Experiment 2: The effect of sweetness on bitterness of caffeine

BITTERNESS

Results from a 3 x 4 (sucrose v caffeine) two-way repeated measures ANOVA with bitterness as the dependent variable revealed there was a significant main effect of sucrose \[F(2,89) = 76, p<0.0005\] and a significant main effect of caffeine \[F(3,88) = 59, p<0.0005\]. There were significant increases in bitterness as caffeine concentration increased from 1.5 to 3 to 4.5 mM (pooled across sucrose concentrations) \(p<0.05\). There were significant decreases in bitterness as sucrose concentration increased from 0 to 125 to 250 mM (pooled across caffeine concentrations) \(p<0.05\). There was a significant interaction between sucrose and
Suppression of caffeine bitterness

caffeine [F(6,85) = 17, p<0.0005] indicating differences in bitterness intensity of specific combinations of caffeine and sucrose. Post-hoc pairwise analysis revealed a significant increase in bitterness between 1.5 mM caffeine and 3 & 4.5 mM caffeine (p<0.0027). Sucrose concentrations did not significantly affect bitterness of 1.5 mM caffeine (p>0.0027). However 250 mM sucrose did significantly inhibit the bitterness of 3 mM and 4.5 mM caffeine (p<0.0027), yet 125 mM sucrose did not (Figure 2A).

SWEETNESS

Results from a 3 x 4 (sucrose v caffeine) two-way repeated measures ANOVA with sweetness as the dependent variable revealed there was a significant main effect of sucrose [F(2,89) = 190, p<0.0005] and a significant main effect of caffeine [F(3,88) = 34, p<0.0005]. There were significant differences in sweetness between 1.5 mM caffeine and 3 & 4.5 mM caffeine (p<0.05) but no difference in sweetness between 3 to 4.5 mM caffeine (pooled across sucrose concentrations) (p=1.0). There were significant increases in sweetness as sucrose concentration increased from 0 to 125 to 250 mM (pooled across caffeine concentrations) (p<0.0005). There was a significant interaction between sucrose and caffeine [F(6,85) = 18, p<0.0005] indicating differences in sweetness intensity of specific combinations of caffeine and sucrose. Post-hoc analysis revealed caffeine concentration had no effect on the sweetness of 125 mM sucrose (p>0.0027). However, 3 & 4.5 mM caffeine significantly inhibited the sweetness of 250 mM sucrose (p<0.0027). All 250 mM sucrose solutions were significantly sweeter than the corresponding 125mM sucrose solution (p<0.0027) (Figure 2B).

Experiment 3: The effect of milk fat on bitterness of caffeine
Suppression of caffeine bitterness

Results from a 3 x 4 (fat v caffeine) two-way repeated measures ANOVA with bitterness as the dependent variable revealed there was a significant main effect of fat [F(2,95) = 20, p<0.0005] and a significant main effect of caffeine [F(3,94) = 39, p<0.0005]. There were significant increases in bitterness as caffeine concentration increased from 1.5 to 3 to 4.5 mM (pooled across levels of fat) (p<0.05). Also, there were significant differences in bitterness between 0% fat and 2% & 4% fat (p<0.05) but no difference in bitterness between 2 and 4% fat (pooled across caffeine concentrations) (p=0.09)). There was a significant interaction among fat and caffeine [F(6,91) = 8, p<0.0005] indicating differences in bitterness intensity of specific combinations of caffeine and fat. Post-hoc analysis revealed no significant differences in bitterness between 0-2% milkfat, however at all caffeine concentrations there were significant differences between 0-4% milkfat (p<0.0027) with 0% milk fat being less bitter at each caffeine concentration (Figure 3).

Experiment 4: The effect of congruent aroma on bitterness of caffeine

Results from a 4 x 3 (aroma v caffeine) two-way repeated measures ANOVA with bitterness as the dependent variable revealed there was a significant main effect of aroma [F(3,91) = 10, p<0.0005] and a significant main effect of caffeine [F(2,92) = 28, p<0.0005]. There were significant increases in bitterness as caffeine concentration increased from 1.5 to 3 to 4.5 mM (pooled across aromas) (p=0.62). Mocha aroma was significantly less bitter than either chocolate or coffee aroma (p<0.05) (pooled across caffeine concentrations) but there was no difference in bitterness between coffee and chocolate aromas (p=0.99). There was no significant interaction between aromas and caffeine [F(6,88) = 1.6, p=0.15] (Figure 4).
DISCUSSION

The organization of the bitter taste system is complex with multiple putative receptor mechanisms (Chandrashekar, Hoon, Ryba, & Zuker, 2006), including a specific G protein coupled receptor for caffeine in *Drosophila* (Moon, Kottgen, Jiao, Xu, & Montell, 2006). Our understanding of how caffeine activates bitter taste is far from complete and this study shows that the bitterness elicited by caffeine can be partially inhibited using both oral peripheral (Na, Zn) and central cognitive strategies. The most effective inhibitor of the bitterness of caffeine appears to be Zn-lactate (71% bitterness inhibition), followed by non-fat milk (49%), 250 mM sucrose (47%), Na-gluconate (31%), and mocha (10%).

*Sodium and zinc ions as bitterness inhibitors*

As previously shown, the utility of zinc ions as a bitterness inhibitor in foods and pharmaceuticals is compromised by the fact that zinc ions are potent inhibitors of sweetness (Keast, Canty, & Breslin, 2004b). However, zinc ions are useful to help our understanding of the bitterness elicited by caffeine. While human psychophysical studies cannot directly test oral peripheral mechanisms of taste, such studies can provide information to help understand the taste system (Schiffman, Booth, Sattely-Miller, Graham, & Gibes, 1999). The influence of zinc ions on bitterness is presumably in the oral periphery (Keast & Breslin, 2005), and zinc ions are known to modulate allosterically trans-membrane receptors (GPCRs and ion channels) and can both activate or inhibit them depending on the receptor system (Swaminath, Lee, & Kobilka, 2003; Zheng, Gingrich, Traynelis, & Conn, 1998). To inhibit bitterness, zinc ions may form a complex with the extracellular portions of the bitter taste receptor/s (TAS2Rs), as zinc ions readily complex with amino acids and proteins and
Suppression of caffeine bitterness

have a high affinity for both thiol and hydroxy groups (Christianson, 1991). If zinc ions did bind to a TAS2R, the native configuration of the receptor could be altered and made unavailable for normal reception. Alternatively, zinc ions could form complexes with the bitter compounds that would render them insoluble and, thus, unable to access receptors; however, visual inspection of all solutions did not reveal any precipitation. It is of interest that there was no increase in bitterness as caffeine concentration increased from 1.5 to 4.5 mM indicating the mechanism responsible for increasing levels of bitterness was affected by Zn-lactate. It is reasonable to suggest the zinc ions are modulating an extracellular GPCR’s, presumably a TAS2R but further studies at a receptor level would be required to verify this. Moreover, there appears to be a portion of caffeine bitterness that is not affected by Zn-lactate; this may be due to intracellular activation of bitter taste by caffeine (Peri et al., 2000; Rosenzweig et al., 1999).

Previous studies have shown sodium ions may or may not inhibit the bitterness of caffeine (Breslin & Beauchamp, 1995b; Kamen, Pilgrim, Gutman, & Kroll, 1961; Pangborn, 1960). This study shows Na-gluconate significantly decreases the bitterness of caffeine and inhibition was greater at 3 and 4.5 mM caffeine than at lower concentrations (similar to zinc ions).

Bitter-sweet interactions

The mutual suppression of sweet and bitter tastes has been known for some years (Calvino & Garrido, 1991; Kroeze & Bartoshuk, 1985; Pangborn, 1960). The suppression is primarily central cognitive and named mixture suppression where both bitter and sweet taste intensities are mutually suppressed when mixed in binary solutions (Keast & Breslin, 2003; Kroeze & Bartoshuk, 1985). In this study the
Suppression of caffeine bitterness

sweetness of sucrose had a greater effect on the bitterness of caffeine than the reverse, similar to the interaction reported by (Kamen et al., 1961). This effect may be due to 250 mM sucrose eliciting a higher intensity of sweetness than the corresponding bitterness elicited by 4.5 mM caffeine. This does not adequately explain why higher levels of bitterness failed to significantly suppress 125mM sucrose sweetness.

Milk-fat and caffeine bitterness

Fat emulsions have been shown to decrease bitterness intensity of quinine-HCl (Metcalf & Vicker, 2002; Valentova & Pokorny, 1998). The authors speculated that quinine-HCl preferentially partitioned into the fat phase of the emulsion thereby diluting the concentration in the aqueous phase; tastants in the aqueous phase are less able to access taste receptors, therefore quinine-HCl was less effective at reaching and activating bitter taste receptors. Specific fatty acids have also been implicated in reduction of quinine bitterness (Koriyama, Kohata, Wananabe, & Abe, 2002; Koriyama, Wongso et al., 2002). Contrary to those previous studies, results from the present study show that as the milk fat content increases from 0 to 4%, the level of caffeine bitterness significantly increases. The milk matrix also contained milk proteins and carbohydrates, so the significant increase in bitterness cannot be directly attributed to the increasing fat content. Alternatively, milk proteins could form complexes with caffeine that would render it insoluble and, thus, unable to access receptors; as the solution was opaque, visual inspection of solutions did not reveal any precipitation. Of further interest, comparing the bitterness of like concentrations of caffeine in water (experiments 1& 2) and milk powder we can see there is a decrease in bitterness (40-50%) when caffeine is in the milk powders: the decrease is greatest when caffeine is in non-fat milk powder. The finding that milk powder, and in
Suppression of caffeine bitterness

particular non-fat milk powder, inhibits the bitterness of caffeine warrants further inspection.

Aromas and caffeine bitterness

All aromas were congruent with caffeine bitterness but coffee, chocolate, or mocha did not significantly inhibit the bitterness of caffeine. Indeed, both chocolate (32%) and coffee (17%) aromas increased the perceived bitterness of caffeine. This was expected as aroma can ‘acquire taste’ after a learning phase (Stevenson, Prescott, & Boakes, 1995), and it is known that congruent aromas can modulate taste (Frank & Byram, 1988; Small & Prescott, 2005). This result supports a study by Labbe et al., where a cocoa flavoured caffeinated beverage was perceived more bitter when tasted with olfactory input than without (Labbe, Damevin, Vaccher, Morgenegg, & Martin, 2006). Chocolate was rated the most bitter of the three aromas at 1.5 & 3 mM caffeine, while coffee was most bitter at 4.5 mM caffeine. Of interest was that mocha (½ chocolate, ½ coffee) was perceived as significantly less bitter than coffee or chocolate aromas indicating that the potential flavour association of mocha with bitterness might not be as strong as with chocolate or coffee aromas.
Suppression of caffeine bitterness

CONCLUSION

Zinc ions were most effective at inhibiting the bitterness of caffeine (presumably the mode of action is in the oral periphery) but the use of zinc in food formulations has additional problems as zinc ions also inhibits sweetness. Other methods to inhibit caffeine bitterness were only partially successful with 0% milk fat milk the next most successful, although the mechanism of bitterness suppression remains unknown. Finally, the cognitive strategy of mixture suppression using the sweetness of 250 mM sucrose also proved successful at inhibiting the bitterness of caffeine. Due to the complexity of the bitter taste system, the bitterness of caffeine remains an ongoing problem for manufacturers wanting to add caffeine to food formulations.
Suppression of caffeine bitterness

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Suppression of caffeine bitterness

REFERENCES


Suppression of caffeine bitterness


Suppression of caffeine bitterness


FIGURES

Figure 1  **The influence of sodium gluconate and zinc lactate on the bitterness of caffeine.**

Each bar represents the average bitterness intensity of the compounds listed along the X-axis. The Y-axis represents the average bitterness rating (arithmetic mean ± standard error) on the gLMS (general Labeled Magnitude Scale) for caffeine solutions. Abbreviations and concentrations of the compounds were: Caffeine (Caff) (1.5, 3, 4.5 mM), Sodium Gluconate (NaGluc) (100 mM), Zinc lactate (Zn) (5 mM). Differences among letters over bars indicate that means are statistically different (p<0.0027) in bitterness intensity among relevant pairwise compounds only. For example, grouping within vertical dashed lines, or the same salt at different caffeine concentrations. Water is not included in the graph as the magnitude of bitterness was <1gLMS unit.

Figure 2A  **The influence of the sweetness of sucrose on the bitterness of caffeine**

Refer to Figure 1 for graph description. Abbreviations and concentrations of the compounds were: Caffeine (Caff) (1.5, 3, 4.5 mM) and Sucrose (Suc) (125, 250 mM).

Figure 2B  **The influence of the bitterness of caffeine on the sweetness of sucrose**

Each bar represents the average sweetness intensity of the compounds listed along the X-axis. The Y-axis represents the average sweetness rating (arithmetic mean ± standard error) on the gLMS (general Labeled Magnitude Scale) for sucrose solutions. Abbreviations and concentrations of the compounds were: Caffeine (Caff) (1.5, 3, 4.5 mM) and Sucrose (Suc) (125, 250 mM). Refer to Figure 1 for graph description.
Suppression of caffeine bitterness

Figure 3  **The influence of the milk fat on the bitterness of caffeine**

Refer to Figure 1 for graph description. Abbreviations and concentrations of the compounds were: Caffeine (Caff) (1.5, 3, 4.5 mM) and Milk Fat (Fat) (0, 2, 4%).

Figure 4  **The influence of congruent flavours on caffeine bitterness**

Refer to Figure 1 for graph description. Abbreviations and concentrations of the compounds were: Caffeine (Caff) (1.5, 3, 4.5 mM) and coffee (coff), chocolate (choc).
Suppression of caffeine bitterness
Suppression of caffeine bitterness
Suppression of caffeine bitterness

[Bar chart showing the suppression of caffeine bitterness with differentSuc concentrations. Bars labeled with letters a, b, and c indicating significant differences.]
Suppression of caffeine bitterness

The graph shows the gLMS bitterness intensity for different concentrations of caffeine (Caff) and fat content. The x-axis represents different concentrations: 1.5mM, 3mM, 4.5mM, and 3.5mM Caff with 0%, 2%, and 4% fat levels. The y-axis represents the bitterness intensity on a scale from 0 to 10.

- For 1.5mM Caff 0%, the bitterness intensity is slightly above 2.
- For 1.5mM Caff 2%, the bitterness intensity is significantly lower than the baseline.
- For 1.5mM Caff 4%, the bitterness intensity is similar to the baseline.
- For 3mM Caff 0%, the bitterness intensity is slightly above 4.
- For 3mM Caff 2%, the bitterness intensity is significantly lower than the baseline.
- For 3mM Caff 4%, the bitterness intensity is similar to the baseline.
- For 4.5mM Caff 0%, the bitterness intensity is slightly above 6.
- For 4.5mM Caff 2%, the bitterness intensity is significantly lower than the baseline.
- For 4.5mM Caff 4%, the bitterness intensity is similar to the baseline.

Different letters (a, b) indicate statistical significance between groups.
Suppression of caffeine bitterness

![Graph showing suppression of caffeine bitterness intensity at different concentrations. The x-axis represents different concentrations of caffeine: 1.5mM, 3mM, 4.5mM, 1.5mM, 3mM, 4.5mM, and 4.5mM for milk, coffee, chocolate, and mocha drinks, respectively. The y-axis represents the gLMS bitterness intensity. Each bar represents the mean bitterness intensity with error bars showing the standard deviation. The graph illustrates the decrease in bitterness intensity with increasing caffeine concentration.]