12-2020

Efficacy of a Mushroom Derived Saltiness Enhancer in Increasing Saltiness and Consumer Acceptance in Low Sodium Applications

Lindsay Jenkinson
ljenki22@vols.utk.edu

Recommended Citation

Jenkinson, Lindsay, "Efficacy of a Mushroom Derived Saltiness Enhancer in Increasing Saltiness and Consumer Acceptance in Low Sodium Applications. " Master's Thesis, University of Tennessee, 2020.
https://trace.tennessee.edu/utk_gradthes/5841

To the Graduate Council:

I am submitting herewith a thesis written by Lindsay Jenkinson entitled "Efficacy of a Mushroom Derived Saltiness Enhancer in Increasing Saltiness and Consumer Acceptance in Low Sodium Applications." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science.

Curtis R. Luckett, Major Professor

We have read this thesis and recommend its acceptance:

John P. Munafo, Tao Wu

Accepted for the Council:
Dixie L. Thompson

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

Efficacy of a Mushroom Derived Saltiness Enhancer in Increasing Saltiness and Consumer Acceptance in Low Sodium Applications

A Thesis Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Lindsay Jenna Jenkinson
December 2020

ACKNOWLEDGEMENTS

First, I would like to thank my advisor Dr. Curtis Luckett for his mentorship and allowing me to conduct research in his lab. I am very appreciative of his willingness to share his knowledge of sensory science and statistics, as well as providing me with ample opportunities to learn and grow. I would also like to acknowledge Dr. John Munafo and Dr. Tao Wu, both of whom served as members of my committee. Their counsel has helped strengthen my abilities as a student and a scientist.

I would like to thank the members of my lab, specifically Sara Burns and Bob Pellegrino, for their support throughout the last two years. They have played a critical role in my work by helping facilitate sensory tests, constantly boosting morale, and providing guidance and suggestions for improvement. A special thanks to Andrew Moore, who spent countless hours preparing materials for my experiments. Additionally, I cannot overstate how much I appreciate all of the undergraduate lab technicians who have helped prepare and serve samples during the many sensory tests involved in my research. Their hard work and attention to detail directly contributed to the success of these experiments.

Lastly, I am incredibly thankful for my friends and family for their endless patience, empathy, and encouragement throughout this process. My deepest gratitude goes to my parents and my brother, who continually inspire and motivate me to succeed. Without their unwavering faith and support in my academic career and overall wellbeing, this would not be possible.

ABSTRACT

The average American consumes sodium at excessive levels resulting in a multitude of adverse health effects. To reduce these risks, it is imperative to lower consumption rates. When sodium is reduced in a product, the main effect is decreased saltiness and often corresponds with reduced consumer acceptance. In addition to addressing the issue of sodium reduction, attenuating effects such as reduced consumer acceptance is also of importance.

Consuming and perceiving food is a multimodal experience, involving tastes, smells, and trigeminal sensations to produce a singular percept of flavor. This is an example of multisensory integration. When stimuli through different sensory input are associated with one another based on previous experiences, this results in a psychological effect known as cross-modal correspondence. These can be leveraged to enhance saltiness perception and mitigate negative side effects of sodium reduction. Odorants specifically have shown promise for enhancing taste perception. In terms of saltiness, meaty, brothy aromas are considered salt-congruent and have been shown elicit enhancement of salty taste.

When mushrooms are enzymatically hydrolyzed and thermally treated, aroma active compounds are generated that can improve the palatability of reduced sodium foods. The resulting proteins (eHMP) increased perceived saltiness of low-sodium chicken broth, with the enhancement compounded by the addition of cysteine (eHMP + cys). An aroma model was developed to simulate eHMP + cys and demonstrated a similar saltiness enhancing effect to that of eHMP + cys.

The eHMP + cys (mushroom-derived saltiness enhancer; MDSE) was then tested for effectiveness in a beef-mushroom blend meatball. Using hedonic threshold methodology (HTM), samples of varying concentrations of MDSE and salt were rated by panelists to determine the compromised acceptance threshold (CAT). The MDSE enhanced saltiness perception though

samples with no MDSE or low levels of MDSE were liked significantly more. Saltiness does not necessarily correspond with increased acceptance in this case, possibly due to an off-flavor at high concentrations of MDSE. HTM resulted in inconsistent CATs between MDSE levels, reflecting a limitation of the procedure in assessing acceptance with multiple variables. Consistent with our previous studies, MDSE is optimized at low concentrations in a complex food matrix.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I – Introduction

1. Why Is Sodium Reduction Necessary?

Sodium is required to carry out physiological functions of the human body and does not pose any health risks when consumed at tolerable levels (Liem et al., 2011). Preference for salty taste likely developed from a need for sodium ions, which cannot be stored in high amounts, to regulate electrolyte balance and maintain homeostasis (Dötsch et al., 2009). The minimum amount of sodium necessary for these functions to occur is between 180 and 230 milligrams per day, though both the World Health Organization and the United States Department of Agriculture recommend consuming no more than 2 grams of sodium—5 grams of salt—daily (World Health Organization, 2012; United States Department of Agriculture, 2015). However, exceeding this recommended level may cause adverse effects. Excessive salt consumption is associated with hypertension, an increased risk of cardiovascular disease, and stroke (Ha, 2014). With the average American adult consuming well over this limit at approximately 3400 milligrams of sodium per day, it is imperative to reduce dietary salt intake (*Sodium and the Dietary Guidelines*, 2017).

There are several contributing factors to humans' elevated sodium intake. Beyond a physiological need for sodium ions, humans have developed a preference for salty taste. While newborns have been found to be indifferent or reject salt, by 4 to 6 months of age, infants have demonstrated a preference for saline solutions (Roper, 2015). This may be a result of both exposure levels and maturation of salt detection ability. In terms of food supply, processed foods are one of the main contributors to elevated sodium intake, which is exacerbated by the transition from preserved foods to modern processed foods (Bernstein & Willett, 2010). Up to 75% of sodium intake comes from processed foods or restaurant foods, and frequent dining outside of the home further adds to this effect. With home cooking declining, the CDC estimates this statistic to be even higher, at 77% (*Sodium and the Dietary Guidelines*, 2017; Andersen et al., 2009). More specifically, according to the World Health Organization’s report, in the United States over 50% of estimated sodium intake can be attributed to cereals and cereal products alone, including bread, breakfast cereals, pastries, and cakes, among others (World Health Organization, 2012).

Sodium serves many roles in food production and preservation, the most basic of which is salty taste. The addition of sodium to products affects other properties of foods as well, including water activity and water-holding capacity of proteins, in turn impacting sensory characteristics like aroma, mouthfeel, and texture (Hoppu et al., 2017). Sodium plays a crucial part in food processing, as well, due to its preservation ability. By lowering water activity, sodium aids in the prevention of microbial growth for foods ranging from meats and fish to fermented products, sauces, and baked goods. Additionally, sodium ions help influence the activity of other microorganisms and enzymes, aid in the development of gluten in baked goods, and regulate fermentation in a host of products (Dötsch et al., 2009).

When salt is reduced in food products, the main effect is a reduction in saltiness. However, because overall flavor is a combination of tastes, smells, and other chemical sensations, removing salt tends to have effects beyond reduced saltiness (Israr et al., 2016). Other taste qualities (e.g. bitterness) become more pronounced, appetitive aromas may be perceived at lower intensities, and overall liking may decrease (Liem, Miremadi, & Keast, 2011; Hoppu et al., 2017). As a result, sodium reduction generally has negative effects on taste quality and flavor perception, and market success in these products is limited (Keast et al., 2007).

While the World Health Organization suggests reducing sodium intake through diet, this task can be difficult given the prevalence of salt in food products (World Health Organization, 2012). Studies have shown that people struggle to maintain low-sodium diets, as they call for major changes in consumer eating behavior (Hooper et al., 2002). In a community-based intervention trial where participants attempted to reduce sodium intake below the recommended upper limit of 5.8 mg NaCl per day, only 20 to 40% were successful, despite employing intense counseling (Karanja et al., 2007).

The United Kingdom implemented a stealth reduction strategy, where sodium was gradually and unnoticeably reduced in food products (Kilcast & den Ridder, 2007). This method both allowed the food industry to meet sodium content targets over several years and consumers to maintain their eating behavior, which historically is difficult to alter (Liem et al., 2011). While this approach was moderately successful in the United Kingdom population, achieving a sodium reduction intake of 1 gram per day, the same may not be feasible for larger nations such as the United States due to size, cost, and legislature ensuring adherence.

This review will consider sensory-led cross-modal approaches to sodium reduction strategies. Because taste perception can be influenced at different levels—peripherally and centrally—focusing on specific sensory modalities has the potential to identify methods of sodium reduction that maintain overall flavor profile and liking of food items (Keast et al., 2007). Various tastants, odorants, and texture manipulations have all been shown to influence saltiness and will be further examined in this review.

2. Multisensory Integration and Cross-modal Correspondence

Food perception is a multimodal experience, involving not just tastes and smells, but a diverse array of sensations. Even in its most basic scenario, taste can only occur through oral-tactile stimulation (Small, 2012). The process of consuming and perceiving food involves gustation and olfaction, in combination with trigeminal sensation, to produce a singular percept of flavor. Flavor, then, is inherently a result of multisensory perception, as the aforementioned sensory input occurs simultaneously from congruent parts of the body (Small, 2012). While there is ample evidence that flavor can be modified by intrinsic sensations such as food texture (Tournier et al., 2007; Slocombe et al., 2016) or extrinsic factors such as the color of the package (Piqueras-Fiszman & Spence, 2011; Barnett & Spence, 2016), it is still unclear whether flavor perception involves audio, visual, and tactile stimuli as well, or if these senses merely serve as modulators. Gustatory and olfactory stimuli are initially transmitted to different parts of the brain, but both eventually are projected in the orbitofrontal cortex (Rolls, 2000; Small, 2012). The orbitofrontal cortex is responsible for interpreting the pleasantness of a food, or hedonic response, which is heightened when the sensory input is congruent or "matches" based on previous experiences (Spence, 2015).

When stimuli of different modalities, such as taste and smell, are associated with one another, this can lead to cross-modal correspondence. Cross-modal correspondence refers to the "compatibility effect between attributes or dimensions of a stimulus in different sensory modalities" (Spence, 2011). This psychological effect has been theorized as a means of consolidating redundant sensory input and can occur between all pairings of sensory modalities. When pairings are congruent, multisensory integration has been found to be more pronounced (Parise & Spence, 2009). This can potentially be leveraged to attenuate flavor imbalance as a side effect of sodium reduction by adding other stimuli, specifically salt-congruent odorants, to heighten saltiness perception.

3. Taste-Taste Interactions

Gustation

The sensations of sweet, sour, salty, bitter, and umami are the result of gustation. The taste modality serves to help humans identify nutrients for consumption or foods to avoid (Chaudhari & Roper, 2010). Each of the basic tastes is theorized to represent either a nutritional need or a hazardous health risk. Sweet taste receptors are stimulated by mono- and disaccharide carbohydrates which fulfill physiological needs for energy. Umami receptors are activated by specific L-amino acids, indicating the presence of protein (Galindo et al., 2012). Bitter and sour tastes are cautionary sensations, potentially identifying the presence of poisons, toxins, or spoilage (Roper, 2013). Salt taste indicates the presence of sodium ions and will be explored further in a subsequent section. It is proposed that there are more than five basic tastes, such as fatty, that would be produced by the detection of long chain fatty acids (Galindo et al., 2012).

When a food or beverage enters the oral cavity, taste-active compounds are released and, after dissolving into the saliva, come into contact with taste buds in the mouth. There is estimated to be approximately 2000 to 5000 taste buds located throughout the oral cavity. Taste buds are found predominantly on the tongue, but are also found on the epiglottis, pharynx, and larynx (Roper, 2013). Within each taste bud is a community of about 100 taste receptor cells which can be broken down into three categories. Type I taste receptors act like supportive glial cells and have shown some evidence of being involved in salty taste perception (Vandenbeuch et al., 2008). Type II are integral in perception of sweet, bitter, and umami taste through expression of G protein-coupled receptors (Roper, 2013). These receptors are specific to singular tastes and release ATP to stimulate both sensory afferent fibers and the surrounding Type III presynaptic cells. Type III receptors combine multiple taste sensations, in that they respond to sour stimuli as isolated single cells, as well as sweet, bitter, and umami stimuli (Chaudhari & Roper, 2010).

Salt Taste Perception

Sodium is the predominant ion responsible for eliciting salty taste (Keast & Roper, 2007). Unlike sweet, bitter, and umami tastes, which are transduced through G protein-coupled receptors, salty taste perception utilizes membrane ion channels (Roper, 2015). The sensation of saltiness is initiated when sodium is released from the food matrix during mastication, dissolved in saliva, and binds to taste receptors located throughout the oral cavity, activating amiloride sensitive epithelial sodium channels (ENaCs; Keast & Breslin, 2003; Liem, 2017). These channels are involved in transepithelial sodium transport (Pilic et al., 2020). ENaCs allow sodium ions to move from the saliva outside the taste receptor cell into the cell, triggering the release a neural signal to the brain to identify salty taste (Henney et al., 2010). In addition to being located in the oral cavity, ENaCs are found in other areas of the body, including the distal nephron, distal colon, and airway epithelia. Liem (2017) postulated that these remote ENaCs likely play a role in the reabsorption of sodium ions, rather than taste perception.

Little is known regarding the mechanism for salt taste perception via amiloride insensitive channels, and studies focusing on the transient receptor potential vanilloid 1 (TRPV-1) channel in mice have not found much success (Roper, 2015). Channels found in taste receptor cells that are related to capsaicin perception have been proposed as part of the mechanism for amiloride insensitive salty taste (McCaughey, 2019). Although the salty taste transduction pathway is highly selective for sodium ions, the amiloride insensitive channels are nonselective and allow for passage of potassium and calcium cations. Additionally, there is also recent evidence that Type III taste receptors aid in salt taste transduction for amiloride insensitive pathways (Lewandowski et al., 2016).

The interaction between sodium ions and the ENaCs in the oral cavity results in a chemical signal which is converted to an electrical signal and then sent to regions of the brain that process gustatory sensations (Roper, 2015). If the electrical signal is strong enough, perception occurs when the brain decodes the electrical impulses and identifies the appropriate taste quality to sodium, which is saltiness, among other aspects of taste (Liem et al., 2011). Again, although

ENaCs are not the only method of salt taste perception, the other cellular and molecular processes that likely occur are not well understood.

One of the aspects of taste that the brain decodes is taste intensity, which is correlated to strength of the taste sensation. In other words, the strength of the electrical signal increases as the concentration of sodium increases. This in turn intensifies the perception of saltiness, which when plotted against concentrations, forms a psychophysical function (Keast & Breslin, 2003).

Normally, humans do not ingest single taste stimuli, but rather mixtures of tastants. The interactions between saltiness and other tastes result in suppression and enhancement effects (Keast et al., 2007). There are three levels of taste interactions. The first of those, chemical interactions, typically occurs in the food matrix when sodium ions bind to other molecules and therefore are not available to attach to the receptor. Similarly, oral physiological interactions occur when compounds interfere with taste receptors that correspond with other compounds and tastes. Unlike chemical interactions, these happen at the epithelial or cellular level, though both are considered peripheral interactions. Cognitive interactions occur centrally, as they occur after the electrical signals are sent to the taste processing regions of the brain (Keast & Breslin, 2003).

Mineral Salts

The use of various tastants as a method of sodium reduction employs both peripheral and central interactions to be successful. The most frequently used of which are mineral salts where the sodium cation is replaced by other ions, such as potassium, magnesium, calcium, ammonium, and lithium (Israr et al., 2016). These salt replacers contribute to salty taste in food products, but do not contain sodium, theoretically reducing the daily sodium intake (Busch, Yong, & Goh, 2013).

Potassium chloride is the most widely used mineral salt to achieve saltiness in reduced-salt products. This particular mineral salt is ideal, as its benefits are two-fold; in addition to contributing to saltiness without the use of sodium, it also increases potassium in the diet (Doyle & Glass, 2010). Potassium chloride (KCl) behaves similarly to sodium chloride in terms of functionality; its rheological and antimicrobial properties are comparable to that of salt (Bidlas & Lambert, 2008). However, KCl has been found to impart other less than desirable tastes, such as bitter, metallic, and chemical, particularly at levels above 30% (Brandsma, 2006). Those with heightened sensitivity to bitterness may be more perceptive of this negative side effect of KCl, though large variation has been shown between individuals (Wilson, Komitopoulou, & Incles, 2012). These negative attributes can potentially be masked when potassium chloride is used in conjunction with other taste enhancers, such as yeast extract. Partial replacement of NaCl may be a viable option to both reduce sodium and maintain consumer acceptance (Campagnol, dos Santos, Wagner, Terra, & Pollonio, 2011; Israr et al., 2016).

Calcium chloride and magnesium chloride are both mineral salts that produce salty taste as well. However, in a model reduced-sodium cheddar cheese, these salts were found to impart bitter, metallic, and soapy off-flavors (Grummer et al., 2012). Other mineral salts, such as ammonium chloride and magnesium sulfate, also tend to yield an unpleasant flavor in food products (Heidolph, 2011).

Mineral salts have been extensively shown to yield undesirable bitter, metallic, and astringent aftertastes, all of which negatively correspond with consumer acceptance (Reddy & Marth, 1991; Lawless et al., 2003). Without including sodium, it is extremely difficult to create salty taste. Because epithelial sodium channels (ENaCs) are sodium-specific, it is ideal to identify a mineral that elicits that same pure saltiness. The closest known mineral to induce such sensation is lithium, though toxicity and neurological side effects remain an issue (Liem et al., 2011; Doyle & Glass, 2010; Van Der Klaauw & Smith, 1995).

While mineral salts attempt to mimic saltiness, other tastants can enhance the effects of salt, allowing for lower sodium usage levels while still achieving the same saltiness intensity. When the intensity of a mixture of tastants is greater than the sum of the individual components, taste enhancement occurs (Keast et al., 2007).

Acids

Sour acids have been shown to enhance saltiness (Wilkie & Capaldi Phillips, 2014). At low concentrations, salt and sour taste mixtures symmetrically affect the intensity of one another (Keast & Breslin, 2002). Low concentrations of acids such as succinic, adipic, malic, and tartaric acid have been observed to significantly intensify saltiness (Labruni et al., 2003). In bread samples and low intensity salt solutions, increases in both lactic and acetic acid concentration saw increases in perceived saltiness up to a certain point where saltiness began to decrease (Hellemann, 1992). This is consistent with current literature, which suggests that at higher intensities, the salt-sour enhancement effect becomes limited or has no effect, even potentially suppressing saltiness (Keast & Breslin, 2002). Similarly, in tomato soups, saltiness intensity increased with the addition of citric acid. While consumer acceptance initially increased, it dropped rapidly after reaching a point of maximum taste preference (Little & Brinner, 1984). Based on these findings, a reduced-sodium soup with added citric acid may successfully maintain consumer acceptability comparable to that of a high-salt soup. However, the dominant sour taste of acid may not translate well in other food products where sourness is not congruent, thus adversely affecting hedonic response (Hellemann, 1992). Therefore, these results cannot necessarily be generalized to all products.

Umami Compounds

The use of ingredients imparting umami taste has also been shown to enhance saltiness. The flavor profile in sodium-reduced recipes can be rebalanced through the use of the brothy, beefy, or savory taste of umami flavor (Rotola-Pukkila, Pihlajaviita, Kaimainen, & Hopia, 2015; Keast & Breslin, 2003). Studies have shown through various ingredients with natural umami flavor, such as soy sauce, yeast extract, and hydrolyzed vegetable protein, that umami-salt interactions can enhance saltiness perception (Dötsch et al., 2009). In a study where salt was replaced by naturally brewed soy sauce in a variety of foods, taste intensity and acceptance were successfully maintained in salad dressings, stir-fried pork, and soup (Kremer et al., 2009).

Monosodium glutamate (MSG) is frequently used as a flavor enhancer in savory foods, as it is responsible for umami flavor. It has previously demonstrated the ability to reduce sodium in food products without negatively affecting their acceptability or taste intensity (Jinap et al., 2016). In particular, MSG has shown promise in increasing consumer acceptance of plant-based dishes with significant reductions in salt (Halim et al., n.d.). Although MSG contains only one third the amount of sodium found in table salt and does not significantly increase the sodium content of foods, it is only partly effective because it still contains an extra sodium ion that other tastants do not (Busch et al., 2013; Halim et al., n.d.).

Unlike sour and umami tastants, other taste enhancers modify or increase perceived taste intensity without having any taste of their own (Israr et al., 2016). Reduced-salt products are balanced by activation of the taste receptor cells in the mouth and throat by compounds such as nucleotides, glutamates, yeast extracts, and amino acids (Noort et al., 2010). Amino acids and food protein-derived flavor peptides have demonstrated saltiness-enhancing effects on several occasions (Hoppu et al., 2017). The zwitterionic properties of peptides, their charged terminals, and their polar amino and carboxyl groups are all theorized to be responsible for the taste interactions that impart salty flavor (Temussi, 2012). Given its presence in meats, seafood, cheese, and mushrooms, glutamate is one of the most abundant amino acids (Rotola-Pukkila et al., 2015). In addition to glutamate, arginine and lysine have also been shown to impart salty taste, though other additives are needed to mask their residual bitterness (Kilcast & den Ridder, 2007). Further, arginyl dipeptides found in fish protein digests have displayed salt taste enhancing properties (Schindler et al., 2011). However, the concentration of flavor in amino acids is quite low, and using the aforementioned amino acids as saltiness enhancers would likely require concentration prior to any sort of application (Kilcast & den Ridder, 2007). Further investigation of potential safety hazards is necessary before large scale application and implementation. Additionally, applications appear limited to savory food profiles (Noort et al., 2010).

4. Taste-Aroma Interactions

Odors are perceived along two different pathways: orthonasally and retronasally. During orthonasal olfaction, the traditional sense of smell that occurs through the nose, odorants are brought through the nostrils during inhalation to the nasal cavity where they then pass through the olfactory cleft and stimulate olfactory receptors. Unlike orthonasal olfaction, retronasal olfaction occurs within the mouth. When food enters the oral cavity and is chewed, volatile odorants are released and enter the nasal cavity from the rear behind the palate (Bartoshuk et al., 2019). Again, the volatiles bind to the olfactory receptors, though during retronasal olfaction the sensation is referred to the mouth rather than the nose. Both forms of olfaction, but specifically retronasal olfaction, are critical in providing the signals that produce and define flavor (Small, 2012).

It is well evidenced that the odor and taste modalities converge at the cognitive level and are perceived as flavor (Frank & Byram, 1988; Stevenson, 1999; Keast et al., 2007; Small &

Prescott, 2005). These cross-modal interactions have been well documented to enhance sweetness perception (Djordjevic et al., 2004a), and to a lesser degree saltiness perception. For example, strawberry and vanilla aromas have been shown to enhance the sweetness of sucrose solutions (Frank & Byram, 1988). The parameter of congruency, however, is crucial in terms of eliciting taste enhancement. Rather, the odorant and tastant pairing must be consistent with that of previous experiences. Thus, while the effect of sweetness enhancement may have been present in sucrose solutions with sweet-congruent aromas, the same effect did not occur with saltiness of sodium chloride solutions. Djordjevic, Zatorre, and Jones-Gotman (2004b) confirmed this by showing that soy sauce odor enhanced saltiness but not sweetness in aqueous solutions where odorants were delivered orthonasally.

In a similar manner, odor-induced saltiness enhancement (OISE) has only been found to occur in the presence of corresponding savory aromas. Lawrence, Salles, Septier, Busch, and Thomas-Danguin (2009) demonstrated that terms such as anchovy, bacon, smoked salmon, peanuts and sardines were most associated with saltiness, in comparison to those such as vanilla, fig, strawberry, and cinnamon. As a result, the food items associated with saltiness induced the greatest saltiness enhancement of retronasally-evaluated aqueous solutions. A dried bonito aroma fraction, the odor of which comprises a combination of savory sulfur-containing compounds, pyridines, alcohols, and phenols, enhanced saltiness and taste intensity in low-salt solutions (Ogasawara et al., 2016). In a study assessing the effect of savory aroma compounds on saltiness enhancement in chicken bouillons, aromas that were “brothy,” “meaty,” or “roasted” were identified as salt-congruent, and in combination with a salt replacer, able to fully compensate for sodium reduction (Batenburg & Velden, 2011). The use of a single brothy aroma compound was found to potentially allow for up to 15% salt reduction. However, this effect was only seen prior to reaching a maximum concentration at which the flavor profile was altered and saltiness enhancement was diminished (Batenburg & Velden, 2011).

The role of salt concentration or tastant intensity in OISE is inconclusive. Generally, as the intensity of the congruent odorant increases, saltiness intensity also increases (Lawrence et al., 2009). This is consistent with patterns seen in sweetness and sourness, where the degree to which an odor increases predicts enhancement of tastant perception (Stevenson, 1999; Cliff & Noble, 1990). However, this effect may exist only to a certain extent. Djordjevic et al. (2004) found the enhancing effects of soy sauce odor on saltiness to be the most pronounced in the weakest taste conditions. When salt concentration was the highest, enhancement was much less prominent, which may suggest the existence of a ceiling effect beyond which odor perception no longer influences taste perception (Djordjevic et al., 2004b). Nasri, Beno, Septier, Salles, & Thomas-Danguin (2011) observed a similar effect in low-salt solutions; although sardine aroma significantly enhanced salt taste perception, OISE was diminished when saltiness intensity exceeded seven on a linear scale of one to ten.

In a study demonstrating the saltiness enhancing effect of odorants in soy sauce, Zhou et al. (2021) identified five salty taste odorants, three of which were sulfur-containing compounds. The odorant with the strongest OISE, 3-(methylthio)propanal, enhanced perceived saltiness for all concentrations of sodium chloride tested. Consistent with the results of the Nasri study, the OISE was no more significant in the solutions with the highest concentrations of sodium chloride in comparison to the low concentrations. It appears that, while high odor intensity correlates with high saltiness perception, this association exists within certain parameters concerning congruency and overstimulation beyond which there is no effect.

5. Taste-Texture Interactions

Texture involves multiple parameters, including sensations such as pressure, pain, and temperature, which are derived from the structure of the food matrix (Szczesniak, 2002). There is no single, specific receptor to these stimuli, but rather several receptors and tissues (Pflaum et al., 2013). Evaluation of textural properties predominantly occurs within the oral cavity, with less occurring visually. Some parameters are perceived upon initial placement of food in the mouth, though the majority are sensed through mastication, mixing with saliva, and bolus formation. Texture-taste interactions occur due to the corresponding presence of tactile stimulation with taste stimulation that takes place when food is consumed (Szczesniak, 2002). Different aspects of texture may allow for the enhancement of salt taste intensity of food products, though this is likely matrix-dependent.

The general approach to increasing saltiness perception is to optimize the delivery of sodium to the appropriate taste receptor cells (TRCs) by maximizing taste bud stimulation without increasing sodium concentration. A number of factors affect this mechanism of taste, such as a product’s salt distribution, its rate of salt release, its mixing with saliva, and delivery of sodium to taste buds (Busch et al., 2013).

Dissolution of Salt

The physical properties of the salt crystal itself, such as shape, size, and density, impact the delivery of sodium to the TRCs, and in turn, salt perception. Given that saltiness intensity is partially related to the rate of salt release and its dissolution, the physical properties of the salt crystal are inherently related to these mechanisms and therefore, perception of salty taste (Wilson et al., 2012). This section will consider the dissolution of salt in the oral cavity as a parameter of texture perception as it relates to salt taste.

Before saltiness can be detected and perceived, sodium ions must first be dissolved in the oral cavity during mastication (Busch et al., 2013). Sodium chloride forms a crystal that can take on a wide range of shapes, sizes, and morphologies, ranging from concentric, cubic, pyramidal, and even quadrilateral form (Barringer, 2006). Current literature provides contrasting evidence for the optimal size and shape of salt crystals. Salt crystals of lower particle size and hollow shape allowed for desired salty taste with a lower salt content in a popcorn application (Patent No. US20080075813A1, n.d.). Similarly, salt crystals of smaller size yielded faster saltiness perception of crisps in time intensity experiments (Kilcast & den Ridder, 2007).

However, this may not be the case in baked products. In bread, coarse grain salt was found to accelerate sodium release and in turn enhance salty taste, allowing for a 25% reduction of sodium (Konitzer et al., 2013). Mueller, Koehler, and Scherf (2016) demonstrated a similar effect through the late addition of coarse grain salt to pizza dough, to which they attributed accelerated delivery of sodium and the creation of a “taste contrast,” a concept further explored in later sections of this review. Currently, no studies have been conducted that assess the effect of salt crystal size on hedonic response in solid foods, which is a crucial measure in the efficacy of sodium reduction technologies, particularly in snack foods (Kilcast & den Ridder, 2007).

Aqueous Solutions

A food’s matrix has an effect on saltiness perception by influencing its inherent textural characteristics, and ultimately its ability to deliver sodium to the proper channels (Hoppu et al., 2017; Kuo & Lee, 2014). Therefore, it is important to consider foods with varying matrices in sodium reduction studies.

As would be expected, the effect of texture on saltiness perception varies between liquid and solid foods (Busch et al., 2013). In aqueous solutions, there is some agreement in findings that as salt solutions thicken, taste perception declines (Moskowitz & Arabie, 1970; Christensen, 1980; Yamamoto & Nakabayashi, 1999; Cook, Hollowood, Linforth, & Taylor, 2003; Koliandris et al., 2010). However, Moskowitz and Arabie (1970) found that the changes in viscosity were large relative to the elicited effects in perceived saltiness. Yamamoto and Nakabayashi (1999) demonstrated that, in salt solutions thickened with cornstarch, a critical concentration must first be achieved in order for hydrocolloid chains to overlap and increase viscosity before saltiness intensity sharply decreases. In paired comparison tests of salt solutions thickened with hydroxypropyl methylcellulose (HPMC) and lambda-carrageenan, samples with viscosities above this critical concentration exhibited reduced taste perception compared with those below (Cook et al., 2003).

These findings, however, may exist due to the use of thickening agents rather than an underlying cognitive mechanism. In a study analyzing the effect of viscosity on saltiness intensity across thickening agents, soups thickened with xanthan gum were perceived as less salty than those thickened with corn starch, potato starch, Na-CMC gum, locust bean gum, and without thickener. Because of the cationic nature of sodium, anionic substituents of thickeners can bind the sodium ion and make it unavailable for taste perception. Additionally, other agents can impart bitter taste, which due to previously mentioned taste-taste interactions can mask or suppress saltiness (Rosett et al., 1996).

Solid Foods

Less work has been done in assessing the effect of texture on saltiness perception of solid foods. Most literature discusses these effects in gels or semi-solids, and even then findings are limited as salt can cause phase separation behavior in gelatin and other protein and polysaccharide mixed gels (Çakır & Foegeding, 2011).

With all foods, the contact area between the food itself and the taste receptors on the tongue is critical for taste perception. This contact area is maximally achieved by liquids with low viscosities, but in terms of solid foods, those which break down easily and into smaller particulates elicit greater perception (Busch et al., 2013). Koliandris et al. (2010) found that brittle gels with lower strain at fracture had an increased surface area and in turn demonstrated an enhanced release of sodium ions (Koliandris, Lee, Ferry, Hill, & Mitchell, 2008). This effect was confirmed by a study employing an in vitro mouth system in gels constructed with gelatin, gellan, and alginate: gels that broke down into many, discrete fragments had greater salt release compared with those of fewer, larger pieces (Mills et al., 2011).

Though taste intensity has been found to decrease as the amount of gelling agent increases, as with aqueous solutions, the hydrocolloids used to thicken gels should be considered (Moritaka & Naito, 2002). In an earlier study conducted by Pangborn, Trabue, and Szczesniak (1973) using a range of hydrocolloids, the effect on taste intensity was primarily a result of the nature of the thickener rather than viscosity. As previously mentioned, sodium ions in salt solutions may bind with hydrocolloids and reduce saltiness perception. As a result, whether viscosity has an effect on saltiness intensity in semi-solid food systems is inconclusive (Busch et al., 2013).

With most previous work focusing on rheological properties of semi-solid food systems, other aspects of texture-taste interactions in solid foods have been minimal. In a study analyzing the effect of crispiness on flavor intensity of potato chips, the crispiest samples appeared to yield the greatest flavor intensity (Luckett et al., 2016). Crispier chips theoretically fragment into more pieces, and therefore increase contact area with the tongue. Textural attributes such as crispiness can influence and alter mastication patterns as well, factoring into crispiness-taste interactions through modifying particle size. A similar effect has been seen in model cheeses with higher fat content; a larger fat content produced a larger contact area and therefore greater salt release and perception (de Loubens et al., 2011). Contact area appears to be a key criterion in saltiness perception and should be further studied in solid food systems.

Distribution of Salt in Complex Matrices

The distribution of salt within a food product can potentially influence saltiness perception. During mastication, food is broken down into fragments, that when mixed with saliva, create a bolus. These fragments contain different levels of tastant—salt being the tastant of interest—which then come into contact with the TRCs. When a tastant is not evenly distributed within a fragment, the taste receptors receive discontinuous stimulation (Israr et al., 2016). This creates the aforementioned "taste contrast," which enhances saltiness perception. The magnitude of the amplification depends on the intensity of the stimulation and the number of affected receptors (Mosca et al., 2010).

Lim and Green (2008) conducted a study that provided some evidence for the convergence of taste and somatosensory pathways, or those that involve pain, pressure, and temperature that are not localized to part of the body. When participants' tongues were touched simultaneously with three cotton swabs—two on the exterior, one in the middle—the taste of the outer swabs was attributed to the middle swab, despite the middle swab lacking tastant. Thus, taste perception can be associated with tasteless, tactile stimuli, evidencing the prospective effectiveness of heterogeneous salt distribution.

The manipulation of salt distribution within a product and use of taste contrasts has been widely studied in bread, but has also been seen in other food products, such as sandwiches and snack foods (Stieger, Hamer, Bult, & Graaf, 2009). Overall, heterogeneous distribution of salt within bread can yield sodium reductions up to 28% compared to homogeneous salt distribution

(Noort et al., 2010). Compared to breads without, those with taste contrasts have been considered significantly saltier. Likewise, sandwiches with layers of varying salt concentration were perceived as saltier than sandwiches with even salt distribution, which may be a result of perceptual expectation (Dijksterhuis et al., 2014). In layered snack foods, consumers experienced enhanced perception of saltiness in those with heterogeneously distributed salt (Emorine et al., 2013). Though a large contrast in salt concentration was needed to see saltiness enhancement, the heterogeneity of the salt did not affect consumer acceptance and was relatively well-liked by panelists (Emorine et al., 2013).

While heterogeneous distribution of salt shows promise as a method of sodium reduction, it may be difficult to implement in certain food products and logistically in a manufacturing facility. Achieving the production of layered food matrices with varying salt concentrations will require incorporation of additional manufacturing processes and prevention of salt migration within the product over time (Busch et al., 2013). The main advantage of this method is its ability to maintain consumer liking of food products while reducing overall sodium content.

6. Limitations

A cross-modal approach to reducing sodium in food products while maintaining consumer acceptance has demonstrated potential for success. While this review has considered those pertaining to taste, odor, and texture, there are other important considerations to be made.

The best approach to sodium reduction varies depending on the nature of the product. Breads and baked goods, for example, are processed and consumed differently than meats, and therefore will require implementation of different salt reduction technologies. This includes the numerous constraints of sodium replacements or enhancers; sodium is by far the cheapest and most efficient ingredient available that translates to pure saltiness. Replacing sodium chloride in a product formulation may raise raw material cost, a matter of concern for the food industry. In order to successfully reduce sodium content without adversely affecting consumer acceptance or product cost, methods will likely need to be combined. The safety of the technologies will also need to be heavily substantiated prior to widespread implementation.

7. An Overview of Enzymatically Hydrolyzed Mushroom Protein (eHMP)

Protein hydrolysates have been shown to enhance saltiness and flavor (Hoppu et al., 2017). When white button mushrooms are hydrolyzed with protease enzymes and exposed to heat treatment, aroma and taste active compounds are generated (Lopez et al., 2019). The resulting substance will be referred to as enzymatically hydrolyzed mushroom protein, or eHMP. These compounds can aid in the palatability of reduced sodium foods, particularly those with a savory flavor profile.

Prior to cooking, the characteristic flavor of mushrooms can be predominantly attributed to the presence of glutamic and aspartic acids, free amino acids (FAAs), and 5`nucleotides (Phat et al., 2016). Rotola-Pukkila et al. (2019) found that cooking mushrooms reduced FAA content but increased the concentration of 5`nucleotides, with some flavor nucleotides only being detected in cooked samples. It is well evidenced that these non-volatile compounds are responsible for the characteristic umami taste of mushrooms (Sun et al., 2020). When most foods are exposed to heat, a number of chemical reactions occur that generate compounds with taste-enhancing properties that contribute to overall flavor, such as the Maillard reaction. Chen et al. (2018) determined that the Maillard reaction of mushroom hydrolysates leads to the formation of volatiles contributing to caramel-like and meaty flavors, as well as an increased concentration of FAAs and 5`guanosine monophonsphate (5`GMP), a flavor nucleotide.

A previous study compared the saltiness enhancing effects of eHMP and eHMP with cysteine (eHMP + cys) added during thermal processing in a reduced sodium chicken broth. On its own, eHMP had a toasty, mushroom-like, weakly meaty character. With the addition of cysteine, the meaty character was found to become much more pronounced. Sensory analysis was conducted to determine any differences in the effect of eHMP and eHMP + cys in a low-sodium chicken broth, both of which were compared to the control broth without any added enhancer. The samples were rated for saltiness intensity by 9 panelists in triplicate according to 3-point scale traditionally used in flavor chemistry. The broth with eHMP added was rated saltier than the control broth, and broth with eHMP + cys received a significantly higher saltiness rating than the broth with eHMP alone (t_8 = 2.300, p = 0.0252; Figure 1). This suggests that the addition of cysteine to eHMP can heighten the saltiness enhancing effect.

Lopez et al. (2019) further investigated the possibility of odor-induced saltiness enhancement (OISE) by developing an aroma model from seven odorants suspected to be main contributors to the increased saltiness of eHMP + cys in the previous study. Two sensory evaluation sessions were conducted; the first assessed saltiness intensity as in the previous study (n = 96), and the second involved a 2-alternative forced choice (2AFC) task (n = 86). Both eHMP and eHMP + aroma model were dissolved in low-sodium chicken broth and compared to a control broth without any added enhancer. The first session demonstrated that the addition of the aroma model resulted in greater perceived saltiness compared to the model broth with eHMP alone (t_{95} = 3.626, p = 0.0005; Figure 2). The eHMP + aroma model sample also received significantly higher overall flavor ratings (t_{95} = 3.0437, p = 0.0030).

In the second session with the 2-AFC task, the broth with eHMP + aroma model added was selected as saltier than the model broth with eHMP (d` = 0.5647, p = 0.0025). The results of these studies indicate that both eHMP and eHMP + the aroma model were perceived as saltier than the control broth, with the aroma model compounding this effect. These findings suggest that the compounds enhancing perceived saltiness may be aroma-active, given the comparable results of the eHMP + cys and eHMP + aroma model, suggesting the possibility of OISE.

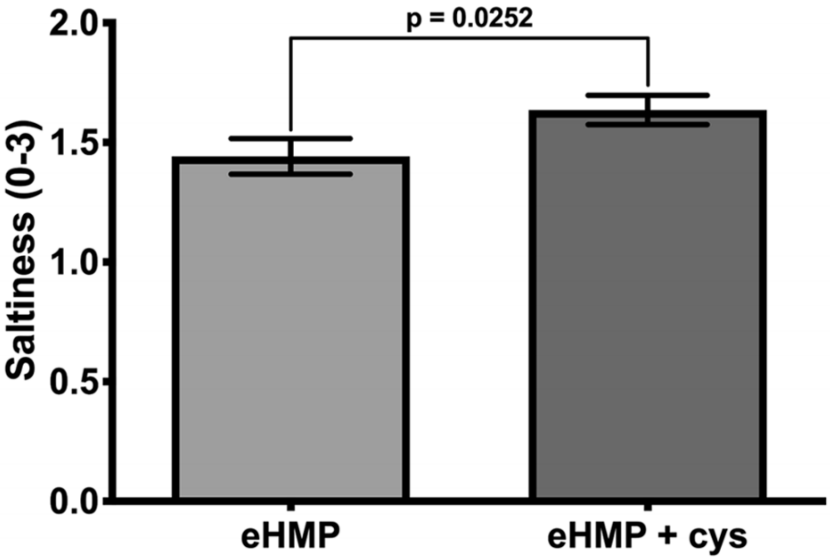

Figure 1. Paired comparison results of low-sodium chicken broth with eHMP and eHMP + cysteine added.

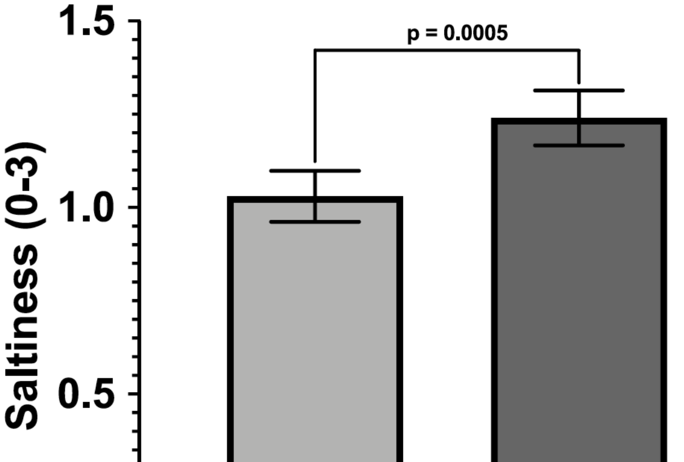

Figure 2. Paired comparison results of low-sodium chicken broth with eHMP and eHMP + the aroma model intended to simulate eHMP + cys.

CHAPTER II – Optimization of Enzymatically Hydrolyzed Mushroom Protein as a Novel Sodium Reduction Technology

A version of this chapter is currently in review with *Journal of Food Science* by Lindsay Jenkinson, Andrew Moore, John P. Munafo, and Curtis R. Luckett.

Abstract

One of the main challenges of reducing sodium in food is a loss in palatability. To address this problem, salt enhancers have been implemented in commercial products to increase the perception of saltiness when sodium is reduced. The increase in saltiness perception is hypothesized to lead to an increase in consumer acceptance. In this study we evaluated a mushroom-derived saltiness enhancer (MDSE) prepared from enzymatically hydrolyzed mushroom protein that was then thermally reacted with cysteine under kitchen-like conditions. Previous research has demonstrated the saltiness enhancing effects of the MDSE; however, the enhancement on consumer acceptance has not been evaluated. In the present study, hedonic threshold methodology (HTM) was employed to identify the compromised acceptance threshold (CAT) in a reduced-sodium beef-mushroom blend meatball with various levels of MDSE. Confirming previous findings in simpler food matrices, low levels of MDSE (1.75 mg MDSE/g meatball) allowed for a 15% reduction in salt with no change in perceived saltiness. Both sodium concentration and MDSE level had a significant effect on acceptance. Within each MDSE level, sodium reduction corresponded with decreased scores in liking. The addition of MDSE at all levels increased saltiness perception, significantly decreasing the proportion of participants describing the samples as "Not Salty Enough." Additionally, we found evidence showing the limitations of HTM in evaluating the effectiveness of saltiness enhancers or salt replacers while simultaneously varying sodium concentration.

Practical Application

Reducing sodium in food typically corresponds with decreased consumer acceptance. A mushroom-derived saltiness enhancer (MDSE) prepared from enzymatically hydrolyzed mushroom protein that was then thermally reacted with cystine under kitchen-like conditions was developed to enhance saltiness perception in reduced-salt and partial meat alternative formulations. This study demonstrates the efficacy of the novel saltiness enhancer and identifies an optimal usage level through a series of acceptance tests as part of hedonic threshold methodology.

Introduction

The average American adult consumes approximately 3.4 grams of sodium per day (*Sodium and the Dietary Guidelines*, 2017). This amount is higher than the recommendations of the World Health Organization and the United States Department of Agriculture, both of whom have recently advised consuming no more than 2 grams of sodium daily (World Health Organization, 2012; United States Department of Agriculture, 2015). Exceeding this recommended level is associated with negative health effects such as hypertension and an increased risk of cardiovascular disease or stroke (Ha, 2014). The WHO has identified reducing salt intake as one of the most cost-effective measures for improving population health. However, reducing salt in food products tends to have effects beyond a lower perceived saltiness. Without sodium, other taste characteristics (e.g., bitterness) become more pronounced, undesirable appetitive aromas can be perceived at lower intensities, and consumer acceptability may decrease (Liem, Miremadi, & Keast, 2011; Hoppu et al., 2017). As a result, sodium reduction generally has a negative influence on flavor quality, leading to little market success for reduced sodium foods (Keast et al., 2007). Accordingly, there is an increasing commercial interest in developing food technologies to lower sodium in food products while maintaining consumer acceptance. One potential solution is the use of saltiness enhancers that increase the perception of saltiness and compensate for the reduced sodium levels.

In addition to dietary concerns centered around sodium, a growing number of consumers are attempting to reduce their meat intake (Neff et al., 2018). While plant-based and cell-cultured meats are rapidly progressing, partially replacing ground meat with non-meat ingredients (i.e., plant protein, mushrooms) is a method for reducing the cost, lowering fat content, adding nutritional value, increasing fiber, and improving the texture of meat products (Burnett, 1951; Pearson, 1976). In recent years, the partial replacement of ground beef with mushrooms has received considerable attention (Miller et al., 2014; Guinard et al., 2016; Patinho et al., 2019). While the partial replacement of meat with mushrooms has shown success, there is a notable discrepancy in the sodium contents of mushrooms and the meat they replace. Mushrooms naturally have a low sodium content, with less than 5 mg/100g, as compared with 72 mg/100g in ground beef (*USDA National Nutrient Database*, 2020). Even though mushrooms are low in sodium, they contain umami tastants, which previous research has shown to mitigate some of the negative effects on flavor imparted by the reduced sodium (Manabe et al., 2009; Miller et al., 2014). Beyond these umami tastants, several key odorants produced from enzymatically hydrolyzed button mushrooms (*Agaricus bisporus* L.) thermally treated with cysteine under kitchen-like conditions have been demonstrated to enhance perceived saltiness (Lopez et al., 2019). This reaction flavor (mushroom-derived saltiness enhancer; MDSE) was demonstrated to significantly increase perceived saltiness in chicken broth. While attenuating decreases in perceived saltiness with decreasing sodium content is important in and of itself, the larger goal should be to reduce sodium without reducing acceptability. More research is needed to better understand how saltiness enhancement modulates acceptability.

Hedonic threshold methodology (HTM; Lima Filho et al., 2015) is a sensory methodology that may provide more insight in to how saltiness enhancement modifies acceptability. HTM has been used in the past to identify the threshold at which sodium reduction alters consumer acceptance, known as the compromised acceptance threshold (CAT) (Lima Filho et al., 2019). HTM has also been shown to effectively evaluate hedonic thresholds as they pertain to specific sensory attributes, such as aroma and texture. To determine the CAT, a series of acceptance tests for taste using the 9-point hedonic scale are conducted, measuring a control against a series of samples with varying intensities of the same stimuli. The present study uses HTM to assess how different levels of sodium reduction and MDSE relate to consumer acceptability. The major objectives were: 1) to investigate the decoupling of saltiness enhancement and consumer acceptance; and 2) to assess HTM for use in comparing the effectiveness of MDSE.

Materials and Methods

Participants

One hundred participants ages 18 and older were recruited through the sensory consumer database at the University of Tennessee at Knoxville. The final consumer group that completed all sessions was comprised of 37 males and 44 females (mean 35.3 years old, standard deviation 11.9). Participants were recruited for regular consumption of beef and mushrooms and were compensated upon completion of the study. This experiment was conducted in compliance with the Declaration of Helsinki for studies on human subjects and approved by the University of Tennessee IRB review for research involving human subjects (IRB# 19-05283-XM).

Sample Preparation

Finely chopped white button mushrooms (*A. bisporus*) were sautéed for 10 minutes until soft, with frequent stirring. The cooked mushrooms were then combined with 80% lean ground beef to make the mixture into a 66% beef, 34% mushroom blend (w/w). The beef-mushroom mixture was portioned into 4 equal parts, with each part receiving different concentrations of the MDSE (0 mg/g, 1.75 mg/g, 3.75 mg/g, 7.5 mg/g). Sodium chloride, commonly referred to as *salt* and will be referred to as such throughout the remainder of this paper, was then added to each MDSE-beef-mushroom mixture (Table 1). Meatballs (19g) were formed from each combination of MDSE and salt concentration. The meatballs were baked at 176.7°C for 13 minutes in separate convection ovens according to MDSE level (i.e., all meatballs with the same level of MDSE were baked in the same oven) and were held no more than 30 minutes in steam trays at 60°C prior to serving.

Preliminary Testing: Determination of Sodium Content

The goal for the salt concentration of the control was to mimic that of commercially produced meatballs. The nutrition facts of four commercial meatball brands were analyzed for their salt content, the arithmetic mean of which informed the salt content of the experimental control (14.81 mg salt/g meatball). However, in preliminary studies, this concentration of salt in combination with the high levels of MDSE was considered aversively high and, as a result, the control salt concentration was lowered to 10.50 g salt/g meatball. In order to develop a range of stimulus intensities that would include the target threshold, salt concentrations for the test samples were reduced in 15% increments until reaching a 60% reduction from the control (Lawless & Heymann, 1999).

Hedonic Threshold Methodology (HTM)

Given that sodium reduction is thought to be associated with reduced consumer acceptance, we assessed the efficacy of the MDSE in attenuating reduced acceptance with sodium reduction. Participants were asked to attend eight separate test sessions. Each session consisted of 5 samples:

Table 1. MDSE and Sodium Levels and Their Corresponding Ratios

Sodium Level (% Reduction)	Ratio (mg salt/g meat mixture)
0 (Control)	10.5
15	8.92
30	7.35
45	5.78
60	4.20
MDSE Level	Ratio (mg MDSE/g meat mixture)
0	0
Low	1.75
Mid	3.75
High	7.5

1 warm-up sample, 2 control samples, and 2 reduced-salt samples. Only two concentrations of enhancer were tested per session. Salt concentration was randomized across sessions, and the stimulus and control positions were randomized within each session. Samples were presented in sequential monadic order. Carrots and water were provided as palette cleansers between samples.

Participants were asked to rate each sample for overall opinion, flavor, and texture according to a 9-point hedonic scale (from 1 = “Dislike Extremely” to 9 = “Like Extremely”). Participants also evaluated saltiness according to a just-about-right scale, with 5 being “Much Too Salty” and 1 being “Not Salty Enough.” Participants were asked about the presence of an off-flavor, for which they were to rate the flavor according to a 15-point scale where 0 represented “Not at all Noticeable” and 15 represented “Very Noticeable.”

Data Analysis

The compromised acceptance threshold (CAT) was determined following the method outlined by Lima Filho et al. (2015). The difference between the hedonic scores of the stimulus sample and those of the control samples (control sample – stimulus sample) was calculated for each individual, then compared via a paired t-test. The t-values were regressed against salt concentration of the stimulus samples for each level of MDSE. The point at which the regression line intersected with the t-value$_{\text{crit}}$ indicated the precise salt concentration at which acceptance was significantly altered.

Additionally, a three-way ANOVA with sodium level and MDSE level as fixed factors and participant as a random factor was conducted to determine the effect of salt concentration and MDSE level on overall liking. Post-hoc contrasts were used to highlight differences among specific samples. Mean liking scores were plotted for each combination of salt concentration and MDSE level and overlaid with saltiness JAR responses to illustrate any patterns within the MDSE levels regarding liking and perceived saltiness. Penalty was calculated from the JAR data by comparing responses of "Just About Right" with "Not Salty Enough" through pairwise t-tests for each salt concentration. An ANOVA and linear regression analysis were conducted to examine the proportion of participants rating samples as "Not Salty Enough" as salt concentration decreased for each MDSE level.

Results

No interaction between salt concentration and MDSE level was found for consumer acceptance ratings ($F_{3,2833} = 1.7$, $p = 0.17$), providing some evidence that the relationship between MDSE level and acceptance was not dependent on salt concentration. Both salt concentration ($F_{1,80.5} = 121.4$, $p < .0001$) and MDSE level ($F_{3,248.3} = 30.2$, $p < .0001$) had significant effects on consumer acceptance. The control (full-salt) samples within the 0, Low, Mid, and High MDSE levels received the highest acceptance scores (mean 6.7 ± 1.6, 7.0 ± 1.4, 6.0 ± 1.9, and 6.0 ± 2.0, respectively). On average, with reduction in salt, overall liking scores decreased. Within each MDSE level, the proportion of participants who rated samples "Not Salty Enough" increased as salt was reduced ($p < 0.01$). More specifically, the 40% salt samples were consistently rated the least salty within each MDSE level. In contrast, the control and 85% salt samples were perceived as the saltiest.

Generally, as MDSE level increased, the perceived saltiness of the samples also increased (Figure 3). However, there was an interaction between MDSE level and salt concentration on saltiness ratings ($F_{12,3279} = 4.81$, $p < .0001$), suggesting that the effect of MDSE on saltiness was dependent on the magnitude of the salt reduction. This effect is most clearly evident in the samples with the lowest salt concentration. For example, when only considering the 40% salt samples, the

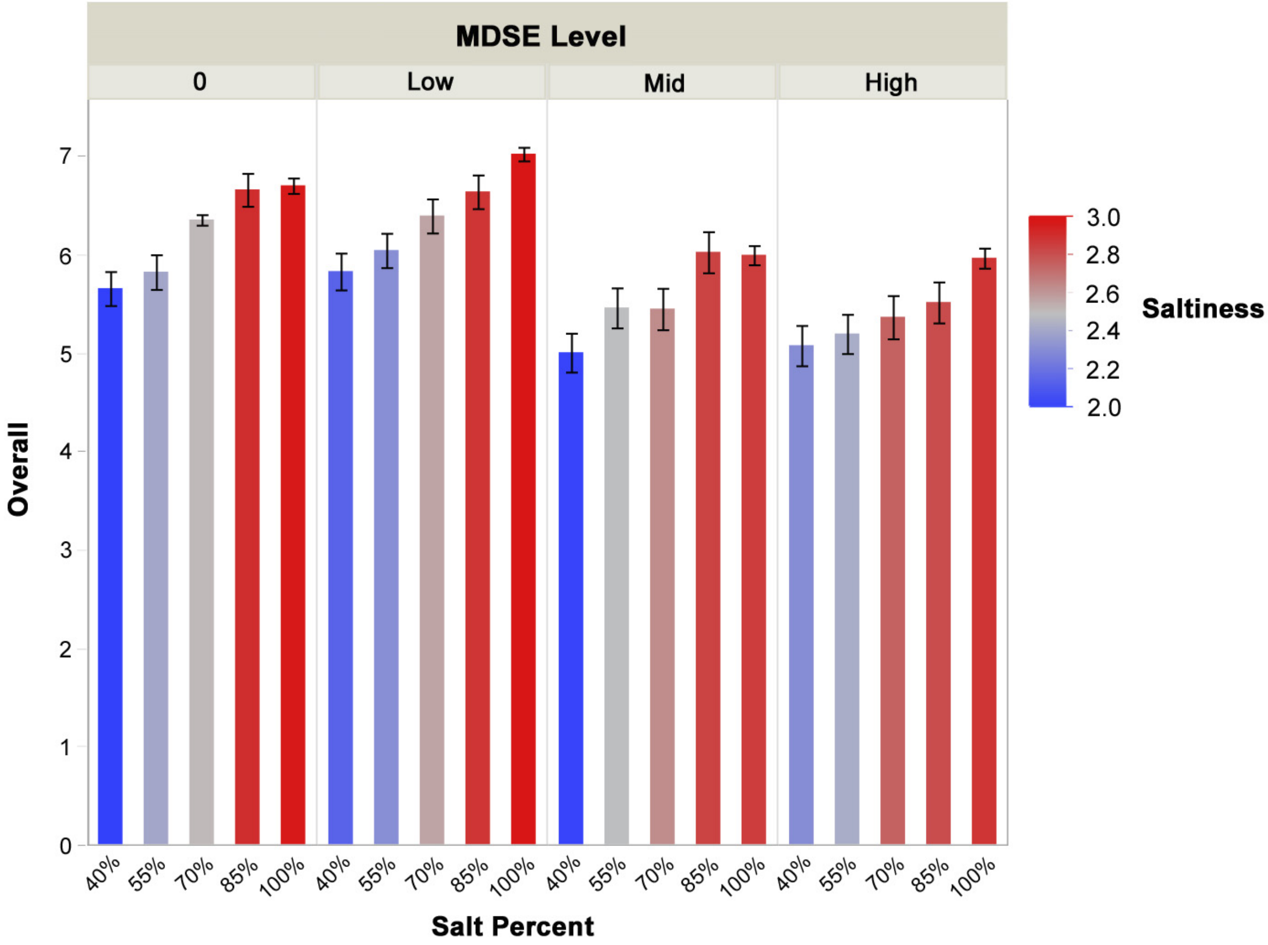

Figure 3. Mean overall liking scores for each condition of salt concentration and MDSE level derived from HTM.

Scores are overlaid with perceived saltiness intensity according to a JAR scale. As bars increase in red coloring, participants rated samples as saltier.

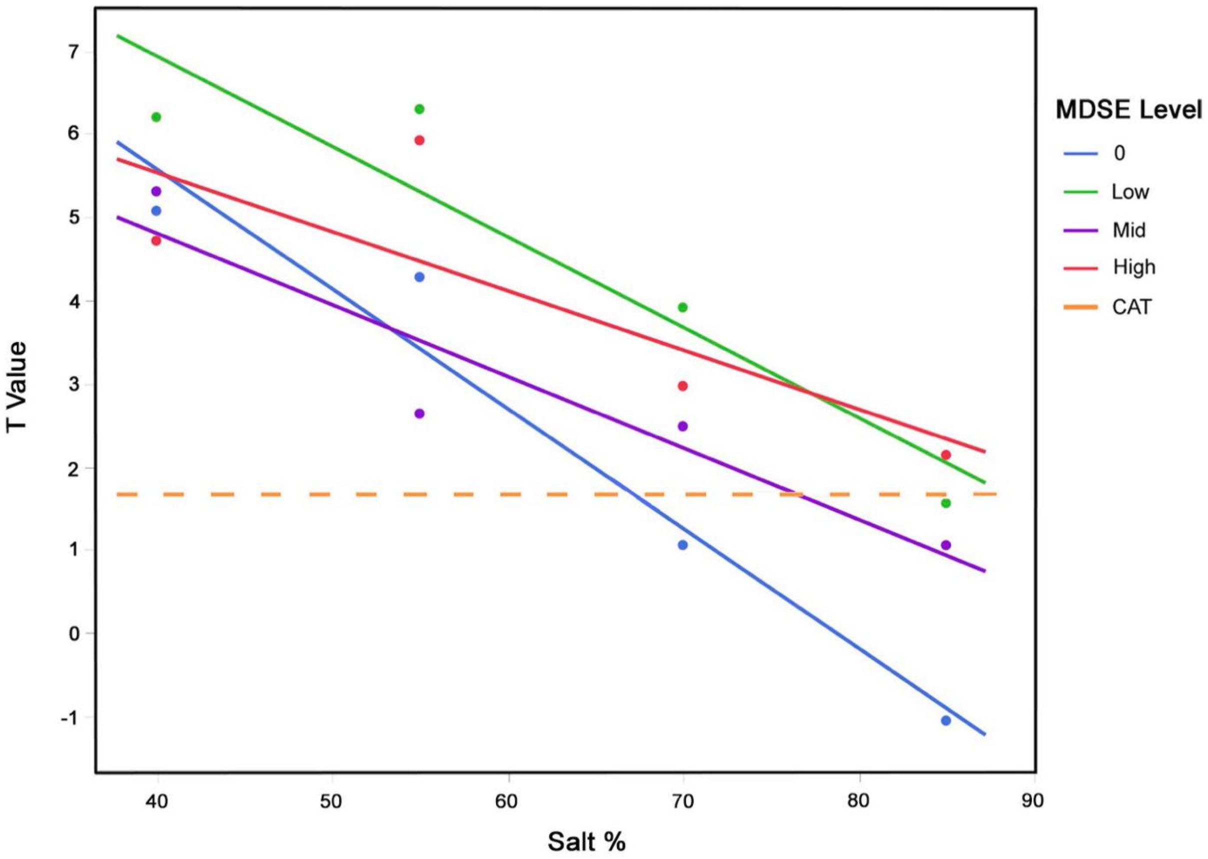

Figure 4. Linear regression analysis of t-value against salt reduction for each MDSE level as a means of determining the compromised acceptance threshold (CAT).

The dotted line corresponds with the t-value 1.664, the threshold beyond which acceptance is compromised.

High MDSE level received a significantly higher saltiness rating than the other MDSE levels ($F_{1,3175}$= 14.94, $p < .0001$). However, this effect is reversed at smaller reductions of sodium. In the 85% salt samples, the Low MDSE level was rated as saltier than the High MDSE level ($F_{1,240.4}$ = 10.04, $p = .0017$).

As seen in Figure 4, the relationship between t-value and salt concentration was linear for only two of the four levels of MDSE. In the samples with 0 MDSE and Low MDSE, there was a significant linear relationship between salt concentration and consumer acceptance ($p < 0.05$). Salt concentration accounted for 89.0% and 87.5% of the variation within overall liking for 0 MDSE and Low MDSE samples respectively (Table 2). With increased concentrations of MDSE, the relationship between salt concentration and acceptance became less clear, as the linear regression model for salt content and overall liking was not significant for either the Mid or High MDSE levels. Figure 5 depicts the variation in overall liking amongst participants through regression of R^2 as a function of MDSE level. The distribution of liking scores is concentrated in the upper range of the liking scale for the 0 and Low MDSE levels, whereas the scores for Mid and High MDSE levels are more dispersed. As MDSE level increased, the distribution of acceptance scores widened. The lower R^2 values that correspond with the higher levels of MDSE also suggest that less of the variation in liking can be attributed specifically to salt reduction. Decreased consumer acceptance in these samples is likely due to additional factors.

The linear regression equations found in Table 2 were used to calculate the compromised acceptance threshold for each level of MDSE by replacing the Y value with 1.664, i.e., the t-value that corresponds with an α of 0.05 and 80 degrees of freedom. The point at which the regression line for each MDSE level crossed this threshold signified the level of salt reduction at which acceptance was significantly altered. For the 0 MDSE samples, a 26.1% reduction in salt was

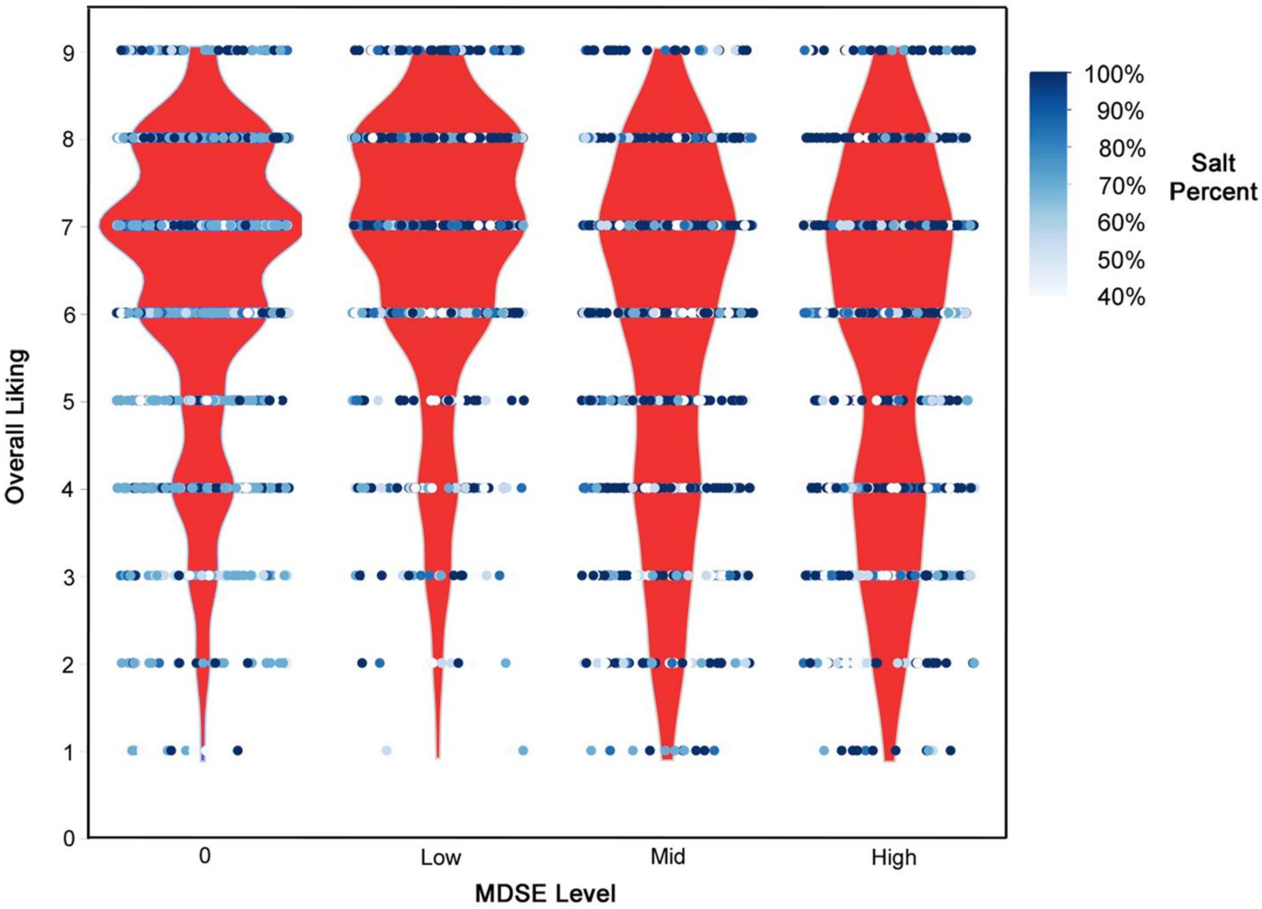

Figure 5. Regression of R^2 as a function of MDSE level.

Violin plots depict the variation in overall liking scores between participants for each MDSE level. Darker blue coloring corresponds with higher salt concentrations.

feasible without a change in acceptance. The Mid level of MDSE allowed for a comparable salt reduction at 23.5%. The Low MDSE and High MDSE samples achieved similar reductions in salt, at 6.8% and 6.7% respectively. Although the results of the CAT analysis are inconsistent, it should be noted that an ANOVA showed no significant difference between 0 MDSE samples with 100% salt and Low MDSE samples with 85% salt. This suggests that even at the Low level, the use of MDSE can compensate for a 15% reduction in salt.

To help us understand any differences between saltiness and overall liking, participants were asked to identify if they detected an off-flavor and provide an intensity score following positive identification. There was an inverse relationship between off-flavor intensity and overall liking ($F_{1,1134} = 289.83$, $p < .0001$). As off-flavor intensity increased, liking decreased (ß = -0.216, $p < .0001$). The off-flavors, as suspected, were most intense in the High and Mid MDSE samples. Where the off-flavor was the least detectable, in the Low MDSE sample, the acceptance scores were the greatest.

Discussion

This study demonstrated the saltiness enhancing effect of an MDSE in a complex, savory food matrix. Samples with higher concentrations of the MDSE were perceived as saltier than samples with lower concentrations or without any MDSE when comparing the same level of sodium. This is consistent with previous studies, which have indicated increased salty taste when MDSE was added to low-sodium chicken broth (Lopez et al., 2019). Naturally occurring umami ingredients have shown promise for enhancing saltiness by increasing perception of salty taste, specifically in meats (Dermiki et al., 2013). Within some of the MDSE levels, we saw a linear relationship between overall liking and salt reduction. As salt was reduced, consumer acceptance

Table 2. Linear models used to determine compromised acceptance threshold.

MDSE Level	Overall Liking	Slope	Y-Intercept	R^2 (Adj)	p-value
0	6.41[a]	-0.143	12.23	0.8898	**0.0374**
Low	6.60[a]	-0.115	12.38	0.8737	**0.0430**
Mid	5.72[b]	-0.089	8.47	0.7878	0.0734
High	5.63[b]	-0.078	8.94	0.5500	0.1635

Levels not connected by the same letter are significantly different.

Y = Calculated t-value used to determine CAT

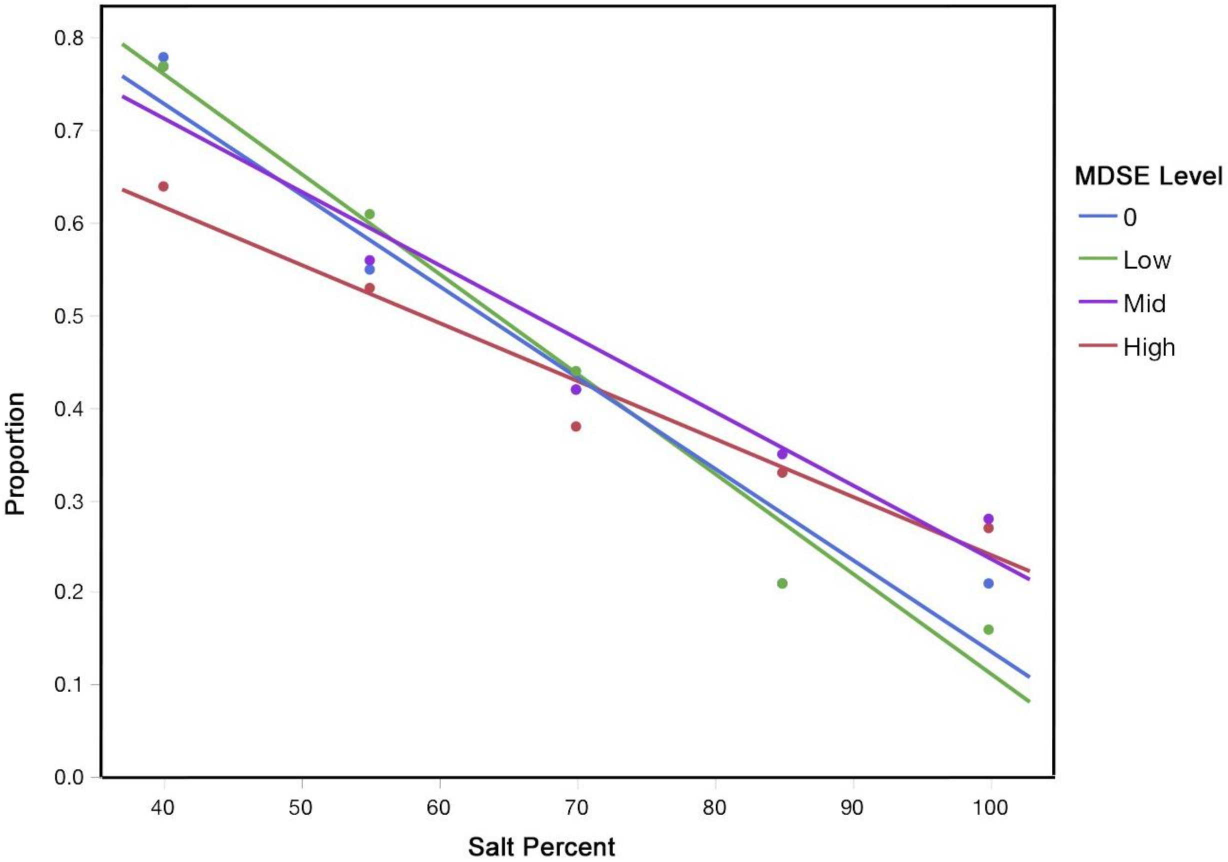

Figure 6. Linear regression analysis of perceived saltiness intensity.

The proportion of participants rating samples as “Not Salty Enough” according to a JAR scale is plotted against salt concentration.

declined. This was expected, as it is well substantiated that saltiness has a significant influence on hedonic response (Tuorila-Ollikainen et al., 1986). With taste being one of the most important influences for liking, large reductions in sodium generally correspond with reduced consumer acceptance (Liem et al., 2011).

However, other studies have suggested that the relationship between saltiness and acceptability is more complex and dependent on physiological, cultural, and environmental factors (Hayes et al., 2010). A meta-analysis examining the effect of sodium reduction on hedonic response did not find a linear relationship between acceptability and salt reduction (Jaenke et al., 2017). This same analysis suggested that the effectiveness of flavor enhancers is variable in processed meats, with few achieving comparable acceptance to their controls (Jaenke et al., 2017).

Consistent with this trend, the inclusion of the MDSE in the meatball formulation produced varying effects. Our hypothesis assumed that perceived saltiness would associate with consumer liking. The results of this experiment indicate otherwise. Although salty taste increased with the addition of MDSE, the enhancer did not appear to also improve consumer acceptance. Based on the linear regression analysis, larger reductions in salt concentration without compromising acceptance were feasible in the samples with no MDSE than with any other level of MDSE. The next largest reduction in salt possible was at the Mid level of MDSE.

There are several possible explanations for this effect. The samples with higher concentrations of MDSE may have imparted a negative off-flavor. Given the inverse relationship between off-flavor intensity and overall liking, perhaps as the off-flavor became more prominent in samples with higher concentrations of MDSE, the overall liking of the samples decreased. The off-flavor that was perceived at the higher MDSE concentrations was described as having a slightly burnt retronasal character. This off-flavor may have been generated during the thermal treatment of the mushroom protein with cysteine. To maximize the effectiveness of the MDSE, research is currently underway in our laboratory to determine the odorants responsible for the off-flavor, to determine the mechanism of their formation, and investigate methods to minimize its aversive effects. Doing so may allow for the use of MDSE at higher concentrations and in turn increase the feasibility of larger salt reductions.

As previously stated, hedonic response can be environmentally dependent. A study examining saltiness and liking of hash browns found that samples served monadically in a sensory booth setting were scored lower than the same samples included as a part of a lunch. When a single bite was consumed, as in a sequential monadic serving, sodium concentration had a much greater effect than when part of a meal (Lucas et al., 2011). In the present study, the inclusion of meatballs in a lunch setting with additional elements may have increased the overall liking. Other studies have considered the effect of companion foods on liking and the ability to discriminate sensory attributes (Nguyen & Wismer, 2020). The studied food pairs included regular and reduced-sodium samples of chips and salsa, cooked rice and soy sauce, and ketchup and tater tots. Meatballs, the food matrix examined in the current study, are generally consumed with pasta, rice, or at minimum, a sauce. Not only may serving of the meatball samples with an accompanying sauce have improved acceptability scores, it may also have allowed for larger reductions in salt without compromising acceptance.

The hedonic response to sodium reduction is suggested to be food specific (Hayes et al., 2010). For example, although cheddar cheese and snack foods have similar levels of sodium, saltiness has been found to predict liking for snack foods but not for cheddar cheese (Lucas et al., 2011). The food matrix used in this study, a beef-mushroom blend meatball, was not generally well-liked, particularly in comparison to the previously mentioned samples that have been used in other sodium-reduction studies. Using a different, more well-liked product with a savory profile may have yielded better acceptance results.

Lastly, there is a possibility that within the participant pool, there were segments of a population with different salt sensitivities. Recently, it has been shown that consumers who prefer foods with higher salt contents are more sensitive to reductions in sodium (Antúnez et al., 2019). Other salt reduction studies have seen two different groups of participants: those who rate full-salt samples with the highest liking scores and decrease liking as salt is reduced, and those who give low scores to full-salt samples, giving the highest ratings to samples with moderate sodium concentrations, which then again decline as sodium is further reduced. This segmentation of participants may account for some of the variation in consumer acceptance in the present study.

Our results highlight some limitations of the hedonic threshold methodology, as outlined by Lima Filho et al. (2015), to address issues that involve multiple variables. In their previous study determining the CAT in reduced-sodium hamburgers, Lima Filho et al. (2019) calculated high coefficients of determination for overall liking and each attribute of interest. Their study, however, manipulated only the amount of salt, with all other ingredient amounts held constant. Though they hypothesized that additional seasonings could lead to even larger reductions in sodium without compromising acceptance, the present study found that this practice can lead to inconsistent hedonic thresholds. Given the widespread use of sodium replacers and saltiness enhancers in reduced-salt food products, this method may not be appropriate for evaluating ingredient efficacy, as it obscures the ability to determine an optimal level of both salt and enhancer to achieve comparable consumer acceptance.

Conclusion

The use of an MDSE in a complex food matrix, such as a beef-mushroom blend meatball, can enhance the saltiness of the product. Hedonic threshold methodology showed 0 MDSE yielded the lowest compromised acceptance threshold, followed by Mid MDSE. The Low MDSE samples received the highest mean liking scores and were found to achieve a 15% reduction in salt with no significant difference in acceptance. However, increased saltiness perception does not necessarily correspond with increased consumer acceptance, as there are other factors involved with hedonic response. More specifically, higher concentrations of MDSE were found to impart a slightly burnt off-flavor, which was negatively associated with liking. The variability in our results indicates hedonic threshold methodology may not be the best method of determining optimal levels of sodium and other additives when consumer acceptability is widely variant.

CHAPTER III – Conclusions

A potential solution for attenuating flavor imbalance as a result of sodium reduction is utilizing sensory input through the different modalities to heighten taste perception. Because flavor is a combination of taste, orthonasal and retronasal olfaction, and trigeminal sensations, we focused on leveraging olfactory input. Mushrooms specifically are comprised of many savory odorants and tastants, some of which become more pronounced upon heat treatment and exposure to the Maillard reaction. When mushroom protein is enzymatically hydrolyzed and thermally treated (eHMP), it produces a reaction flavor that can enhance saltiness perception. This effect is heightened when cysteine is added (eHMP + cys), as a result of a combination of taste- and aroma-active savory compounds that contribute to umami taste and meaty flavor, both of which compensate for reduced sodium in chicken broth. An aroma model was developed from seven volatiles suspected of contributing most to the saltiness enhancing effect. This aroma model, which was intended to simulate eHMP + cys, compounded the saltiness enhancement of eHMP in a similar manner to eHMP + cys. This substantiates not only the saltiness enhancing effect of eHMP and eHMP + cys, but also that the compounds contributing to this effect are likely aroma-active, making this is an example of OISE.

The effectiveness of eHMP + cys, which is a mushroom-derived saltiness enhancer (MDSE), was assessed in a complex food matrix. In beef-mushroom blend meatballs, increased concentration of MDSE was associated with increased saltiness perception. Samples with Mid and High MDSE levels saw a smaller proportion of panelists provide “Not Salty Enough” ratings. However, this did not correspond with increased acceptance; samples with no or low levels of MDSE received significantly higher overall liking scores than samples with mid and high MDSE levels. These results may be due to the presence of a retronasal off-flavor of the MDSE, which becomes more pronounced at high concentrations, the intensity of which was inversely associated with liking. Although the off-flavor may have influenced the directness of the relationship between saltiness and hedonic response, it provides an opportunity for future research and to better improve the efficacy and application of the MDSE.

In addition to the off-flavor, inconsistent compromised acceptance thresholds complicated the determination of an optimal MDSE level. Although hedonic threshold methodology indicated that 0 MDSE and Mid MDSE samples yielded the largest feasible reduction in salt without altering consumer acceptance, Low MDSE samples received the greatest liking scores. An ANOVA further showed that there was no difference between control samples and 85% salt samples at the Low MDSE level, suggesting that a 15% reduction in salt is possible at the low concentration of MDSE. The variability in the CATs may be indicative of the limitations of HTM in terms of evaluating multiple variables, and therefore may not be an appropriate method in evaluating saltiness enhancers in sodium reduction studies. Overall, savory odorants derived from bioactive compounds provide a promising method of compensation for lack of saltiness in reduced-sodium foods.

REFERENCES

Andersen, L., Rasmussen, L. B., Larsen, E. H., & Jakobsen, J. (2009). Intake of household salt in a Danish population. *European Journal of Clinical Nutrition*, *63*(5), 598–604. https://doi.org/10.1038/ejcn.2008.18

Antúnez, L., Giménez, A., Alcaire, F., Vidal, L., & Ares, G. (2019). Consumers' heterogeneity towards salt reduction: Insights from a case study with white rice. *Food Research International*, *121*, 48–56. https://doi.org/10.1016/j.foodres.2019.03.007

Barnett, A., & Spence, C. (2016). Assessing the Effect of Changing a Bottled Beer Label on Taste Ratings. *Nutrition and Food Technology: Open Access*, *2*(4). https://doi.org/10.16966/2470-6086.132

Barringer, S. (2006). Coating Snack Foods. In *Handbook of Food Science, Technology, and Engineering* (Vol. 4, pp. 169-6-169–8). Taylor & Francis Group, LLC.

Bartoshuk, L. M., Sims, C. A., Colquhoun, T. A., & Snyder, D. J. (2019). What Aristotle didn't know about flavor. *The American Psychologist*, *74*(9), 1003–1011. https://doi.org/10.1037/amp0000577

Batenburg, M., & Velden, R. van der. (2011). Saltiness Enhancement by Savory Aroma Compounds. *Journal of Food Science*, *76*(5), S280–S288. https://doi.org/10.1111/j.1750-3841.2011.02198.x

Bernstein, A. M., & Willett, W. C. (2010). Trends in 24-h urinary sodium excretion in the United States, 1957–2003: A systematic review. *The American Journal of Clinical Nutrition*, *92*(5), 1172–1180. https://doi.org/10.3945/ajcn.2010.29367

Bidlas, E., & Lambert, R. J. W. (2008). Comparing the antimicrobial effectiveness of NaCl and KCl with a view to salt/sodium replacement. *International Journal of Food Microbiology*, *124*(1), 98–102. https://doi.org/10.1016/j.ijfoodmicro.2008.02.031

Brandsma, I. (2006). Reducing sodium a European perspective. *Food Technology*, *60*, 24–29.

Burnett, R. S. (1951). Soybean protein food products. *K. S. Markley, Ed. Soybeans and Soybean Products.*, *2*, 949–1002.

Busch, J. L. H. C., Yong, F. Y. S., & Goh, S. M. (2013). Sodium reduction: Optimizing product composition and structure towards increasing saltiness perception. *Trends in Food Science & Technology*, *29*(1), 21–34. https://doi.org/10.1016/j.tifs.2012.08.005

Çakır, E., & Foegeding, E. A. (2011). Combining protein micro-phase separation and protein–polysaccharide segregative phase separation to produce gel structures. *Food Hydrocolloids*, *25*(6), 1538–1546. https://doi.org/10.1016/j.foodhyd.2011.02.002

Campagnol, P. C. B., dos Santos, B. A., Wagner, R., Terra, N. N., & Pollonio, M. A. R. (2011). The effect of yeast extract addition on quality of fermented sausages at low NaCl content. *Meat Science*, *87*(3), 290–298. https://doi.org/10.1016/j.meatsci.2010.11.005

Chaudhari, N., & Roper, S. D. (2010). The cell biology of taste. *The Journal of Cell Biology*, *190*(3), 285–296. https://doi.org/10.1083/jcb.201003144

Chen, X., Yu, J., Cui, H., Xia, S., Zhang, X., & Yang, B. (2018). Effect of Temperature on Flavor Compounds and Sensory Characteristics of Maillard Reaction Products Derived from Mushroom Hydrolysate. *Molecules*, *23*(2), 247. https://doi.org/10.3390/molecules23020247

Christensen, C. M. (1980). Effects of solution viscosity on perceived saltiness and sweetness. *Perception & Psychophysics*, *28*(4), 347–353. https://doi.org/10.3758/BF03204394

Cliff, M., & Noble, A. C. (1990). Time-Intensity Evaluation of Sweetness and Fruitiness and Their Interaction in a Model Solution. *Journal of Food Science*, *55*(2), 450–454. https://doi.org/10.1111/j.1365-2621.1990.tb06784.x

Cook, D. J., Hollowood, T. A., Linforth, R. S. T., & Taylor, A. J. (2003). Oral shear stress predicts flavour perception in viscous solutions. *Chemical Senses*, *28*(1), 11–23. https://doi.org/10.1093/chemse/28.1.11

de Loubens, C., Panouillé, M., Saint-Eve, A., Déléris, I., Tréléa, I. C., & Souchon, I. (2011). Mechanistic model of in vitro salt release from model dairy gels based on standardized breakdown test simulating mastication. *Journal of Food Engineering*, *105*(1), 161–168. https://doi.org/10.1016/j.jfoodeng.2011.02.020

Dermiki, M., Mounayar, R., Suwankanit, C., Scott, J., Kennedy, O. B., Mottram, D. S., Gosney, M. A., Blumenthal, H., & Methven, L. (2013). Maximising umami taste in meat using natural ingredients: Effects on chemistry, sensory perception and hedonic liking in young and old consumers. *Journal of the Science of Food and Agriculture*, *93*(13), 3312–3321. https://doi.org/10.1002/jsfa.6177

Dietary Guidelines for Americans 2015-2020 (No. 8). (2015). United States Department of Agriculture.

Dijksterhuis, G., Boucon, C., & Le Berre, E. (2014). Increasing saltiness perception through perceptual constancy created by expectation. *Food Quality and Preference*, *34*, 24–28. https://doi.org/10.1016/j.foodqual.2013.12.003

Djordjevic, J., Zatorre, R. J., & Jones-Gotman, M. (2004a). Effects of Perceived and Imagined Odors on Taste Detection. *Chemical Senses*, *29*(3), 199–208. https://doi.org/10.1093/chemse/bjh022

Djordjevic, J., Zatorre, R. J., & Jones-Gotman, M. (2004b). Odor-induced changes in taste perception. *Experimental Brain Research*, *159*(3), 405–408. https://doi.org/10.1007/s00221-004-2103-y

Dötsch, M., Busch, J., Batenburg, M., Liem, G., Tareilus, E., Mueller, R., & Meijer, G. (2009). Strategies to Reduce Sodium Consumption: A Food Industry Perspective. *Critical Reviews in Food Science and Nutrition*, *49*(10), 841–851. https://doi.org/10.1080/10408390903044297

Doyle, M. E., & Glass, K. A. (2010). Sodium Reduction and Its Effect on Food Safety, Food Quality, and Human Health. *Comprehensive Reviews in Food Science and Food Safety*, *9*(1), 44–56. https://doi.org/10.1111/j.1541-4337.2009.00096.x

Emorine, M., Septier, C., Thomas-Danguin, T., & Salles, C. (2013). Heterogeneous salt distribution in hot snacks enhances saltiness without loss of acceptability. *Food Research International*, *51*(2), 641–647. https://doi.org/10.1016/j.foodres.2013.01.006

Frank, R. A., & Byram, J. (1988). Taste–smell interactions are tastant and odorant dependent. *Chemical Senses*, *13*(3), 445–455. https://doi.org/10.1093/chemse/13.3.445

Galindo, M. M., Voigt, N., Stein, J., van Lengerich, J., Raguse, J.-D., Hofmann, T., Meyerhof, W., & Behrens, M. (2012). G Protein–Coupled Receptors in Human Fat Taste Perception. *Chemical Senses*, *37*(2), 123–139. https://doi.org/10.1093/chemse/bjr069

Grummer, J., Karalus, M., Zhang, K., Vickers, Z., & Schoenfuss, T. C. (2012). Manufacture of reduced-sodium Cheddar-style cheese with mineral salt replacers. *Journal of Dairy Science*, *95*(6), 2830–2839. https://doi.org/10.3168/jds.2011-4851

Guideline: Sodium Intake for Adults and Children. (2012). World Health Organization.

Guinard, J.-X., Myrdal Miller, A., Mills, K., Wong, T., Lee, S. M., Sirimuangmoon, C., Schaefer, S. E., & Drescher, G. (2016). Consumer acceptance of dishes in which beef has been partially substituted with mushrooms and sodium has been reduced. *Appetite*, *105*, 449–459. https://doi.org/10.1016/j.appet.2016.06.018

Ha, S. K. (2014). Dietary Salt Intake and Hypertension. *Electrolytes & Blood Pressure : E & BP*, *12*(1), 7–18. https://doi.org/10.5049/EBP.2014.12.1.7

Halim, J., Bouzari, A., Felder, D., & Guinard, J.-X. (n.d.). The Salt Flip: Sensory mitigation of salt (and sodium) reduction with monosodium glutamate (MSG) in "Better-for-You" foods. *Journal of Food Science*, *n/a*(n/a). https://doi.org/10.1111/1750-3841.15354

Hayes, J. E., Sullivan, B. S., & Duffy, V. B. (2010). Explaining variability in sodium intake through oral sensory phenotype, salt sensation and liking. *Physiology & Behavior*, *100*(4), 369–380. https://doi.org/10.1016/j.physbeh.2010.03.017

Heidolph. (2011). Looking for My Lost Shaker of Salt…Replacer: Flavor, Function, Future. *Cereal Foods World*. https://doi.org/10.1094/CFW-56-1-0005

Hellemann, U. (1992). Perceived taste of NaCl and acid mixtures in water and bread. *International Journal of Food Science & Technology*, *27*(2), 201–211. https://doi.org/10.1111/j.1365-2621.1992.tb01196.x

Henney, J. E., Taylor, C. L., & Boon, C. S. (2010). Taste and Flavor Roles of Sodium in Foods: A Unique Challenge to Reducing Sodium Intake. In *Strategies to Reduce Sodium Intake in the United States*. National Academies Press (US). https://www.ncbi.nlm.nih.gov/books/NBK50958/

Hooper, L., Bartlett, C., Smith, G. D., & Ebrahim, S. (2002). Systematic review of long term effects of advice to reduce dietary salt in adults. *BMJ*, *325*(7365), 628. https://doi.org/10.1136/bmj.325.7365.628

Hoppu, U., Hopia, A., Pohjanheimo, T., Rotola-Pukkila, M., Mäkinen, S., Pihlanto, A., & Sandell, M. (2017). Effect of Salt Reduction on Consumer Acceptance and Sensory Quality of Food. *Foods*, *6*(12), 103. https://doi.org/10.3390/foods6120103

Israr, T., Rakha, A., Sohail, M., Rashid, S., & Shehzad, A. (2016). Salt reduction in baked products: Strategies and constraints. *Trends in Food Science & Technology*, *51*, 98–105. https://doi.org/10.1016/j.tifs.2016.03.002

Jaenke, R., Barzi, F., McMahon, E., Webster, J., & Brimblecombe, J. (2017). Consumer acceptance of reformulated food products: A systematic review and meta-analysis of salt-reduced foods. *Critical Reviews in Food Science and Nutrition*, *57*(16), 3357–3372. https://doi.org/10.1080/10408398.2015.1118009

Jinap, S., Hajeb, P., Karim, R., Norliana, S., Yibadatihan, S., & Abdul-Kadir, R. (2016). Reduction of sodium content in spicy soups using monosodium glutamate. *Food & Nutrition Research*, *60*(1), 30463. https://doi.org/10.3402/fnr.v60.30463

Karanja, N., Lancaster, K. J., Vollmer, W. M., Lin, P.-H., Most, M. M., Ard, J. D., Swain, J. F., Sacks, F. M., & Obarzanek, E. (2007). Acceptability of Sodium-Reduced Research Diets, Including the Dietary Approaches to Stop Hypertension Diet, among Adults with Prehypertension and Stage 1 Hypertension. *Journal of the American Dietetic Association*, *107*(9), 1530–1538. https://doi.org/10.1016/j.jada.2007.06.013

Keast, R. S. J., & Breslin, P. A. S. (2002). Modifying the Bitterness of Selected Oral Pharmaceuticals with Cation and Anion Series of Salts. *Pharmaceutical Research*, *19*(7), 1019–1026. https://doi.org/10.1023/A:1016474607993

Keast, R. S. J., & Breslin, P. A. S. (2003). An overview of binary taste–taste interactions. *Food Quality and Preference*, *14*(2), 111–124. https://doi.org/10.1016/S0950-3293(02)00110-6

Keast, R. S. J., Dalton, P. H., & Breslin, P. A. S. (2007). Flavor Interactions at the Sensory Level. In *Flavor Perception* (pp. 228–255). John Wiley & Sons, Ltd. https://doi.org/10.1002/9780470995716.ch8

Keast, R. S. J., & Roper, J. (2007). A Complex Relationship among Chemical Concentration, Detection Threshold, and Suprathreshold Intensity of Bitter Compounds. *Chemical Senses*, *32*(3), 245–253. https://doi.org/10.1093/chemse/bjl052

Kilcast, D., & den Ridder, C. (2007). 10—Sensory issues in reducing salt in food products. In David Kilcast & F. Angus (Eds.), *Reducing Salt in Foods* (pp. 201–220). Woodhead Publishing. https://doi.org/10.1533/9781845693046.2.201

Koliandris, A., Lee, A., Ferry, A.-L., Hill, S., & Mitchell, J. (2008). Relationship between structure of hydrocolloid gels and solutions and flavour release. *Food Hydrocolloids*, *22*(4), 623–630. https://doi.org/10.1016/j.foodhyd.2007.02.009

Koliandris, A.-L., Morris, C., Hewson, L., Hort, J., Taylor, A. J., & Wolf, B. (2010). Correlation between saltiness perception and shear flow behaviour for viscous solutions. *Food Hydrocolloids*, *24*(8), 792–799. https://doi.org/10.1016/j.foodhyd.2010.04.006

Konitzer, K., Pflaum, T., Oliveira, P., Arendt, E., Koehler, P., & Hofmann, T. (2013). Kinetics of Sodium Release from Wheat Bread Crumb As Affected by Sodium Distribution. *Journal* *of Agricultural and Food Chemistry, 61*(45), 10659–10669. https://doi.org/10.1021/jf404458v

Kremer, S., Mojet, J., & Shimojo, R. (2009). Salt Reduction in Foods Using Naturally Brewed Soy Sauce. *Journal of Food Science, 74*(6), S255–S262. https://doi.org/10.1111/j.1750-3841.2009.01232.x

Kuo, W.-Y., & Lee, Y. (2014). Effect of Food Matrix on Saltiness Perception—Implications for Sodium Reduction. *Comprehensive Reviews in Food Science and Food Safety, 13*(5), 906–923. https://doi.org/10.1111/1541-4337.12094

Labruni, T., Henry, S., Affolter, M., & Schlichterle-Cerny, H. (2003). *Seasoning Compositions*.

Lawless, H. T., & Heymann, H. (1999). *Sensory Evaluation of Food: Principles and Practices*. Springer Science & Business Media.

Lawless, H. T., Rapacki, F., Horne, J., & Hayes, A. (2003). The taste of calcium and magnesium salts and anionic modifications. *Food Quality and Preference, 14*(4), 319–325. https://doi.org/10.1016/S0950-3293(02)00128-3

Lawrence, G., Salles, C., Septier, C., Busch, J., & Thomas-Danguin, T. (2009). Odour–taste interactions: A way to enhance saltiness in low-salt content solutions. *Food Quality and Preference*, *20*(3), 241–248. https://doi.org/10.1016/j.foodqual.2008.10.004

Lewandowski, B. C., Sukumaran, S. K., Margolskee, R. F., & Bachmanov, A. A. (2016). Amiloride-Insensitive Salt Taste Is Mediated by Two Populations of Type III Taste Cells with Distinct Transduction Mechanisms. *Journal of Neuroscience, 36*(6), 1942–1953. https://doi.org/10.1523/JNEUROSCI.2947-15.2016

Liem, Djin G. (2017). Infants’ and Children’s Salt Taste Perception and Liking: A Review. *Nutrients, 9*(9), 1011. https://doi.org/10.3390/nu9091011

Liem, Djin Gie, Miremadi, F., & Keast, R. S. J. (2011). Reducing Sodium in Foods: The Effect on Flavor. *Nutrients*, *3*(6), 694–711. https://doi.org/10.3390/nu3060694

Lim, J., & Green, B. G. (2008). Tactile Interaction with Taste Localization: Influence of Gustatory Quality and Intensity. *Chemical Senses*, *33*(2), 137–143. https://doi.org/10.1093/chemse/bjm070

Lima Filho, T., Della Lucia, S. M., Minim, L. A., Gamba, M. M., Lima, R. M., & Minim, V. P. R. (2019). Directional hedonic thresholds for sodium concentration in hamburger. *Food Quality and Preference*, *78*, 103722. https://doi.org/10.1016/j.foodqual.2019.103722

Lima Filho, T., Minim, V. P. R., Silva, R. de C. dos S. N. da, Della Lucia, S. M., & Minim, L. A. (2015). Methodology for determination of two new sensory thresholds: Compromised acceptance threshold and rejection threshold. *Food Research International*, *76*, 561–566. https://doi.org/10.1016/j.foodres.2015.07.037

Little, A. C., & Brinner, L. (1984). Taste responses to saltiness of experimentally prepared tomato juice samples. *Journal of the American Dietetic Association*, *84*(9), 1022–1027.

Lopez, J., Kerley, T., Jenkinson, L., Luckett, C. R., & Munafo, J. P. (2019). Odorants from the Thermal Treatment of Hydrolyzed Mushroom Protein and Cysteine Enhance Saltiness Perception. *Journal of Agricultural and Food Chemistry*, *67*(41), 11444–11453. https://doi.org/10.1021/acs.jafc.9b04153

Lucas, L., Riddell, L., Liem, G., Whitelock, S., & Keast, R. (2011). The Influence of Sodium on Liking and Consumption of Salty Food. *Journal of Food Science*, *76*(1), S72–S76. https://doi.org/10.1111/j.1750-3841.2010.01939.x

Luckett, C. R., Meullenet, J.-F., & Seo, H.-S. (2016). Crispness level of potato chips affects temporal dynamics of flavor perception and mastication patterns in adults of different age groups. *Food Quality and Preference*, *51*, 8–19. https://doi.org/10.1016/j.foodqual.2016.02.013

Manabe, M., Ishizaki, S., Yoshioka, T., & Oginome, N. (2009). Improving the Palatability of Salt-Reduced Food Using Dried Bonito Stock. *Journal of Food Science*, *74*(7), S315–S321. https://doi.org/10.1111/j.1750-3841.2009.01283.x

McCaughey, S. A. (2019). 2 - Dietary salt and flavour: Mechanisms of taste perception and physiological controls. In C. Beeren, K. Groves, & P. M. Titoria (Eds.), *Reducing Salt in Foods (Second Edition)* (pp. 45–70). Woodhead Publishing. https://doi.org/10.1016/B978-0-08-100890-4.00002-0

Miller, A. M., Mills, K., Wong, T., Drescher, G., Lee, S. M., Sirimuangmoon, C., Schaefer, S., Langstaff, S., Minor, B., & Guinard, J.-X. (2014). Flavor-Enhancing Properties of Mushrooms in Meat-Based Dishes in Which Sodium Has Been Reduced and Meat Has Been Partially Substituted with Mushrooms. *Journal of Food Science*, *79*(9), S1795–S1804. https://doi.org/10.1111/1750-3841.12549

Mills, T., Spyropoulos, F., Norton, I. T., & Bakalis, S. (2011). Development of an in-vitro mouth model to quantify salt release from gels. *Food Hydrocolloids*, *25*(1), 107–113. https://doi.org/10.1016/j.foodhyd.2010.06.001

Moritaka, H., & Naito, S. (2002). Agar and Gelatin Gel Flavor Release. *Journal of Texture Studies*, *33*(3), 201–214. https://doi.org/10.1111/j.1745-4603.2002.tb01345.x

Mosca, A. C., Velde, F. van de, Bult, J. H. F., van Boekel, M. A. J. S., & Stieger, M. (2010). Enhancement of sweetness intensity in gels by inhomogeneous distribution of sucrose. *Food Quality and Preference*, *21*(7), 837–842. https://doi.org/10.1016/j.foodqual.2010.04.010

Moskowitz, H. R., & Arabie, P. (1970). Taste Intensity as a Function of Stimulus Concentration and Solvent Viscosity. *Journal of Texture Studies*, *1*(4), 502–510. https://doi.org/10.1111/j.1745-4603.1970.tb00748.x

Mueller, E., Koehler, P., & Scherf, K. A. (2016). Applicability of salt reduction strategies in pizza crust. *Food Chemistry*, *192*, 1116–1123. https://doi.org/10.1016/j.foodchem.2015.07.066

Nasri, N., Beno, N., Septier, C., Salles, C., & Thomas-Danguin, T. (2011). Cross-modal interactions between taste and smell: Odour-induced saltiness enhancement depends on salt level. *Food Quality and Preference*, *22*(7), 678–682. https://doi.org/10.1016/j.foodqual.2011.05.001

Neff, R. A., Edwards, D., Palmer, A., Ramsing, R., Righter, A., & Wolfson, J. (2018). Reducing meat consumption in the USA: A nationally representative survey of attitudes and behaviours. *Public Health Nutrition*, *21*(10), 1835–1844. https://doi.org/10.1017/S1368980017004190

Nguyen, H., & Wismer, W. V. (2020). The influence of companion foods on sensory attribute perception and liking of regular and sodium-reduced foods. *Journal of Food Science*, *85*(4), 1274–1284. https://doi.org/10.1111/1750-3841.15118

Noort, M. W. J., Bult, J. H. F., Stieger, M., & Hamer, R. J. (2010). Saltiness enhancement in bread by inhomogeneous spatial distribution of sodium chloride. *Journal of Cereal Science*, *52*(3), 378–386. https://doi.org/10.1016/j.jcs.2010.06.018

Ogasawara, Y., Mochimaru, S., Ueda, R., Ban, M., Kabuto, S., & Abe, K. (2016). Preparation of an Aroma Fraction from Dried Bonito by Steam Distillation and Its Effect on

Modification of Salty and Umami Taste Qualities. *Journal of Food Science, 81*(2), C308–C316. https://doi.org/10.1111/1750-3841.13194

Pangborn, R. M., Trabue, I. M., & Szczesniak, A. S. (1973). Effect of Hydrocolloids on Oral Viscosity and Basic Taste Intensities *. *Journal of Texture Studies*, *4*(2), 224–241. https://doi.org/10.1111/j.1745-4603.1973.tb00666.x

Parise, C. V., & Spence, C. (2009). ‘When Birds of a Feather Flock Together’: Synesthetic Correspondences Modulate Audiovisual Integration in Non-Synesthetes. *PLOS ONE, 4*(5), e5664. https://doi.org/10.1371/journal.pone.0005664

Patinho, I., Saldaña, E., Selani, M. M., de Camargo, A. C., Merlo, T. C., Menegali, B. S., de Souza Silva, A. P., & Contreras-Castillo, C. J. (2019). Use of Agaricus bisporus mushroom in beef burgers: Antioxidant, flavor enhancer and fat replacing potential. *Food Production, Processing and Nutrition*, *1*(1), 7. https://doi.org/10.1186/s43014-019-0006-3

Pearson, A. M. (1976). Meat Extenders and Substitutes. *BioScience*, *26*(4), 249–256. https://doi.org/10.2307/1297347

Pflaum, T., Konitzer, K., Hofmann, T., & Koehler, P. (2013). Influence of Texture on the Perception of Saltiness in Wheat Bread. *Journal of Agricultural and Food Chemistry*, *61*(45), 10649–10658. https://doi.org/10.1021/jf403304y

Phat, C., Moon, B., & Lee, C. (2016). Evaluation of umami taste in mushroom extracts by chemical analysis, sensory evaluation, and an electronic tongue system. *Food Chemistry*, *192*, 1068–1077. https://doi.org/10.1016/j.foodchem.2015.07.113

Pilic, L., Lubasinski, N. J., Berk, M., Ward, D., Graham, C. A.-M., Da Silva Anastacio, V., King, A., & Mavrommatis, Y. (2020). The associations between genetics, salt taste perception and salt intake in young adults. *Food Quality and Preference*, *84*, 103954. https://doi.org/10.1016/j.foodqual.2020.103954

Piqueras-Fiszman, B., & Spence, C. (2011). Crossmodal correspondences in product packaging. Assessing color–flavor correspondences for potato chips (crisps). *Appetite*, *57*(3), 753–757. https://doi.org/10.1016/j.appet.2011.07.012

Reddy, K. A., & Marth, E. H. (1991). Reducing the Sodium Content of Foods: A Review. *Journal of Food Protection*, *54*(2), 138–150. https://doi.org/10.4315/0362-028X-54.2.138

Rolls, E. T. (2000). The Orbitofrontal Cortex and Reward. *Cerebral Cortex*, *10*(3), 284–294. https://doi.org/10.1093/cercor/10.3.284

Roper, S. D. (2013). Taste buds as peripheral chemosensory processors. *Seminars in Cell & Developmental Biology*, *24*(1), 71–79. https://doi.org/10.1016/j.semcdb.2012.12.002

Roper, S. D. (2015a). The taste of table salt. *Pflugers Archiv : European Journal of Physiology*, *467*(3), 457–463. https://doi.org/10.1007/s00424-014-1683-z

Roper, S. D. (2015b). The taste of table salt. *Pflügers Archiv - European Journal of Physiology*, *467*(3), 457–463. https://doi.org/10.1007/s00424-014-1683-z

Roper, S. D. (2015c). The taste of table salt. *Pflügers Archiv - European Journal of Physiology*, *467*(3), 457–463. https://doi.org/10.1007/s00424-014-1683-z

Rosett, T. R., Kendregan, S. L., Gao, Y., Schmidt, S. J., & Klein, B. P. (1996). Thickening agents effects on sodium binding and other taste qualities of soup systems. *Journal of Food Science*, *61*(5), 1099–1104. https://doi.org/10.1111/j.1365-2621.1996.tb10939.x

Rotola-Pukkila, M. K., Pihlajaviita, S. T., Kaimainen, M. T., & Hopia, A. I. (2015). Concentration of Umami Compounds in Pork Meat and Cooking Juice with Different

Cooking Times and Temperatures. *Journal of Food Science*, *80*(12), C2711–C2716. https://doi.org/10.1111/1750-3841.13127

Rotola-Pukkila, M., Yang, B., & Hopia, A. (2019). The effect of cooking on umami compounds in wild and cultivated mushrooms. *Food Chemistry*, *278*, 56–66. https://doi.org/10.1016/j.foodchem.2018.11.044

Schindler, A., Dunkel, A., Stähler, F., Backes, M., Ley, J., Meyerhof, W., & Hofmann, T. (2011). Discovery of Salt Taste Enhancing Arginyl Dipeptides in Protein Digests and Fermented Fish Sauces by Means of a Sensomics Approach. *Journal of Agricultural and Food Chemistry*, *59*(23), 12578–12588. https://doi.org/10.1021/jf2041593

Slocombe, B. G., Carmichael, D. A., & Simner, J. (2016). Cross-modal tactile–taste interactions in food evaluations. *Neuropsychologia*, *88*, 58–64. https://doi.org/10.1016/j.neuropsychologia.2015.07.011

Small, D. M. (2012). Flavor is in the brain. *Physiology & Behavior*, *107*(4), 540–552. https://doi.org/10.1016/j.physbeh.2012.04.011

Small, D. M., & Prescott, J. (2005). Odor/taste integration and the perception of flavor. *Experimental Brain Research*, *166*(3–4), 345–357. https://doi.org/10.1007/s00221-005-2376-9

Sodium and the Dietary Guidelines. (2017). Centers for Disease Control and Prevention.

Spence, C. (2011). Crossmodal correspondences: A tutorial review. *Attention, Perception, & Psychophysics*, *73*(4), 971–995. https://doi.org/10.3758/s13414-010-0073-7

Spence, C. (2015). Multisensory Flavor Perception. *Cell*, *161*(1), 24–35. https://doi.org/10.1016/j.cell.2015.03.007

Stevenson, R. J. (1999). Confusing Tastes and Smells: How Odours can Influence the Perception of Sweet and Sour Tastes. *Chemical Senses*, *24*(6), 627–635. https://doi.org/10.1093/chemse/24.6.627

Stieger, M. A., Hamer, R. H., Bult, J. H. F., & Graaf, C. de. (2009). *Food products with inhomogeneous tastant bulk distribution and method for making such products*. https://research.wur.nl/en/publications/food-products-with-inhomogeneous-tastant-bulk-distribution-and-me

Sun, L., Zhang, Z., Xin, G., Sun, B., Bao, X., Wei, Y., Zhao, X., & Xu, H. (2020). Advances in umami taste and aroma of edible mushrooms. *Trends in Food Science & Technology*, *96*, 176–187. https://doi.org/10.1016/j.tifs.2019.12.018

Szczesniak, A. S. (2002). Texture is a sensory property. *Food Quality and Preference*, *13*(4), 215–225. https://doi.org/10.1016/S0950-3293(01)00039-8

Temussi, P. A. (2012). The good taste of peptides. *Journal of Peptide Science*, *18*(2), 73–82. https://doi.org/10.1002/psc.1428

Tournier, C., Sulmont-Rossé, C., & Guichard, E. (2007). *Flavour perception: Aroma, taste and texture interactions*. *1*(2). https://hal.inrae.fr/hal-02823959

Tuorila-Ollikainen, H., Salovaara, H., & Kurkela, R. (1986). Effect of saltiness on the liking and consumption of bread and butter. *Ecology of Food and Nutrition*, *18*(2), 99–106. https://doi.org/10.1080/03670244.1986.9990916

USDA National Nutrient Database. (2020). https://fdc.nal.usda.gov/

Van Der Klaauw, N. J., & Smith, D. V. (1995). Taste quality profiles for fifteen organic and inorganic salts. *Physiology & Behavior*, *58*(2), 295–306. https://doi.org/10.1016/0031-9384(95)00056-O

Vandenbeuch, A., Clapp, T. R., & Kinnamon, S. C. (2008). Amiloride-sensitive channels in type I fungiform taste cells in mouse. *BMC Neuroscience*, *9*(1), 1. https://doi.org/10.1186/1471-2202-9-1

Wilkie, L. M., & Capaldi Phillips, E. D. (2014). Heterogeneous binary interactions of taste primaries: Perceptual outcomes, physiology, and future directions. *Neuroscience & Biobehavioral Reviews*, *47*, 70–86. https://doi.org/10.1016/j.neubiorev.2014.07.015

Wilson, R., Komitopoulou, D. E., & Incles, M. (2012). *Evaluation of Technological Approaches to Salt Reduction*. Food and Drink Federation (FDF) and British Retail Consortium (BRC).

Yamamoto, Y., & Nakabayashi, M. (1999). Enhancing Effect of an Oil Phase on the Sensory Intensity of Salt Taste of Nacl in Oil/Water Emulsions. *Journal of Texture Studies*, *30*(5), 581–590. https://doi.org/10.1111/j.1745-4603.1999.tb01409.x

Zhou, T., Feng, Y., Thomas-Danguin, T., & Zhao, M. (2021). Enhancement of saltiness perception by odorants selected from Chinese soy sauce: A gas chromatography/olfactometry-associated taste study. *Food Chemistry, 335*, 127664. https://doi.org/10.1016/j.foodchem.2020.127664

VITA

Lindsay Jenna Jenkinson was born in Mount Kisco, New York to parents Brian and Evelyn Jenkinson. She was raised in Bethel, Connecticut with her brother, Brett. After high school, she attended Clemson University and earned a Bachelor of Science degree in Food Science and Technology with a minor in Chemistry. Following her undergraduate studies, Lindsay completed an internship with Bimbo Bakeries USA in Analytical Services. She then chose to pursue a Master's degree in Food Science with a minor in Statistics at the University of Tennessee, Knoxville. Lindsay's research interests focus on various sensory methodologies and leveraging cross-modal interactions to aid sodium reduction initiatives in the food industry.