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A high-throughput plasmonic tongue using an aggregation assay and nonspecific interactions: classification of taste profiles in maple syrup†

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A simple colorimetric test detects off-flavour profiles of maple syrups in minutes, which are detectable by the naked eye. As flavour profiles are due to complex mixtures of molecules, the test uses nonspecific interactions for analysing the aggregation and color change of Au nanoparticles (AuNPs) induced by the different organic molecules contained in off-flavour maple syrup. The test was optimal with 13 nm citrate-capped AuNPs reacting 1 : 1 with pure maple syrup diluted 10 times. Under these conditions, normal flavour maple syrups did not react and the solution remained red, while off-flavoured maple syrups aggregated the AuNPs and the solution turned blue. Different classes of molecules were then tested to evaluate the types of compounds typically found in maple syrups reacting in the test, showing that sulfur- and amine-containing amino acids and aromatic amines caused aggregation of the AuNPs. The test was validated with 1818 maple syrup samples from the 2018 harvest in Quebec and 98% of the off-flavoured maple syrups were positively identified against the standard taste test. Preliminary tests were performed on site in maple sugar shacks to validate the applicability of the test on the production site.

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Introduction

Maple syrup, one of the most widely recognized products of Canada, is produced by boiling sap from *Acer saccharum* (sugar maple tree) harvested during the snow melt days in the Spring season. Organic molecules are transformed in the boiling process that concentrates the sap about 40 times to yield maple syrup. Flavour profiles are associated with broad classes of organic molecules[1] including phenols,[2] pyrazines,[3–6] minerals,[1] organic acids,[1] and amino acids[7] that account for only 1% of the content of maple syrup, where the bulk is 66% sugar (mostly sucrose) and 33% water.[1] The taste of maple syrup can change depending on environmental factors,[8] sap pre-treatment, the boiling process and on how late in the season the sap is harvested among other factors.[9] The different climate profiles of recent years led to the occurrence of a taste defect associated with the emergence of buds in *Acer saccharum*.[10] If this taste defect is present, maple syrup can be downgraded to industrial quality with economic impacts.[10]

To ensure that the best quality maple syrup enters the retail market, its production is quality controlled by a series of tests to determine the colour (transmittance), presence of lead, °Brix (sugar content by refractometry) and complemented with a taste test performed by trained technicians to assure that commercial maple syrups are properly classified and has the normal flavour expected for maple syrup.[10] In some cases, technicians are assisted in the classification with fluorescence spectroscopy to evaluate the profile of the fluorescent organic molecules in maple syrup, which is used to classify it into normal or off-flavour categories (including buddy taste defects).[11] However, there is still no rapid test that can be used in high-throughput 96-well plates or at maple sugar shacks (production sites) by producers to assess the presence of off-flavours in maple syrup.

Colorimetric tests are simple and effective to parallelize and perform analysis using multi-well plates or on site by untrained personnel. However, classical organic dyes for colorimetric tests are unsuited to monitor the complex changes in flavour profiles of maple syrup. One could use extensive chromatographic and mass spectrometric analysis of syrups, but it is impractical in the context of rapid classification of a yearly production of 300 000 barrels in a few months or for on-site evaluation of maple syrup at sugar shacks. As an alternative for organic dyes for colorimetry, plasmonic sensors are especially interesting as they can be configured for naked eye detection[12] or are easily amenable to 96 well plate assays.[13,14]

Plasmonic sensors are based on the resonant interaction of light with the conducting electrons of a noble metal nanostructure leading to strong absorption band(s) in the visible to

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near infrared spectrum.[15] When two or more nanoparticles cluster, plasmonic coupling occurs and leads to a stark change in resonance wavelength, and thus in colour. This optical phenomenon has been extensively studied for targeted analytes by specifically functionalizing AuNPs to capture the analyte and cause aggregation.[16] However, in the context of detecting changes in the flavour profiles of a broad range of molecules, a non-specific colorimetric test is more appropriate than a molecularly-targeted analytical test.

The use of nonspecific interactions for chemical analysis with plasmonics is highly interesting to analyse complex mixtures. The nonspecific interactions with arrays of surfaces can lead to patterns that are distinctive of a sample, a method often used in artificial noses/tongues.[17] Plasmonic-based noses/tongues can differentiate between types of samples, for example normal cells from cancer cells,[18] proteins,[19,20] among others. A recent paper demonstrated the use of different surface chemistries on an array of plasmonic substrates to detect the different signatures of Scottish whisky,[21] showing the potential of this approach for flavour profiling. We extend here the concept of non-specific interaction monitoring to use the aggregation of a AuNP suspension to discriminate normal and off-flavour profiles to classify maple syrup.

Experimental

Au nanoparticle synthesis

AuNPs of different shapes and sizes were synthesized. In all cases, all glassware was first cleaned with *aqua regia* (**Caution**! *Aqua regia* is highly corrosive) and thoroughly rinsed with water until the pH of wash water becomes neutral.

Spherical AuNPs were synthesized according to the Turkevitch method.[22] To a volume of 200 mL of ultrapure water, 68 mg of gold chloride trihydrate (99.9% pure, metal basis, Sigma-Aldrich Canada) was added and brought to a boiling point under vigorous stirring. Then, 20 mL of sodium citrate dihydrate (20 mg mL^{-1}) was added under continuous boiling and stirring for 10 minutes. Heating was then removed and the solution was left to cool to room temperature.

Gold nanostars were prepared according to a published procedure[23] by adding 40 mL of a 100 mM HEPES pH 7.4 aqueous solution to 100 mL of ultrapure water and stirred slowly at room temperature. Then, 80 μL of an aqueous gold chloride solution (250 mM) was added and stirred until reagents were mixed. Stirring was then stopped and the nanostars were left to grow at room temperature for two hours. The reaction was completed when a turquoise blue colour appeared.

Au nanoraspberries were synthesized according to an existing protocol.[24] In this case, 3 mL of the 100 mM HEPES pH 7.4 solution was added to 100 mL of ultrapure water. The solution was stirred at room temperature momentarily and 100 μL of a 250 mM gold chloride solution was added under continuous stirring for 2 hours at room temperature. The reaction was completed when the solution became burgundy-brown in diffusion and blue in transmission. The UV-Vis spectra of all nanoparticles were then acquired. All AuNPs were stored at 4 °C until use.

Test optimization

The AuNPs were diluted in an equal volume of water before use. The stability of the different shapes of AuNPs in maple syrup was evaluated with UV-Vis spectroscopy at a dilution ratio of 1 : 9 (syrup : water) and then an equal volume of the AuNP suspension was added. The UV-Vis spectra were acquired from 400 to 800 nm at different times for up to 30 minutes. The dilution factor, reaction time, and pH were then optimized under otherwise identical conditions.

Selectivity test

To evaluate the molecular classes potentially causing AuNP aggregation, a panel of 25 molecules was tested in water, a 66°Brix sucrose solution and in good grade maple syrup. Most of these molecules were previously detected in maple syrup, while others are putative compounds of maple syrup. These included amino acids, aromatic amines, polyphenols, and other organic molecules. Aggregation was tested at 500 nM, 5 μM, 50 μM and 500 μM for each of these molecules in water, sugar solution and maple syrup.

To handle a large number of samples, the test was adapted to a multi-well plate reader, where 96 well plates were employed to provide high-throughput. The UV-Vis absorbance was then measured on a wavelength scanning plate reader at 520 and 625 nm (Infinite M200 Pro, Tecan Group Ltd.) and then scanned to obtain a photograph of the plate (Perfection V500 Photo, Epson).

Validation of the test

The test was validated with a series of 1818 maple syrup samples provided by Quebec Maple Syrup Producers (QMSP), coming from different production regions, with different darkness and flavour profiles. Once again, the tests were carried out in 96-well plates and measured with a plate reader under identical conditions to those stated above. Prior to the test, these samples were graded by the Centre ACER (Inspection division), where the transparency of the maple syrup was measured at 560 nm, the °Brix was measured with refractometry and a taste test was performed by trained technicians to identify the different flavour profiles of the maple syrup samples. The colorimetric test results were compared with the grade assigned to each maple syrup to evaluate the predictability of the colorimetric test to detect off-flavours in maple syrup.

Results and discussion

Maple syrup contains ppb to ppm levels of a series of organic compounds.[1] Among these, it has been revealed that amino acid[25] and small organic thiol[10] levels can be increased in the late harvest. Some of these molecules have been putatively associated with the emergence of buddy or off-flavour maple syrups, more specifically dimethyldisulfide (DMDS).[10] In addition, maple syrups can contain aromatic amine molecules that are associated with the pyrazine family.[3,4] Molecules that contain thiols and other types of sulphur compounds,[26] as well as aliphatic[27] and aromatic[28] amines,

albeit less strongly than thiols, can bind to gold surfaces. If hydrophobic molecules containing thiols or amines, among others, bind to aqueous AuNP suspensions, they can cause the aggregation of AuNPs leading to a stark colour change of the solution. Hence, the elevated presence of such molecules in off-flavour maple syrups could be used to detect this taste defect.

Stability of AuNPs in maple syrups

The stability of different AuNPs was tested in normal flavour maple syrups under different conditions to evaluate the potential of using AuNPs in this matrix. The Au nanoparticles were synthesized according to our standard protocols[29] based on published procedures.[22–24] To avoid rapid aggregation of the AuNPs in maple syrup, a 10 times dilution was necessary prior to adding an equal volume of AuNP suspension (Fig. 1), which corresponds to a sugar concentration of 3% in the solution analysed (approximately 3 °Brix). Otherwise, a large response in the UV-Vis spectra was observed at wavelengths greater than 600 nm for smaller dilution factors, indicative of aggregation of the solution. Greater dilution factors would lead to further dilution of the organic molecules, decreasing the sensitivity of the aggregation test for detecting compounds associated with off-flavours. Thus, maple syrup was always diluted 10 times for the remaining experiments.

Nanoparticles of different shapes were then tested to evaluate the impact of shape on the stability in maple syrup. Au nanospheres of 13 nm were very stable for at least 30 minutes in maple syrup (Fig. 1). However, nanoraspberries showed partial aggregation, which is confirmed by the presence of a significant shoulder in the UV-Vis peak at longer wavelength (black dotted line in Fig. 1). The nanostars rapidly precipitated out of solution under these conditions and a nearly null absorbance spectrum was measured after 30 minutes. Thus, Au nanospheres were selected for the remainder of the experiments.

The pH of maple syrup typically varies between 5.6 and 7.9 depending on the composition in organic acids,[30] while most maple syrups have a relatively narrow pH range between 6 and 7. The stability of the nanoparticles was thus measured for 10 minutes in water, 66% sucrose, and maple syrup for which the pH was adjusted from 2.5 to 10 (Fig. 2). While the AuNP suspensions in water and sucrose remained stable for all pHs, the pH of maple syrup played a role in the stability of the AuNPs. A low pH of 2.5 and 4 or a high pH of 10 induced mild aggregation of the AuNPs, as seen from the emergence of a shoulder on the plasmon resonance of the AuNPs. At the pHs normally encountered for maple syrups, the AuNPs were stable in the 10× diluted maple syrup samples. This experiment provides an indication that the aggregation of AuNPs is partly driven from acidic and basic molecules contained in the maple syrup and that sucrose does not react with the AuNPs.

Detection of off-flavour in maple syrups

The emergence of off-flavours due to buddy sap is important to control for the maple syrup industry. Currently, the detection of these off-flavours is performed by fluorescence spectroscopy and tasting by trained technicians. These steps are performed in the context of classification after the production in centralized facilities. Providing the producers a simple tool for detecting off-flavours could help them manage production and facilitate the classification by the trained technicians by providing them another objective tool to assess the presence of off-flavours.

A 10× diluted maple syrup sample classified as off-flavoured with the presence of a buddy taste was reacted with the AuNPs. A rapid rise of the absorbance at 625 nm was observed within seconds with a concomitant change of colour from red to blue of the sample (Fig. 3). The Au nanoparticles remained stable in a normal flavoured sample and the solution stayed red. While the test can be evaluated with the naked eye, a normal flavoured maple syrup sample is red and off-flavoured with buddy taste is blue coloured, we anticipated early on that the test would lead to a series of different shades of colours between red and blue.

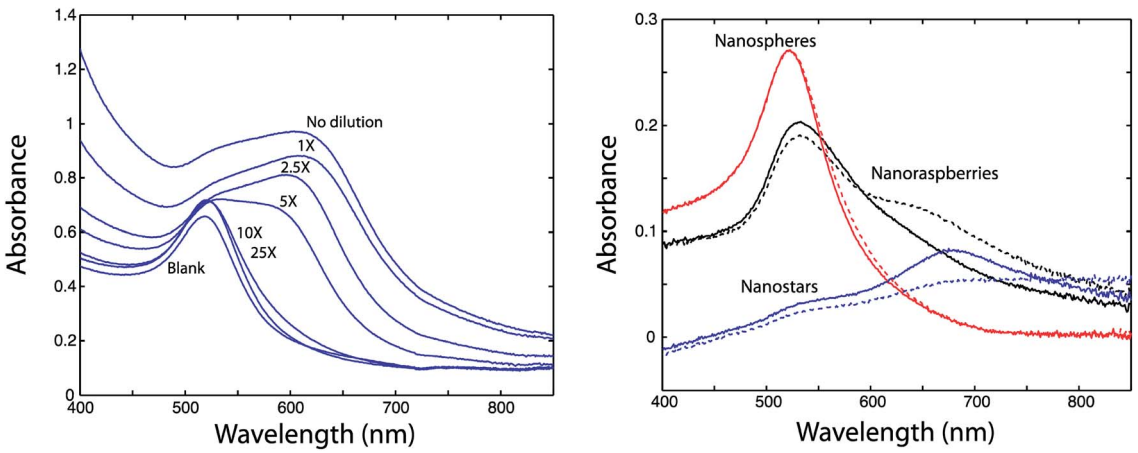

Fig. 1 (Left) Stability of good flavour maple syrup at different dilution factors with spherical Au nanoparticles. The appearance of a large peak at >600 nm is indicative of aggregation. (Right) UV-Vis spectra of AuNPs of different shapes in water (solid lines) and mixed with a good maple syrup at a dilution of 10× with an equal volume of AuNPs (dashed lines).

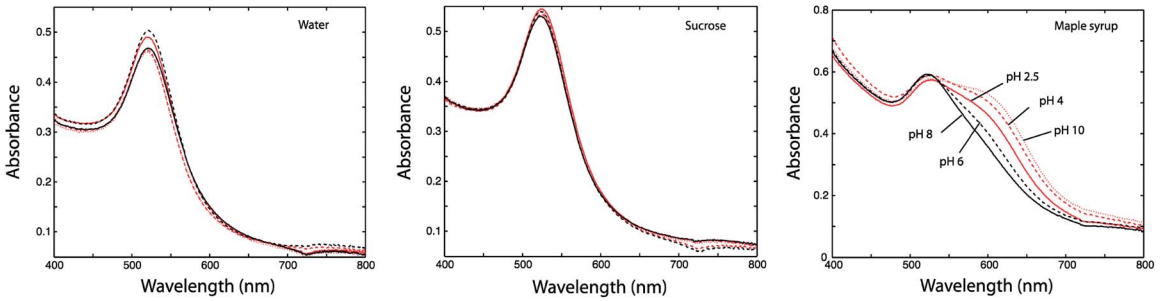

Fig. 2 Stability of AuNPs at different pHs in water (Left), 66% aqueous sucrose solution (Center) and maple syrup (Right). The sucrose and maple syrup samples were diluted 10× and mixed 1 : 1 with AuNPs. pH 2.5: solid red trace; pH 4: dashed red trace; pH 6: dashed black trace; pH 8: solid black trace; and pH 10: dotted red trace.

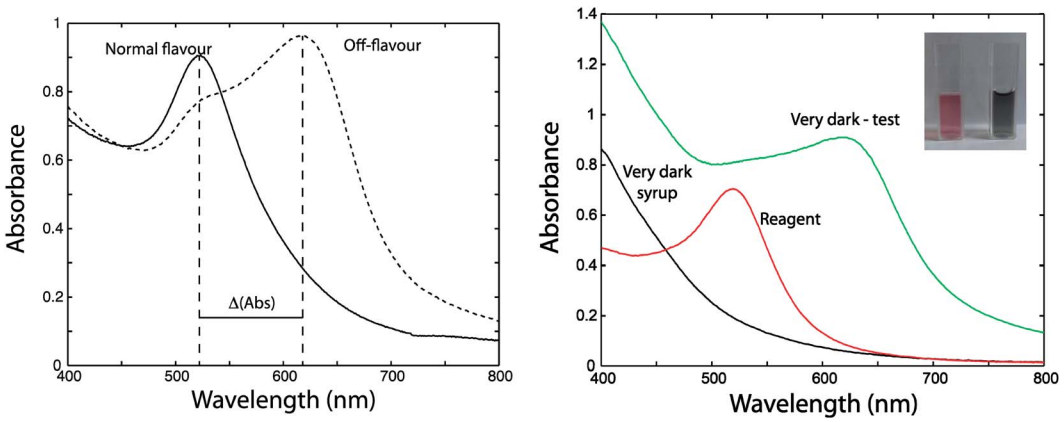

Fig. 3 (Left) UV-Vis spectra of the AuNP aggregation tests in the presence of normal (solid line) and off-flavour (dashed line) maple syrup samples. The large spectral shift in the presence of the off-flavour maple syrup and colour change of the solution are indicative of strong aggregation of the AuNPs with off-flavour maple syrup samples. (Right) UV-Vis spectra of a very dark, strong taste maple syrup (black trace, diluted 10× – original transmission of 15% at 560 nm), the AuNP reagent (red trace) and the result of the colorimetric test (green trace) for the very dark, strong taste maple syrup. The presence of a strong absorption background from the maple syrup causes the test to turn a greyish green colour and cause uncertainty of the test.

As such, a simple numerical value derived from the difference in absorbance at 625 and 520 nm is proposed. Strongly aggregated AuNPs lead to an absorbance higher than the one at 520 nm, while unaggregated AuNPs lead to an opposite result. Hence, the difference in absorbance at those wavelengths would be positive for an off-flavour maple syrup sample (positive test) and negative for a normal maple syrup sample (negative test). Other wavelengths were tested with no improvement in the performance of the test.

To validate that the aggregation of AuNPs and absorbance difference could be used in detecting the presence of off-flavours in maple syrups, we conducted a small-scale test with a few tens of normal and off-flavour maple syrup. These samples were previously graded by trained technicians. Maple syrups from the province of Quebec are primarily classified by the transmittance at 560 nm and their taste profile.[31] Syrups with greater than 75% transmittance are labelled golden, delicate taste, the ones between 50% and 75% are amber, rich taste, from 25% to 50% are classified dark, robust taste and the ones of less than 25% transmittance are labelled very dark, strong taste. As the transmittance from the maple syrup could lead to a change in absorbance, we carried out the colorimetric test with different grades of maple syrups. Golden, delicate taste, amber, rich taste, and dark, robust taste maple syrups classified as normal flavoured did not react in the test as these samples had negative values for the differential absorbance and the solution remained red (Fig. 4). However, all samples classified with flavour defects associated with the buddy taste showed extensive aggregation of the AuNPs (solution turned blue) and the differential absorbance was positive.

Very dark, strong taste maple syrup samples were challenging to detect, as the residual absorbance is non-negligible even with a 10× dilution (Fig. 3). Hence, the differential absorbance value of these maple syrups is skewed from the high background and the colour observed from very dark, strong taste maple syrups with defects had a more greyish green hue. As these samples only represent a small fraction of the yearly production in Québec (less than 5%), these samples were classified separately from other categories as sappy taste – green.

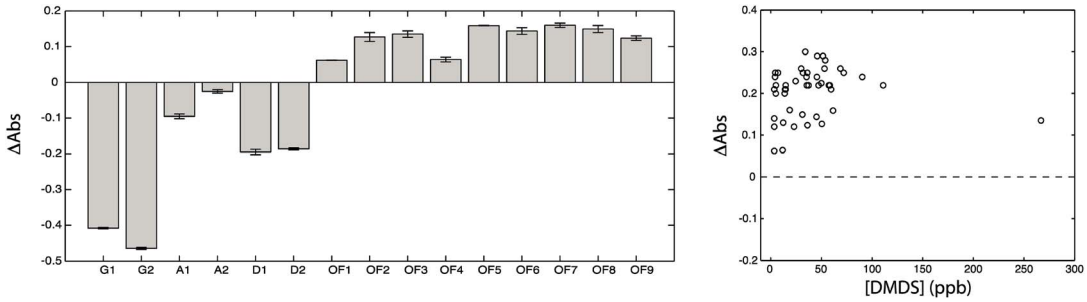

Fig. 4 (Left) Analysis of normal and off-flavour maple syrup samples with the colorimetric test. The absorbance difference was read by subtracting the absorbance at 520 nm from the absorbance at 625 nm. A normal flavour syrup sample led to higher absorbance at 520 nm than at 625 nm, while the opposite was observed for off-flavoured maple syrup samples. G: golden, delicate taste, A: amber, rich taste, D: dark, robust taste, and OF: off-flavour. (Right) Correlation plot of the concentration of DMDS in maple syrup samples and the absorbance difference read with the colorimetric test. Tests were replicated 3 times ($n = 3$).

Putative molecular origins of off-flavours and the test reaction

In an attempt to explain the molecular origins of the aggregation of nanoparticles, we evaluated a series of different molecules within broad classes of molecules that were identified in maple syrup samples. DMDS has been identified as one of the putative sources of the emergence of the buddy taste in maple syrup.[10] As a disulphide, DMDS can react at low concentrations with AuNPs, displacing the citrate capping agent and potentially causing their aggregation. A series of head space solid-phase microextraction (HS-SPME) coupled to GC-MS analysis was performed to quantify DMDS in a series of off-flavoured maple syrups according to a recently published procedure.[10] All the off-flavoured maple syrup samples led to a positive colorimetric test with the AuNPs, while the DMDS concentration detected in these syrup samples varied from low ppb to more than 100 ppb (Fig. 4). However, no correlation between the colorimetric test and GC-MS analysis could be established.

To validate whether DMDS reacted in the colorimetric test, normal flavoured maple syrup samples were spiked with increasing concentrations of DMDS (0.5, 5, 50 and 500 μM – 47 ppb, 0.47 ppm, 4.7 ppm and 47 ppm, respectively, for DMDS). The colorimetric test was also performed in water and 66% sucrose solution for comparison. The minimal concentration required to induce a change in the colorimetric test was 4.7 ppm or 50 μM for DMDS (Fig. 5K and Table 1), a concentration nearly 20 times higher than the largest concentration reported in maple syrup samples. It is interesting to note that the test turned blue in water, sucrose and maple syrup, albeit less strongly in maple syrup (a more purplish hue was observed instead of a greyish blue hue). Therefore, maple syrup seems to decrease the reaction of DMDS on AuNPs and is not the main molecule causing the aggregation of the AuNPs with off-flavoured maple syrup samples.

A more extensive survey of molecules was then undertaken. We attempted to measure the SERS response of aggregated AuNPs, but only a weak carbon signal with no distinctive features was obtained (Fig. S1†). We measured the zeta potentials of the blank AuNPs (-22 ± 7 mV), of the AuNPs reacted with normal flavor (-12 ± 4 mV) and off-flavour (-22.4 ± 0.5 mV). This indicated that the molecular mechanism of aggregation of off-flavour compounds may involve charged molecules to a greater extent than normal flavor maple syrups. Then, a series of amino acids were spiked at the same molar concentrations as for DMDS in normal flavour maple syrup samples. A shift in amino acid profiles was recently seen in off-flavoured maple syrup samples.[9] Glutamine, glycine, glutamic acid, and serine did

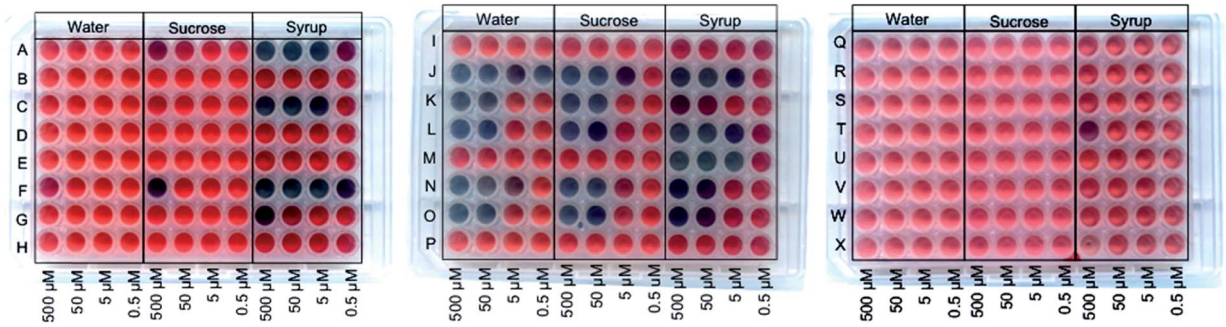

Fig. 5 Images of the multi-well plates for the evaluation of the aggregation from different organic molecules: (A) methionine, (B) glutamine, (C) histidine, (D) glycine, (E) glutamic acid, (F) cysteine, (G) lysine, (H) serine, (I) pyrazine, (J) 4-mercaptopyrazine, (K) DMDS, (L) adenosine, (M) purine, (N) imidazole, (O) pyridine, (P) glucose, (Q) homovannilic acid, (R) mandelic acid, (S) benzylmalonic acid, (T) 2-pyrazine carboxylic acid, (U) phenol, (V) 3-methoxy phenylacetic acid, (W) 4-aminophenylacetic acid, and (X) vannilin.

Table 1 Screening assay for different organic molecules inducing aggregation of the AuNPs

		Concentration inducing aggregation/solution		
Compound	Detected in maple syrup	Water	Sucrose	Maple Syrup
Methionine	Yes	Stable	500 μM	0.5 μM
Glutamine	Yes	Stable	Stable	Stable
Histidine	Yes	Stable	Stable	5 μM
Glycine	Yes	Stable	Stable	Stable
Glutamic acid	Yes	Stable	Stable	Stable
Cysteine	Yes	500 μM	500 μM	0.5 μM
Lysine	Yes	Stable	Stable	500 μM
Serine	Yes	Stable	Stable	Stable
Pyrazine	Yes	Stable	Stable	Stable
4-Mercapto-pyrdine	No	5 μM	5 μM	5 μM
DMDS	Yes	50 μM	50 μM	50 μM
Adenosine	Yes	50 μM	50 μM	5 μM
Purine	Derivatives only	Stable	Stable	5 μM
Imidazole	No	5 μM	50 μM	50 μM
Pyridine	No	5 μM	50 μM	50 μM
Glucose	Yes	Stable	Stable	Stable
Mandelic acid	No	Stable	Stable	Stable
Benzylmalonic acid	No	Stable	Stable	Stable
2-Pyrazine carboxylic acid	No	Stable	Stable	500 μM
Phenol	No	Stable	Stable	Stable
3-Methoxy phenylacetic acid	No	Stable	Stable	Stable
4-Amino-phenyl acetic acid	No	Stable	Stable	Stable
Vannilin	Yes	Stable	Stable	Stable
Homovanillic acid	No	Stable	Stable	Stable
Allantonin	Yes	Stable	Stable	Stable

not react with the AuNPs in any solutions. Methionine and cysteine reacted at concentrations of 0.5 μM, histidine at 5 μM and lysine at 500 μM in maple syrup samples. The concentration at which the test reacts follows the same trend as the strength of adsorption on Au, where thiols and sulfur compounds react strongly with Au, more than aromatic amines and followed by aliphatic amines. In addition, combined with the fact that other amino acids tested did not react suggest that the side chains of the amino acids are the primary point of contact with the AuNPs. In addition, it is interesting to note that the concentrations required for the test to react were significantly greater in sucrose and in water, where only the sulphur-containing amino acids reacted at 500 μM. Methionine did not even react in water. This also suggests a synergistic effect in the aggregation of the AuNPs caused by the numerous constituents of maple syrup. In addition, the concentrations of these amino acids in maple sap are on the order of mid-nanomolar to low micromolar in maple sap,[9] strengthening the argument that these amino acids can play a role in the detection of off-flavours in maple syrups.

Aromatic amines were then tested to evaluate their impacts on the colorimetric test (Fig. 5 and Table 1). Pyrazine, 4-mercaptopyridine, adenosine, purine, imidazole, and pyridine were doped into normal flavour maple syrup samples and to water and 66% sucrose solutions. Most of these compounds were previously detected in maple syrups,[32] but were only qualitatively identified.[33] From these compounds, only pyrazine did not react with the AuNPs. 4-Mercaptopyridine, adenosine, and purine caused the aggregation of the AuNPs at a concentration of 5 μM, while imidazole and pyridine needed a concentration of 50 μM for inducing the colour change of the test. In this case, almost all of these compounds (except purine) reacted also in water and 66% sucrose solution in similar concentrations, and in some cases more strongly in water. Hence, the aromatic amines can also be a putative class of molecules causing aggregation in the test. It is interesting to note that these compounds are found in thermally processed food products and contribute to flavour.[34]

A series of oxygen-containing molecules including organic acids were then tested (Fig. 5 and Table 1). All these molecules did not react with the AuNPs, with the exception of 2-pyrazine carboxylic acid, which reacted at 500 μM with AuNPs only in maple syrup. It is interesting to note that this compound is also an aromatic amine, and thus is expected to react at that concentration range. Thus, the organic acids, other sugars (glucose) or other oxygen-containing organic molecules do not seem to react in the test.

The colorimetric test reacts to a series of amino acids and aromatic amines that can synergistically bind with the AuNPs to aggregate them. While these compounds are identified to react in the test, this does not constitute evidence of their implication in the buddy taste, even though they were previously detected in maple syrup samples and in some cases in maple syrup samples with the buddy taste defect. More in-depth molecular studies will be needed to provide further causal effects of these molecules and others yet to be identified in the maple syrups with buddy defects.

Table 2 Results from the analysis of 1818 maple syrup samples (samples were selected to have a high proportion of off-flavour samples for validation)

Colorimetric test classification	Off-flavor (VR5) samples according to Centre ACER (%) ($n = 1056$)	Good tasting samples according to Centre ACER (%) ($n = 762$)
Negative	2 ($n = 21$)	69 ($n = 530$)
Positive	83 ($n = 878$)	17 ($n = 129$)
Uncertain/transition point	5 ($n = 56$)	8 ($n = 60$)
Sappy taste (green)	10 ($n = 101$)	6 ($n = 43$)

Large scale test of the colorimetric test with maple syrup samples from the 2018 season

To validate the colorimetric test, approximately 1818 samples of maple syrup were obtained from the Quebec Maple Syrup Producers (QMSPs). The samples contained a large number of off-flavour maple syrup samples of different grades (transmittance) to validate the test under a broad range of conditions and to obtain statistically relevant data. Hence, these samples do not represent the normal distribution of maple syrups from the harvest and the results presented in Table 2 should not be interpreted other than as a validation tool for the test.

To ensure the analysis of such a number of samples, the test was adapted on a 96-well plate and a plate reader was used to increase the speed of analysis. Hence, a single user could analyze between 100 and 200 samples per day, mainly limited by the large number of manual pipetting steps. This could be largely increased by automating this process. We estimate the cost of the test to be on the order of dollars per test. The plate reader was set to read the absorbance at 520 and 625 nm to obtain the differential absorbance. Samples with negative differential absorbance were classified as normal flavour with the colorimetric test, samples with differential absorbance between 0 and 0.05 were deemed uncertain or within the transition point and samples with higher than 0.05 differential absorbance were classified as positive for off-flavour maple syrup samples (Fig. 6). The samples were classified as green from the analysis of the scans of the 96-well plates by two independent researchers.

The maple syrup samples from the 2018 harvest were first classified by the mandated agency for maple syrup classification in Québec. Barrels were then sampled and the maple syrup samples were then sent to Université de Montréal for analysis with the colorimetric test. Out of the 1818 maple syrup samples sampled, only 2% ($n = 21$) of the off-flavour maple syrup samples with the buddy taste tested negative, while 83% tested positive ($n = 878$). 530 normal flavor maple syrup samples tested negative with the test, while 129 tested positive. A number of samples tested as uncertain or as green for both the normal and off-flavour maple syrup samples. Further investigations will be conducted to better understand these samples.

Preliminary tests on site at maple sugar shacks

The colorimetric test was deployed to a number of maple sugar shacks to evaluate the potential of using the colorimetric test during production of maple syrups. The test was run by a series of producers and non-technical staff with success, but it was rapidly noted that the visual reading of colours, especially near the transition point, could be subjective and can induce false interpretations of the test's result (Fig. 6). Hence, a portable (UV-Vis) spectrophotometer was used, on some samples, by a trained user to read the absorbance spectra between 400 nm and 800 nm for more accurate results. In the near future, maple syrup producers could be equipped with a similar spectrophotometer with a user-friendly software program for data interpretation. Thus, producers could perform the colorimetric test with a quality similar to those performed in a laboratory. This constitutes one of the few examples where plasmonic tests were deployed to the field for the analysis of real samples.[35–38]

Fig. 6 (Top) Photograph of the different test results (negative: red; uncertain: purple; positive: blue; green: greyish green). (Bottom) Photograph showing a test carried out in a maple sugar shack during the 2018 harvest.

Conclusions

A colorimetric test was successfully developed using the principles of nonspecific interactions of the low abundance organic

molecules in maple syrup samples with AuNPs. The test leads to a colour change of the AuNP suspension, which could be observed with the naked eye, with a portable UV-Vis spectrophotometer or with a 96-well plate reader. Hence, the test can be deployed to the field or be performed in high-throughput in centralized laboratories. A molecular study revealed that the test was sensitive to thiol-containing and amine-containing amino acids and to aromatic amines at concentrations on the order of micromolar concentrations. These molecules induced the aggregation of the AuNPs. Then, other molecules such as sugars, oxygen-containing organic molecules or other amino acids did not react in the test. Finally, 1818 maple syrup samples were analyzed from the 2018 harvest. This article provides evidence for the use of nonspecific interactions in the analysis of food products with plasmonic sensors.

Conflicts of interest

No conflict of interest is declared.

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References

1 D. W. Ball, The Chemical Composition of Maple Syrup, *J. Chem. Educ.*, 2007, **84**(10), 1647.
2 L. Li and N. P. Seeram, Maple Syrup Phytochemicals Include Lignans, Coumarins, a Stilbene, and Other Previously Unreported Antioxidant Phenolic Compounds, *J. Agric. Food Chem.*, 2010, **58**(22), 11673–11679.
3 E. AkochiK, I. Alli and S. Kermasha, Characterization of the pyrazines formed during the processing of maple syrup, *J. Agric. Food Chem.*, 1997, **45**(9), 3368–3373.
4 E. Akochik, I. Alli, S. Kermasha, V. Yaylayan and J. Dumont, Quantitation of alkylpyrazines in maple syrup, maple flavors and non-maple syrups, *Food Res. Int.*, 1994, **27**(5), 451–457.
5 H. Sabik, J. Fortin and N. Martin, Identification of pyrazine derivatives in a typical maple syrup using headspace solid-phase microextraction with gas chromatography-mass spectrometry (vol 133, pg 1006, 2012), *Food Chem.*, 2012, **134**(3), 1711.
6 H. Sabik, J. Fortin and N. Martin, Identification of pyrazine derivatives in a typical maple syrup using headspace solid-phase microextraction with gas chromatography-mass spectrometry, *Food Chem.*, 2012, **133**(3), 1006–1010.
7 T. D. Perkins and A. K. van den Berg, Chapter 4 Maple Syrup—Production, Composition, Chemistry, and Sensory Characteristics, in *Advances in Food and Nutrition Research*, Academic Press, 2009, vol. 56, pp. 101–143.
8 Y. T. Kim and R. H. Leech, Effects of Climatic Conditions on Sap Flow in Sugar Maple, *For. Chron.*, 1985, **61**(4), 303–307.
9 G. Q. N'guyen, N. Martin, M. Jain, L. Lagacé, C. R. Landry and M. Filteau, A systems biology approach to explore the impact of maple tree dormancy release on sap variation and maple syrup quality, *Sci. Rep.*, 2018, **8**(1), 14658.
10 M. Camara, M. Cournoyer, M. Sadiki and N. Martin, Characterization and Removal of Buddy Off-Flavor in Maple Syrup, *J. Food Sci.*, 2019, **84**(6), 1538–1546.
11 B. Panneton, A. Clément and L. Lagacé, Potential of fluorescence spectroscopy for the characterisation of maple syrup flavours, *J. Sci. Food Agric.*, 2013, **93**(13), 3279–3285.
12 X. M. Ma, S. He, B. Qiu, F. Luo, L. H. Guo and Z. Y. Lin, Noble Metal Nanoparticle-Based Multicolor Immunoassays: An Approach toward Visual Quantification of the Analytes with the Naked Eye, *ACS Sens.*, 2019, **4**(4), 782–791.
13 M. Couture, K. K. Ray, H. P. Poirier-Richard, A. Crofton and J. F. Masson, 96-Well Plasmonic Sensing with Nanohole Arrays, *ACS Sens.*, 2016, **1**(3), 287–294.
14 K. S. McKeating, M. Couture, M. P. Dinel, S. Garneau-Tsodikova and J. F. Masson, High throughput LSPR and SERS analysis of aminoglycoside antibiotics, *Analyst*, 2016, **141**(17), 5120–5126.
15 M. Couture, S. S. Zhao and J. F. Masson, Modern surface plasmon resonance for bioanalytics and biophysics, *Phys. Chem. Chem. Phys.*, 2013, **15**(27), 11190–11216.
16 K. Saha, S. S. Agasti, C. Kim, X. Li and V. M. Rotello, Gold Nanoparticles in Chemical and Biological Sensing, *Chem. Rev.*, 2012, **112**(5), 2739–2779.
17 Y. Geng, W. J. Peveler and V. M. Rotello, Array-based "Chemical Nose" Sensing in Diagnostics and Drug Discovery, *Angew. Chem., Int. Ed.*, 2019, **58**(16), 5190–5200.
18 A. Bajaj, O. R. Miranda, I.-B. Kim, R. L. Phillips, D. J. Jerry, U. H. F. Bunz and V. M. Rotello, Detection and differentiation of normal, cancerous, and metastatic cells using nanoparticle-polymer sensor arrays, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**(27), 10912–10916.
19 J. L. Rogowski, M. S. Verma and F. X. Gu, Discrimination of Proteins Using an Array of Surfactant-Stabilized Gold Nanoparticles, *Langmuir*, 2016, **32**(30), 7621–7629.
20 J. L. Rogowski, M. S. Verma, P. Z. Chen and F. X. Gu, A "chemical nose" biosensor for detecting proteins in complex mixtures, *Analyst*, 2016, **141**(19), 5627–5636.
21 G. Macias, J. R. Sperling, W. J. Peveler, G. A. Burley, S. L. Neale and A. W. Clark, Whisky tasting using a bimetallic nanoplasmonic tongue, *Nanoscale*, 2019, **11**, 15216–15223.
22 J. Kimling, M. Maier, B. Okenve, V. Kotaidis, H. Ballot and A. Plech, Turkevich Method for Gold Nanoparticle Synthesis Revisited, *J. Phys. Chem. B*, 2006, **110**(32), 15700–15707.
23 J. Xie, J. Y. Lee, D. I. C. Wang and S. Seedless, High-Yield Synthesis of Branched Gold Nanocrystals in HEPES Buffer Solution, *Chem. Mater.*, 2007, **19**(11), 2823–2830.
24 J. Xie, Q. Zhang, J. Y. Lee and D. I. C. Wang, The Synthesis of SERS-Active Gold Nanoflower Tags for In Vivo Applications, *ACS Nano*, 2008, **2**(12), 2473–2480.

25 M. F. Morselli and M. L. Whalen, Amino-acids increase in xylem sap of acer saccharum prior to bud break, *Am. J. Bot.*, 1986, **73**(5), 722–723.
26 J. C. Love, L. A. Estroff, J. K. Kriebel, R. G. Nuzzo and G. M. Whitesides, Self-Assembled Monolayers of Thiolates on Metals as a Form of Nanotechnology, *Chem. Rev.*, 2005, **105**(4), 1103–1170.
27 C. D. Bain, J. Evall and G. M. Whitesides, Formation of monolayers by the coadsorption of thiols on gold: variation in the head group, tail group, and solvent, *J. Am. Chem. Soc.*, 1989, **111**(18), 7155–7164.
28 A. G. Brolo, D. E. Irish and J. Lipkowski, Surface-Enhanced Raman Spectra of Pyridine and Pyrazine Adsorbed on a Au(210) Single-Crystal Electrode, *J. Phys. Chem. B*, 1997, **101**(20), 3906–3909.
29 J. F. Masson and H. Yockell-Lelievre, Spectroscopic and Physical Characterization of Functionalized Au Nanoparticles: A Multiweek Experimental Project, *J. Chem. Educ.*, 2014, **91**(10), 1557–1562.
30 J. G. Stuckel and N. H. Low, The chemical composition of 80 pure maple syrup samples produced in North America, *Food Res. Int.*, 1996, **29**(3), 373–379.
31 http://ppaq.ca/en/producer/retail-sales/classification, accessed September 5th, 2019.
32 I. Alli, K. E. Akochi- and S. Kermasha, Flavor Compounds in Maple Syrup, in *Developments in Food Science*, ed. Charalambous, G., Elsevier, 1992, vol. 29, pp. 131–140.
33 I. Alli, J. Bourque, R. Metussin, R. Liang and V. Yaylayan, Identification of pyrazines in maple syrup, *J. Agric. Food Chem.*, 1990, **38**(5), 1242–1244.
34 J. A. Maga and I. Katz, Pyrazines in foods: An update, *CRC Crit. Rev. Food Sci. Nutr.*, 1982, **16**(1), 1–48.
35 T. Chinowsky, A. Naimushin, S. Soelberg, C. Spinelli, P. Kauffman, S. Yee and C. Furlong, Airborne surface plasmon resonance biosensing, in *Environmental Monitoring and Remediation Iii*, ed. VoDinh, T.; Gauglitz, G.; Lieberman, R. A.; Schafer, K. P.; Killinger, D. K., 2004, vol. 5270, pp. 182–188.
36 Y. C. Kim, J. Cramer, T. Battaglia, J. A. Jordan, S. N. Banerji, W. Peng, L. L. Kegel and K. S. Booksh, Investigation of in Situ Surface Plasmon Resonance Spectroscopy for Environmental Monitoring in and around Deep-Sea Hydrothermal Vents, *Anal. Lett.*, 2013, **46**(10), 1607–1617.
37 S. S. Zhao, N. Bukar, J. L. Toulouse, D. Pelechacz, R. Robitaille, J. N. Pelletier and J. F. Masson, Miniature multi-channel SPR instrument for methotrexate monitoring in clinical samples, *Biosens. Bioelectron.*, 2015, **64**, 664–670.
38 S. A. Kim, S. J. Kim, H. Moon and S. B. Jun, In vivo optical neural recording using fiber-based surface plasmon resonance, *Opt. Lett.*, 2012, **37**(4), 614–616.