A Project Report on Taste sensor

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i. Introduction
As is well known humans have five senses such as sight, hearing, touch, smell and taste. Humans act after receiving information from the outside world and this is why these senses are very important. Fig. 1 illustrates the relevance between the biological system and the artificial system in the process of reception and consequent action. The ability of five organs, i.e., eye, ear, skin, nose and tongue, respectively, in the senses of sight, hearing, touch, smell and taste is organized by a sensor. We often use the term sensor in the global sense with combination of the data-processing part and the receptor part (i.e., the sensor in the narrow sense) and this phenomenon is practical due to computer development. So, the sensor plays roles of recognition as well as reception. This is the trend by which development of intelligent sensors is moving ahead.

Odor sensor and taste sensor are addressed as the senses of smell and taste, respectively. This is expected that these two kinds of sense can be realized at the reception level provided that good sensing materials are used satisfactorily. The quality of taste and chemical substances is perceived in gustatory and olfactory cells, respectively, to produce smell as a discrimination tool (Toko, 2006).

Nevertheless, taste or smell sense cannot be measured if many chemical sensors with high selectivity are developed for different chemical substances since we can extract more than 1000 in one kind of foodstuff. The original role of smell and taste was to detect and get
information within an enormous mass of external information (large numbers of chemicals). There exist too many types of chemical substance included in producing taste and smell, therefore, it sounds important to get most important information quickly instead of discrimination a single chemical species among others. This attitude is seen in unicellular living organisms, which have no sight sense. There is only a very limited correlation between the principles used to solve problems in technical artifacts and in biological systems. Vincent et al. (2006) have shown that only 12% similarity is between biology and technology fields in the principles that connect solutions to problems. This indicates the prosperity of inspiration in nature for how to solve technical problems. Bonser and Vincent (2007) has counted up the number of “biology-inspired” patents and pointed out that it has increased from 0 to 1200 in the last 20 years. It is feasible to directly facsimile solutions from nature, in particular for engineering purposes it is often more useful to use nature as inspiration source.

ii. Biomimetic systems
Biomimetics is known as the ‘abstraction of convenient design from nature’. Its central philosophy is that novel solutions have arisen in the natural world and these can be used as the basis for new technologies. Because nature has a tendency to be very economical with energy, bioinspired technologies have the potentials to create cleaner and greener solutions. It has to be mentioned that biomimetics does not try to copy nature. Biomimetics tries to apply processes and designs, constructional or developmental principles for technical applications. The subject of copying and learning from biology was introduced as Bionics by Jack Steele, of the US Air Force in 1960 at a meeting at Wright-Patterson Air Force Base in Dayton, Ohio (Vincent, 2001) and Otto H. Schmitt made the term Biomimetics in 1969 (Schmitt, 1969). This field is increasingly identified with emerging subjects of science and engineering. The term is derived from bios, meaning life, and mimesis, meaning to imitate. This new science opens a window to the studies and imitation of nature’s methods, designs and processes. Although some of nature’s basic configurations and designs can be copied, there are many ideas from nature that are best adapted if they are to serve as inspiration using human-made capabilities.

iii. Taste sensor (electronic tongue)
Taste sensor or electronic tongue is an analytical tool including an array of non-specific, low selective chemical sensors with partial specificity (cross-sensitivity) to different components in solution accompanied by an appropriate method of pattern recognition and/or multivariate calibration for the data-processing. The stability of sensor behavior and enhanced cross-sensitivity is a critical criterion, which is understood as reproducible response of a sensor to
as many species in solution as possible. If properly configured and trained (calibrated), the electronic tongue has the potential to determine quantitative composition (the content on multiple components) and to recognize (distinguish, classify, identify) complex liquids of different nature. The sense of taste may have two meanings. One aspect devotes to the five basic tastes of the tongue; sour, salt, bitter, sweet, and ‘umami’. These tastes are sensed from different, discrete regions on the tongue including specific receptors known papillae. The other aspect denotes the perception obtained when food enters the mouth. The basic taste is then combined with the information from the olfactory receptors, when aroma from the food enters the nasal cavities via the inner passage. A unique feature in application of taste sensor is the possibility to maintain a correlation between the output of the electronic tongue and human perception. After calibration as acceptable as possible, the electronic tongue can produce results in the same manner a human sensory panel does: as marks or assessments of various simple and complex features of taste and flavor of different products. The electronic tongue can easily taste raw substances, and also new entities that maybe have the hazards for human consumption.

iv. Conclusion

Biomimetic systems such as electronic nose and electronic tongue could be used extensively in food research and technology. These systems are known as human sense inspired sensor array technologies. As a summary, it can be said that the sensors used for smell and taste should have a fabrication principle different from the physical sensors or the conventional chemical sensors. Nowadays, developed taste and odor sensors have outputs well correlated with the human sensory panels, and the taste sensor especially has an intelligent capability to break down the information included in chemical substances to the basic information of taste quality. It is expected odor sensors (electronic noses) and taste sensors (electronic tongues) as the biomimetic systems would be increasingly of interest in food science and technology. Currently, the biggest market for such systems is in the food industry so that the applications of odor and taste sensors in food industry are numerous. They include: Inspection of food by odor and taste, grading quality and safety of food by odor and taste, checking mayonnaise for rancidity, fish inspection, automated flavor control, beverage container inspection, fermentation control monitoring cheese ripening, microwave over cooking control and so on.

(b) Fabrication

Fabrication of the Nanosensors

The sense of taste consists of an extremely complex system. Technology created by humans has attempted to imitate taste and use it in practical applications like domestic security, medical
detectors, etc. This work discusses several nanosensors used in the area of nanotechnology for imitating taste. This section presents the steps involved in the fabrication of such nanosensors. The fabrication of these sensors involves complex and expensive techniques drawn from the greater nanotechnology area.

a. Carbon Nanotube DNA Sensor (Adrian et al., 2005):
Researchers at the Department of Physics at the University of Pennsylvania have developed a carbon nanotube sensor with potential for detecting odor or taste. The fabrication of this nanosensor consists in attaching single-stranded DNA to single-walled carbon nanotubes. The carbon nanotubes are arranged in arrays, which are set in the transistor geometry. A single-stranded DNA is manipulated to recognize a specific target molecule like a protein or a variety of compounds commonly found in food. The single-stranded DNA works as a "detector" while the carbon nanotube works as the "transmitter". A functionalized carbon nanotube is shown on Figure 1. The carbon nanotubes are set so that the attachment of a target molecule causes an electrical disturbance suitable for detection. The materials are tested so that detection is possible in air or water media. The sensor functions by detecting the ionization of the target molecule. Furthermore, the nanosensor is engineered with a self-regenerating mechanism, where a voltage pulse drives off the target molecule and refreshes the surface of the sensor.

b. Gold Nanofinger Sensor Chip (Kim et al., 2012):
Researchers at the Cognitive Systems Lab at the Hewlett-Packard Laboratories have developed nanosensors for detecting traces of the toxic organic compound, melamine, in
milk. This sensor imitates taste and it is applied in food safety. The nanosensors are integrated on a chip and then installed in a portable sensor system based on surface-enhanced Raman Scattering (SERS). Melamine bonds in the gold nanofinger surfaces and causes disturbances on the SERS signal, thus, allowing detection. The prototype is shown in Figure 2. The nanosensor is fabricated with gold nanofingers.

![Gold Nanofinger sensor system](source: Kim et al., 2012)

Kim et al. (2005) described the fabrication procedure: the gold nanofinger chips are fabricated on Si wafers using nanoimprint lithography (NIL). Each nanometer has a typical diameter of 140 nm and height of 530 nm. Gold was deposited over polymer nanofingers by e-beam evaporation. Then, the nanofingers were diced into chips and mounted on strips. The chip and the strips are later integrated into the portable Raman spectrometer.

c. **Plasmonically Active Gold Nanodisks Biosensor (Guerreiro et al., 2014):** Researchers at the Biomark Sensor Research Group at the Porto Institute of Engineering have developed a nanosensor for detecting polyphenols; compounds commonly present in wine. This sensor can have applications in road safety and food quality testing. The nanosensor are fabricated with plasmonically active gold nanodisks. These gold nanodisks have binding affinity for the polyphenols present in wine. The polyphenol is a glucose molecule linked to five gallic acids. The enzyme/protein normally found in saliva, R-amylase (AMY) was integrated into the nanodisks for functionalization. The binding of the polyphenol is detected by optical properties based on localized surface plasmon resonance (LSPR). The prototype is shown in Figure 3. The Au nanodisks are fabricated on glass substrated by hole mask colloidal lithography. This created specific spots for the immobilization of the AMY enzymes. The nanodisks were fabricated with a cylindrical shape with diameters close to 99nm. The surface of the disks was chemically modified in order to allow them to be used as sites for attachment of the polyphenols to the salivary enzymes. The enzymes used were amylase and the polyphenol pentagalloyl glucose PGG was used as a test analyte molecule.

![Gold nanodisk sensor](source: Figure 3- Gold nanodisk sensor)
Food labeling has become a significant issue for food manufacturers, and more consumers are choosing to avoid foods containing additives such as monosodium glutamate (MSG) and 5'-ribonucleotides like inosine 5'-monophosphate (IMP) and guanosine 5'-monophosphate (GMP). But MSG, IMP, and GMP are the principal components for the umami taste. As awareness of health issues surrounding these additives increases, MSG substitutes that contain high levels of natural glutamic acid (GA) like enzymehydrolyzed vegetable protein (EVP), enzyme-hydrolyzed animal protein (EAP), hydrolyzed vegetable protein (HVP), and hydrolysed animal protein (HAP), and natural nucleotide-like yeast extracts, represent ideal alternatives for manufacturers seeking flavor enhancement without the use of MSG, IMP, and GMP when producing crisps, snacks, noodles, and seasonings that meet consumer demands for natural ingredients and indulgent taste profiles. These substances impart an umami taste and an appropriate mouthfeel, and they can deliver taste improvement, balance flavor, mask offnotes, and round off-flavor.

Among the natural GAs, HVP and HAP are dissolved in acid and neutralize acid with base, and have a more intense umami taste than EVP dissolved by enzymes. However, the reaction between glycerol and HCl (which is produced when protein-containing lipids are hydrolyzed) produces 3-chloro-1, 2-propanediol (MCPD), which is a carcinogen that also impairs fertility in females and reduces the sperm count in males (Olsen 1993; Lynch and others 1998). The use of an animal protein (for example, HAP or EAP) produces a strong bitter taste when dissolved, whereas vegetable protein does not, making yeast extract and EVP the preferred sources among the natural GAs.

ii. Materials and Methods
Through a preliminary sensory evaluation, we chose 3 commercially available products, which are ARO, KJ, and MX, with similar sensory characteristics and strong umami taste among 14 MSG substitutes. The specifications of each product as provided by the manufacturer are listed in Table 1.
ARO is torula yeast made by Kojin (Tokyo, Japan). It is made by, after the main fermentation, separating extract and yeast residue of yeast cream, adding enzymes to the extract, dissolving, and then pasteurization. ARO is abundant in natural IMP and GMP and is capable of enhancing the flavor of foods. Also, the high natural- GA content of ARO can increase good tastes and mask unpleasant tastes. The odor and bitterness peculiar to yeast is reduced to a minimum in ARO, which does not contain MCP or DCP.

KJ is a combination of nucleotide-rich yeast extract, fermented wheat gluten, and maltodextrin. It is made by Ajinomoto (Tokyo, Japan). It has a high content of glutamic acid (22%) and is mixed with EVP, which has wheat gluten as its main ingredient, and yeast extract with a high RNA content. EVP is made by mixing a sterilized protein solution made from wheat gluten dissolved in water with aseptic Aspergillus culture fluid at a certain ratio. A separate yeast extract is mixed and an excipient such as maltodextrin is added before being spray dried to produce the final product. The watersoluble, light-yellowish powder is low in sodium, although it does contain glutamates. It is free of genetically modified organisms and MCPDs, and is highly stable against heat and low pH. The KJ is designed to be suitable in a wide variety of applications, including meat and poultry products, seafood, vegetables (especially tomato) dry soup mixes, canned items (for example, cream-based soups with cheese), and fermented foods such as soy and miso sauces.

MX is a yeast extract made by DSM (Heerlen, The Netherlands). It is high in natural 5'-nucleotide (10%) and with 5% GA. This product is a mildly sweet, light-tan, water-miscible powder that has the functional attributes associated with MSG. It enhances flavor or can be used as a clean-label alternative to MSG. It enhances seasonings, spices, and salt perception by imparting a flavor profile and mouthfeel factors that mimic the umami taste of MSG.
iii. Sample preparation and presentation for sensory evaluation

Ten samples were prepared as 0.5% solutions in deionized water (Milli-Q water purification system, Millipore SAS, Molsheim, France) consisting of one or two of the ingredients (MSG and MSG substitutes) selected in the preliminary experiment (Table 2). A 0.5% MSG solution was presented as a reference. Aliquots (30 mL) of sample, which equilibrated with room temperature (20 ± 2 °C), were presented to the panelists in a 100-mL disposable plastic cup (Seoulpack, Cheongiu, Chuncheongbuk-do, Korea) coded with 3-digit random numbers. The presentation order of the 10 samples was randomized to minimize the presentation order effect.

iv. Sensory evaluation procedure

The sensory attributes of MSG and MSG substitutes were evaluated using a quantitative descriptive analysis method (Stone and Sidel 1992). The subjects trained until consensus was reached to evaluate 1 odor attribute (burnt), 4 taste attributes (sweet, salty, bitter, and umami), 7 aroma attributes (beef, potato, squid/seafood, mushroom, soy sauce, fishy, and medicine), and 4 mouthfeel attributes (heavy/viscous, astringent, biting, and metallic). Training sessions were held on 2 d/wk for 6 wk, and each took approximately 2 h.

The sensory evaluation was performed with the panelists in individual booths under red lighting to avoid bias due to differences in the appearance of samples. The subjects had a 5-min break after tasting the 1st 5 samples, and then the other 5 samples were evaluated.
The subjects rinsed their mouths with filtered water before tasting and between tasting samples. The subjects rated the intensity of stimulus using a 15-point category scale whose left and right ends were labeled "weak" and "strong," respectively. Each sample was evaluated 3 times in different sessions.

v. Sample preparation for electronic tongue analysis
The samples used for sensory evaluation (Table 2) were diluted 10-fold with water, to reach a salinity range of 0.05 to 0.1%, according to the recommendation of the analyzer manufacturer (Make-Sense Technology 2000).

<table>
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<tr>
<th>Abbreviation</th>
<th>Samples</th>
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<tbody>
<tr>
<td>MSG</td>
<td>0.5% MSG</td>
</tr>
<tr>
<td>ARO</td>
<td>0.5% Aromild</td>
</tr>
<tr>
<td>KJ</td>
<td>0.5% Koji-Aji</td>
</tr>
<tr>
<td>MX</td>
<td>0.5% Maxarome premium</td>
</tr>
<tr>
<td>ARO-KJ</td>
<td>0.5% Aromild + 0.5% Koji-Aji</td>
</tr>
<tr>
<td>KJ-MX</td>
<td>0.5% Koji-Aji + 0.5% Maxarome premium</td>
</tr>
<tr>
<td>MX-ARO</td>
<td>0.5% Maxarome premium + 0.5% Aromild</td>
</tr>
<tr>
<td>MSG-ARO</td>
<td>0.5% MSG + 0.5% Aromild</td>
</tr>
<tr>
<td>MSG-KJ</td>
<td>0.5% MSG + Koji-Aji</td>
</tr>
<tr>
<td>MSG-MX</td>
<td>0.5% MSG + 0.5% Maxarome premium</td>
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(d) Applications.

Over the present work we have seen different king of nanosensors develop, al about the different ways of sensing and measurement, so the application for every type of them is:

i. Nanosensors with Raman scattering

The types on nanosensors using a Raman scattering with gold nonofinger structures can detect melamine with the limit of detection (LOD) of 120 parts per trillion for melamine in DI water without any sample pre-treatment. The main application is for melamine sensing in commercial milk products, already FDA regulated level using our portable sensor system.

The one-step sampling process based on either dialysis or gel-filtration was easy to use and fully compatible for field applications in a limited-resource environment, such as for consumer applications. Using the gel filtration method, we found the limit of detection for melamine in infant formula and whole milk is 100 ppb, which is well below the FDA regulated level of 1 ppm in infant formulas. The demonstration of the high performance of our portable sensor system
for melamine sensing opens new opportunities for developing other applications that can provide simple, rapid, and inexpensive chemical and biological sensing. Those could be such as in medical diagnosis, with their portability could be used by the doctor in a regular medical check up.

ii. **Gold nanodisk**

One of the main applications for LSPR Sensor Applied to Real Samples. Are that obtained results strongly suggest that the interaction of proteins with small molecules taking place at the LSPR sensor may be employed to assess this phenomenon in specific contexts, such as that for protein/polyphenols. For example, red wines are rich in polyphenols due to their extraction from grape seeds and skin during the fermentation process or oak contact during aging, while white wines usually shows lower polyphenol content because its production does not involve these stages. Both wine types were tested by estimating the interaction of immobilized AMY on the surface of the sensor and polyphenols from real wine samples (complex matrix). The main drawback of current optical sensors is the color interference of some samples in the detection mode, especially in the visible region. Overall, the estimation of polyphenol concentration and its correlation with astringency levels can be extremely useful as a process control parameter during wine production in order to fit the characteristics of the final product and consequently the consumer's satisfaction. LSPR sensors have the potential to provide rapid and valuable information on astringency in wine as an alternative to time-consuming and expensive sensorial analysis.

iii. **Carbon tubes with DNA**

Nano-sized carbon tubes with strands of DNA could potentially detect molecules on the order of one part per million by sniffing molecules out of the air or taste them in a liquid, suggesting applications ranging from domestic security to medical detectors and each sensor will last for more than 50 exposures to the targeted substances. Therefore, the sensors would not need to be replaced frequently. The best characteristic of this sensors as that the array of over a 100 sensors could identify a weak known odour that could include detection of trace amounts of explosive gases and chemical warfare agents, as well as analysis of breath for diagnosis of infections and cancer in the lung.

iv. **Intravital microscopy**
Intravital microscopy has been successfully adapted to a variety of organs\textsuperscript{13–17}, but the tongue poses a significant technical challenge due to its anatomical constraints. In the natural state, the tongue is positioned inside the oral cavity, which makes it inaccessible by conventional microscopy. In addition, functional calcium imaging requires mechanical stabilization of the tissue at a subcellular scale, which is difficult to achieve in the tongue in vivo due to physiologic respiratory and cardiac motions. Here, we report cellular imaging of the dorsal surface of the tongue in live mice, for the first time to our knowledge. This was made possible by using a custom-made suction holder that externalizes the tongue from the oral cavity noninvasively and a tongue stabilizer that suppresses the tissue motion while allowing optical and chemical access simultaneously. Using these tools in combination with a video-rate two-photon microscope, we investigated the 3-dimensional structure and physiological calcium activity of taste cells in response to tastants that are administered orally or intravenously.

v. Conclusions

The portable sensor system that can detect a trace amount of melamine based on surface enhanced Raman scattering with gold nanofinger structures has shown the demonstration of the high performance of our portable sensor system for melamine sensing opens new opportunities for developing other applications that can provide simple, rapid, and inexpensive chemical and biological sensing.

The plasmonically active gold nanodisks can function as multifunctional sensors of small molecule protein interaction. It has been demonstrated the quantification of a model molecule (PGG) binding to AMY by using the LSPR peak shift calibrated by FDTD calculations and correlated the level of binding to the protein structure. In situ measurement of conformation changes for bound AMY were carried out indirectly via plasmonically enhanced CD spectroscopy using gold nanodisks as chiral sensors. The chirality changes of the bound protein layer were correlated to structural alterations of the protein observed upon PGG binding. The potential to carry out both quantification of molecular binding and monitor associated protein structural changes in a sensor format has application in a range of drug discovery and drug mechanistic studies as well as for industrial application in biotechnology and food processing. This kind of sensors has a lot of applications in different fields that affected the health such as food and drugs industry and also in knowing how the tongue actually works, identifying substances and someday could also heal it from harm like burning with hot beverages and preserved the taste sense.
Beyond taste sensation, intravital tongue imaging is expected to provide a wide range of applications, particularly for pathogenesis and homeostatic maintenance, by allowing longitudinal observation of cellular dynamics over prolonged period of time. The lingual keratinized epithelial cells constituting the filiform papillae are one of the most rapidly regenerating cells in the body, with a typical turn over time of 10 days in human. Their rapid proliferation is closely associated with the genesis of squamous cell carcinoma and oral mucositis after cancer therapy. Observing cellular dynamics during the disease progression and therapeutic interventions would facilitate deeper understanding on cellular mechanisms. Moreover, dynamic repopulation of the taste cells, and their renewed connectivity to the afferent nerve fibers should offer an exciting model to study highly orchestrated cellular maintenance and plasticity. Structural and functional mapping of vascular network in the taste bud may also be useful to elucidate the functional role of vascular perfusion in peripheral taste sensation and to measure the potential spatiotemporal correlation (i.e. neurovascular coupling) between neuronal activity and vascular perfusion in the tongue.

Key challenges with respect to commercialization of the carbon nanotube DNA sensors include manufacturability, and the pattern recognition algorithms for e-nose applications. But it's believe that the commercialization of a carbon nanotube DNA e-nose sensor array is essentially reasonable.

vi. References

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