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Short Communication

D-Allulose as a flavoring substance with flavor modifying properties

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Flavoring substances are essential in the production of hedonically pleasant foods and beverages. A group of flavoring substances that is garnering increased interest is those that modify other flavors. In this study a rare sugar, D-allulose, also known as D-psicose, when used below its sweetness threshold of 2.70% in solution, was shown to possess flavor modifying properties in a beverage matrix. In particular, 1.50% D-allulose was shown to modify 0.20% cotton candy and 0.20% salted caramel flavors in water. This suggests that D-allulose acts as a flavoring substance with flavor modifying properties (FMP) when used at a level below its sweetness threshold.

Key words: D-Allulose, flavoring substance, flavor modifying

Abbreviations: Flavor and Extract Manufacturers Association (FEMA); flavoring substance with flavor modifying properties (FMP); two-alternative forced choice (2-AFC)

INTRODUCTION

D-Allulose is Generally Recognized as Safe (GRAS) in the USA for use in foods as a sweetener in beverages and other applications. It is a naturally occurring epimer of D-fructose that has been isolated from *Itea ilicifolia, Itea virgginica and Itea yunnanensis* (Hough and Stacy, 1963). Allulose is also formed during the production of many common foods such as coffee, raisins, figs, molasses and baked goods (Oshima et al., 2006; Bradbury, 2001; Zerban and Sattler, 1942). Aside from sources where it occurs naturally, allulose can also be made via enzymatic and base catalyzed epimerization of fructose (Beveridge et al., 1982; Itoh et al., 1995; Doner, 1979). Allulose is not readily metabolized and is known to have a caloric value of less than or equal to 0.2 kcal/g (Tatsuhiro et al., 2002). It is also reported to be about 70% as sweet as sugar (Chung et al., 2012). These facts suggest that allulose can serve as an effective calorie reduced sweetener.

Some sweeteners have flavor modifying properties when used below their sweetness threshold. An example is erythritol, which when used at 1% was found to reduce the astringency of tea and grapefruit juice (DeCock, 2012).In order to qualify as a flavoring substance with flavor modifying properties(FMP)according to the Flavor and Extract Manufacturers Association (FEMA)sensory testing guidance, a prospective flavoring substance must be shown not to possess an inherent taste (e.g. sweetness) and must modify flavor attributes at the level used for flavor modification (Harman and Hallagan, 2013). FEMA convenes an independent Expert Panel to review the safety and appropriateness of such uses, and if the Expert Panel determines that the substance is safe under its conditions of intended use as a flavoring substance, it is designated as FEMA GRAS for a target use(e.g. to impart or modify flavor) at specified use levels.

The current study focuses on the establishment of sub-sweetening levels of allulose and the investigation of the flavor modifying properties of allulose when used below this sweetness threshold.

MATERIALS AND METHODS

Samples

The allulose used in this study was derived from a commercial product known as Dolcia Prima® allulose syrup, sourced from Tate & Lyle (Hoffman Estates, Illinois) as a 72% dry solids syrup. Cotton candy flavor was sourced from Givaudan Flavors (Elgin, Illinois) and salted caramel flavor was sourced from Flavor and Fragrance Specialties (Essex, Maryland). Both flavors are highly concentrated, liquid aromatic flavors used in the manufacturing of foods and beverages. The samples were stored at68^{o}Ffor the duration of the experiments.

Panel selection

FEMA sensory testing guidance permits the use of trained or untrained panelists, with or without duplicate serving of samples, as long as the total number of observations exceeds 30 for each test administered (Harman and Hallagan, 2013). The manner in which panelists are chosen is likely to impact the results and thus requires consideration to align with research goals. The use of trained panelists could lower threshold values due to heightened awareness among trained panels in detecting differences (Ennis et al., 2017). A small subset of highly trained panelists using duplicate measures could skew taste threshold measurements based on differences in inter-individual sensitivities (Civille and Carr, 2015). The effect can be so significant that prior researchers have suggested use of 20-30 panelists for sensitivity studies. For the above reasons, the current study used 25-44 untrained panelists per test.

Panelists were recruited for the descriptive evaluation of the samples among employees at the Tate &Lyle US headquarters (Hoffman Estates, Illinois), excluding individuals who were pregnant, lactating, or had known food allergies. The panel consisted of 40-60% males/females on any given test date, and ages of the panelists ranged from 20 to 65 years old. Panelists participated in only one sensory test per day.

Test Design and Sample Evaluation

Two studies were performed to demonstrate: first, the sweetness recognition threshold of allulose and second, the ability of allulose to enhance or modify flavor consistent with previously published FEMA sensory testing guidance (Harman and Hallagan, 2013).

In Test 1, a two-alternative forced choice (2-AFC) test was used to compare the sweetness of allulose to a sweetness threshold of 1.50% sucrose solution. The reference (control)solution was a simple solution of 1.50% sucrose (commercial Domino® brand sugar) dissolved in water. Test solutions were made from Tate & Lyle Dolcia Prima® allulose syrup standardized to w/w concentrations of 1.93%, 2.31%, 2.70%, 2.89%, or 3.08% allulose solids in water. Duplicate pairs of samples of a single test concentration were chosen from the allulose concentration series and served on a given day. Panelists were required to compare pairs of the control vs. test samples and identify which sample was sweeter.

In Test 2, a 2-AFC test was used to determine the flavor modifying effects of allulose on two flavors: cotton candy and salted caramel. In Test 2a, 0.20% cotton candy flavor in water was presented to panelists with and without 1.50% allulose. In Test 2b, 0.20% salted caramel flavor in water was presented to panelists with and without 1.5% allulose. For each comparison, panelists had to identify which solution was stronger for the specific flavor attribute being measured.

All testing was performed in the Tate &Lyle sensory testing facility, which has a series of 16 modern isolation booths in a quiet yet accessible area. As is typical in commercial sensory facilities, standard ambient fluorescent lighting was used. The sensory facility was devoid of visual, olfactory, or acoustic distractions during testing. The temperature of the facility was held at 68^{o}F throughout all testing intervals. For both tests, samples were prepared the morning of testing, held and served at room temperature. Prior to each test, subjects were instructed on the method of evaluating the samples. Products were presented to panelists in random digit coded 2 oz. deli cups containing approximately 1.5 oz. of liquid. They were served in sets of two using standard directional difference test methodology (ASTM, 2016). Serving order was based on a complete randomized block design, such that the order of samples was randomized among panelists and among duplicates when they were used. Panelists were provided with a water rinse and unsalted crackers for palate cleansing. A forced 60 second break was implemented when there were duplicate sets of samples.

Statistical Analysis

Test 1 involved the presentation of duplicate samples, therefore beta-binomial statistics were used to analyze the data, consistent with recommended analysis of duplicate 2-AFC tests (Bi, 2015). Tests 2a and 2b had no duplicates and therefore standard binomial statistics were used. The alpha statistic was set at 5% using a two-tailed distribution.

RESULTS AND DISCUSSION

In order to demonstrate that a test substance does not have inherent sweetness under conditions of intended use, FEMA sensory testing guidance requires the demonstration that the test substance is significantly less sweet than the sweetness threshold for sucrose, a 1.50% sucrose solution (Harman and Hallagan, 2013).Test 1 provides this data, as summarized in Table 1. The results

Table 1a: Two-alternative force choice test to assess the inherent sweetness of allulose compared to sucrose threshold[1]

Number of Panelists	Control % Sucrose[2]	% Allulose Solids[2]	Substance Selected as More Sweet	Counts Observed/ Total	2-tailed p-value	Beta Binomial λ
25	1.50%	1.93%	Control	45/50	<0.0001	-0.11
29	1.50%	2.31%	Control	49/58	<0.0001	-0.18
30	1.50%	2.70%	Control	40/60	0.0067	-0.05
22	1.50%	2.89%	Allulose	26/44	0.1456	0.06
30	1.50%	3.08%	Allulose	34/60	0.1831	0.19

[1]Test 1
[2]Aqueous solution

Table 1b: Two-alternative force choice test of flavor alone versus flavor plus allulose in demonstrating a flavor modifying effect

Test ID	Number of Panelists	Flavor Alone[1]	Flavor + Allulose[1]	Substance Selected as More Intense Flavor	Counts Observed/Total	2-tailed p-value
Test 2a	30	0.20% Cotton Candy	0.20% Cotton Candy + 1.50% allulose	0.20% Cotton Candy + 1.50% allulose	27/30	<0.0001
Test 2b	30	0.20% Salted Caramel	0.20% Salted Caramel + 1.50% allulose	0.20% Salted Caramel + 1.50% allulose	28/30	<0.0001

[1]Aqueous solution

indicate that solutions containing 1.93% ($p < 0.0001$), 2.31% ($p < 0.0001$) and 2.70% ($p=0.0067$) dry allulose solids were considered significantly less sweet than the control 1.50% sucrose solution. At these levels, the beta binomial λ value was negative. On the other hand, concentrations of 2.89% and 3.08%dry allulose solids showed signs of being perceived as equal or sweeter than the control 1.50% sucrose solution. Therefore, a concentration of 2.70% dry allulose solids is the maximum use level that falls below the recognition threshold concentration of a 1.50% sucrose solution.

An unexpected finding of this study is that panelists found the 2.89% solution of allulose to have confusable sweetness with a 1.50% sucrose solution. Previously published research has indicated that allulose is about 70% as sweet as sugar, thus it would be expected that a 2.14% of allulose would be equal in sweetness to a 1.50% solution of sucrose (Chung et al., 2012). However, it is necessary to take into account that sweetener dose responses plot to the sigmoidal Hill equation (Antenucci & Hayes, 2015). In other words when near the minimal and maximal responses, it can be expected that allulose sweetness perception is not directly proportional to that of sucrose.

In order to show FMP effects, FEMA sensory testing guidance requires demonstration that a flavored solution containing the test substance be perceived as stronger or weaker in a given taste attribute than a reference product that does not contain the test substance (Harman and Hallagan, 2013; ASTM, 2016). Tests 2a and 2b provide this data, as summarized in Table 1b. The results show that when allulose is added at 1.50% solids in solution, in combination with cotton candy ($p < 0.001$) or salted caramel flavor ($p < 0.001$), there is a highly statistically significant impact on the intensity of both flavors. Given the strength of the response when using 1.50% allulose, it is likely that this effect would also be perceived at lower use rates, although levels below 1.50% allulose were not examined in this study.

CONCLUSIONS

This study demonstrates that allulose acts as a flavoring substance with flavor modifying properties in beverage systems when used at a level below its sweetness threshold. Allulose may also have utility as a FMP in other food matrices and flavor systems in addition to beverages when used below its sweetness threshold. This may be an area that warrants further research.

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Conflicts of Interest

All authors are employed by Tate & Lyle. Tate & Lyle manufactures allulose as well as a variety of other ingredients for use in food and industrial applications.

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