University of São Paulo
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Development and application of flavor enhancer ingredient from shiitake mushroom (*Lentinula edodes*) byproducts

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Thesis presented to obtain the degree of Doctor in Science.
Area: Food Science and Technology

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Summary

RESUMO

Desenvolvimento e aplicação de ingrediente realçador de sabor obtido de subprodutos de cogumelo shitake (*Lentinula edodes*)

A redução do consumo de sódio é uma das preocupações da atualidade, já que a alta ingestão desse micronutriente pode levar ao desenvolvimento de problemas cardiovasculares e hipertensão. Concomitantemente a isso, cresce a demanda por alimentos com menor/ou sem a adição de aditivos sintéticos, alinhada ao movimento *clean label*. Frente a esse panorama, faz-se necessária a pesquisa por novos ingredientes de origem natural que tenham potencial para substituir ou reduzir o uso de aditivos convencionais em alimentos. A utilização de cogumelos como realçadores de sabor em formulações com redução de sódio já vem sendo pesquisada. Contudo, estudos sobre o aproveitamento dos subprodutos da produção de cogumelos (estipes), com este mesmo objetivo, ainda são escassos. Sendo assim, este trabalho teve como objetivo principal o desenvolvimento de um ingrediente em pó rico em compostos umami, obtido a partir de subprodutos (estipes) de cogumelo shitake (*Lentinula edodes*). Para otimizar o processo de extração dos compostos umami foi utilizada a metodologia de Superfície de Resposta e estipes de shitake seco com ar aquecido. O Delineamento Composto Central Rotacional foi aplicado com duas variáveis independentes (proporção soluto:solvente (m:v) e temperatura de extração), tendo como variável dependente a intensidade de gosto umami, avaliada por um painel sensorial. A proporção soluto:solvente apresentou efeito sobre a intensidade de gosto umami dos extratos, ao contrário da temperatura. A partir da condição otimizada de extração, foi avaliado o efeito do método de desidratação (secagem com ar aquecido e liofilização) aplicado aos estipes de shitake previamente à extração dos compostos umami. Os principais compostos umami (aminoácidos livres e 5'-nucleotideos) foram avaliados por cromatografia líquida de alta eficiência. A partir da composição química foi calculada a Concentração Equivalente de Umami, evidenciando que a secagem com ar aquecido favoreceu a maior recuperação de compostos umami quando comparada à liofilização. O extrato líquido produzido nas condições otimizadas (proporção de 1:20 soluto:solvente (m:v), 30 min, independentemente da temperatura), a partir de estipes desidratadas com ar aquecido, foi utilizado para produção do ingrediente em pó por secagem em *spray dryer*. O ingrediente obtido *(Umami Ingredient*) apresentou coloração clara, alta solubilidade em água, baixo conteúdo de sódio (83,52 mg Na/100g) e umidade (2,76%), além de apresentar alta retenção de ácido glutâmico (97%). A fim de verificar o potencial do *Umami Ingredient* como realçador de sabor em alimentos com teor reduzido de sódio, bem como seu impacto nas propriedades tecnológicas, foi realizada a aplicação do ingrediente em *snacks* extrusados de milho e também em hambúrgueres bovinos com teor de sódio reduzido. Na avaliação sensorial dos *snacks* extrusados, o *Umami Ingredient* (1 e 1.5%) apresentou resultados similares aos da amostra com glutamato monossódico (MSG) para os atributos sabor de tempero, gosto salgado e gosto umami, além de não ter afetado as propriedades físicas (densidade e força de corte) dos snacks. Os hambúrgueres foram avaliados quanto às propriedades físico-químicas e de textura instrumental. As formulações testadas (1 e 2% de *Umami Ingredient*) tiveram impacto mínimo nas propriedades tecnológicas dos hambúrgueres (pequenas alterações de cor, pH, aw e coesividade em alguns tratamentos). Sendo assim, o *Umami Ingredient* mostrou-se uma potencial alternativa para substituir o MSG em alimentos com teor de sódio reduzido, atuando como realçador de sabor e reduzindo o uso de aditivos nas formulações dos produtos. Além disso, a proposta do novo ingrediente contribui para o aproveitamento de um resíduo agroindustrial, agrega valor a esse subproduto, além de atender a demanda do mercado consumidor por aditivos naturais.

Palavras chave: Estipe de cogumelo; Resíduo agroindustrial; Gosto umami; Redução de sódio; *Snack* extrusado de milho; Hambúrguer bovino.

ABSTRACT

Development and application of flavor enhancer ingredient from shiitake mushroom (*Lentinula edodes*) byproducts

The reduction of sodium consumption is one of the current concerns, since the high intake of this micronutrient can lead to the development of cardiovascular problems and hypertension. Concomitant to this, the demand for food with less/ or without the addition of synthetic additives, in line with the clean label movement, is growing. Given this panorama, it is necessary to search for new natural ingredients that have the potential to replace or reduce the use of conventional additives in foods. The use of mushrooms as flavor enhancers in formulations with sodium reduction has been researched. However, studies focused on the use of byproducts from mushrooms production (stipes), with this same objective, are still scarce. Thus, this study aimed to develop a powder ingredient rich in umami compounds, obtained from shiitake mushroom (*Lentinula edodes*) byproducts. To optimize the umami compounds extraction process, the Response Surface Methodology and hot-air dried shiitake stipes were used. The Central Composite Rotatable Design was applied with two independent variables (solute:solvent ratio (w:v) and extraction temperature), with the umami taste intensity evaluated by a sensory panel as a dependent variable. The solute:solvent ratio had effect on the umami taste intensity of the extracts, as opposed to temperature. From the optimized extraction condition, the effect of the dehydration method (hot-air drying and freeze-drying) applied to the shiitake stipes prior to the extraction of umami compounds was evaluated. The main umami compounds (free amino acids and 5'-nucleotides) were evaluated by high performance liquid chromatographic. From the chemical composition, the Equivalent Umami Concentration was calculated, evidencing that hot-air drying favored greater recovery of umami compounds when compared to freeze drying. The liquid extract produced under the optimized conditions (1:20 solute:solvent ratio (w:v), 30 min, regardless the temperature), from stipes dehydrated by hot-air drying, was used to produce the powder ingredient by spray drying. The obtained ingredient (Umami Ingredient) had a light color, high water solubility, low sodium (83,52 mg Na/100g) and moisture content (2.76%), in addition to present high glutamic acid retention (97%). In order to verify the potential of the Umami Ingredient as a flavor enhancer in food with reduced sodium content, as well as its impact on technological properties, the ingredient was applied in corn extruded snacks and also in beef burgers with low-sodium content. In the sensory evaluation of the extruded snacks, the Umami Ingredient (1 and 1.5%) presented similar results to those of the sample added of monosodium glutamate (MSG) for the attributes seasoning flavor, salty taste and umami taste; in addition to not having affected the physical properties (density and cutting force) of the snacks. The physicochemical and instrumental texture properties of the beef burgers were evaluated. The tested formulations (1 and 2% of Umami Ingredient) presented a minimum impact on the technological properties of the burgers (little changes in color, pH, aw, and cohesiveness in some treatments). Thus, the Umami Ingredient proved to be a potential alternative to replace MSG in food with reduced sodium content, acting as a flavor enhancer and reducing the use of additives in products formulations. Also, the new ingredient proposal contributes to the use of agro-industrial residue, adds value to this byproduct, in addition to meeting the consumer market demand for natural additives.

Keywords: Mushroom stipe; Agro-industrial residue; Umami taste; Sodium reduction; Corn extruded snack; Beef burger.

1. INTRODUCTION

The use of salt (sodium chloride – NaCl) in food has a long history. In addition to imparting a salty taste to food, salt can also be used as a preservative by limiting microbial growth (Doyle & Glass, 2010). However, worldwide there is a growing concern with excessive sodium intake, as this can lead to the occurrence of chronic non-communicable diseases, such as hypertension, cardiovascular diseases, stroke and kidney problems (Gilbert & Heiser, 2005; WHO, 2014). According to the World Health Organization (WHO), of the total annual deaths from cardiovascular problems in the world, 1.7 million of them were attributed to high sodium consumption (WHO, 2014).

In this context, a series of initiatives to reduce sodium consumption are being developed worldwide and nationally. In order to promote sodium reduction, WHO launched a global voluntary goal of reducing 30% of daily sodium intake by 2025. It is recommended that the daily salt intake does not exceed 5 g/day, which is equivalent to 2 g sodium/day (WHO, 2014). In Brazil, the population consumes, in average, 9.34 g of salt/day (Mill et al., 2019), that is, almost twice the amount recommended by WHO. According to a Brazilian survey based on Household Budgets Research (POF) most of the sodium available for consumption (74,4%) it is from table salt and seasonings with salt. Processed food added with salt contributes with 18,9% for sodium intake (Sarno et al., 2013). For this reason, in 2011, in Brazil, the "National Sodium Reduction Plan for Processed Food" was implemented, which consists of voluntary agreements with the food production sector, based on the establishment of gradual sodium reduction targets for the food categories that most contribute to the high intake of this micronutrient (Brasil, 2018). Anyway, for effective sodium intake reduction it is necessary that consumers change their eating habits.

Although desirable and necessary, the sodium reduction process is a challenge, since the reduction and/or replacement of NaCl causes changes in the physical-chemical, sensory characteristics and in food stability (Dötsch et al., 2009). Among the main alternatives for reducing the sodium content in food, are the replacement of NaCl by salts, such as potassium chloride (KCl) and calcium chloride ($CaCl_2$). However, these salts have the disadvantage of having bitter and metallic taste, which is not desirable from a sensory point of view. In addition to replacing NaCl with other salts, it is possible to act on other tastes to improve the perceived saltiness (Kremer, Mojet, & Shimojo, 2009). The umami taste, for example, interacts with the salty taste, and it has been shown that the presence of umami compounds increases the sensory perception of salty taste (Mojet, Heidema, & Christ-Hazelhof, 2004; Yamaguchi & Takahashi, 1984).

The umami taste, fifth basic taste, is mainly linked to the presence of glutamic acid (L-Glu) and its salt, monosodium glutamate (MSG), that provides a tasty sensation, similar to the meat taste. However, among the most well-known compounds related to umami taste are the aspartic acid (L-Asp) and the 5’-nucleotides, such as 5’- guanosine monophosphate (5’-GMP), 5’- inosine monophosphate (5’-IMP), 5’- adenosine monophosphate (5’-AMP) e 5’- xantosine monophosphate (5’- XMP), which present synergistic effect on the umami taste (Dermiki, Phanphensophon, Mottram, & Methven, 2013; Yamaguchi, 1991). Recently, in addition to these compounds, some peptides such as Arg-Leu (RL), Gly-Glu (GE) and Val-Phe (VF) have been also pointed out as umami substances (Dang et al., 2019; Kong et al., 2019). These compounds can be found in various food, such as: cheeses, seafood, meats, besides vegetables like tomatoes, potatoes, mushrooms, peas and products like fermented soybeans (Ninomiya, 1998; Yoshida, 1998).

Commonly used in the oriental culinary, mushrooms, little by little, have gained space in Brazil. The Brazilian mushroom production is about 12 thousand tons/ year (ANPC, 2013), among the varieties grown in the country are the champignon (*Agaricus bisporus*), shimeji (*Pleurotus ostreatus*) and shiitake (*Lentinula edodes*) mushrooms, the first being the most cultivated variety in Brazil, and the last the most cultivated variety worldwide (Gomes, Akamatsu, Souza, & Figueiredo, 2016; Royse, Baars, & Tan, 2017). Mushroom umami compounds have aroused the interest of the scientific community in recent years (Chen et al., 2015; Cho, Choi, & Kim, 2010; Kong et al., 2019; Manninen, Rotola-Pukkila, Aisala, Hopia, & Laaksonen, 2018; Phat, Moon, & Lee, 2016; Poojary, Orlien, Passamonti, & Olsen, 2017), therefore, they have been used as natural source of flavor enhancers and also to help sodium reduction in some types of food, mainly in meat products (Dermiki et al., 2013; Guinard et al., 2016; Mattar et al., 2018; Miller et al., 2014). The use of mushrooms as a natural source of flavor enhancers is extremely relevant nowadays, since there is a trend to consume food without or with less addition of food additives (such as MSG). In addition, many consumers believe that food added with MSG can cause health problems, although scientific studies have shown that MSG is a safe ingredient for human consumption (Beyreuther et al., 2007; Henry-Unaeze, 2017).

Edible mushrooms have shown their potential for application as umami spices or umami enhancers in food. Nevertheless, further research is needed in this universe, exploring more mushroom species, processing and potential sources available in unexplored mushrooms, as well as research about the mechanisms underlying the variations in umami taste (Sun et al., 2020; Zhang, Venkitasamy, Pan, & Wang, 2013). Mushrooms can be structurally divided into two parts: pileus (cap) and stipe (stem that supports the pileus). However, at harvest, stipes are generally discarded because they do not have the sensory characteristics appropriate to the consumers taste (Li et al., 2018), which may be related to their hard texture. Stipes can represent 25 to 33% of mushrooms fresh weight (Chou, Sheih, & Fang, 2013), considering that mushroom stipes, as the pileus, have umami compounds (Chen et al., 2015; Cho et al., 2010), the utilization of mushroom byproducts for the obtention of these compounds is an excellent alternative to add value to this material, which is usually discarded. Besides that, since these materials have a high concentration of organic matter, if disposed of inappropriately, they would result in environmental contamination. For economic and environmental reasons, find alternatives for byproducts application, adding value to them, is highly desirable. Therefore, the proper processing of mushroom byproducts can represent an economic alternative for producers and agro-industries, nutritional and health for consumers and sustainability for the environment. For this, more research is needed to assess the potential for using shiitake stipes (Van Ba et al., 2016), and also from other mushroom species.

1.1. GENERAL AND SPECIFIC OBJECTIVES

The major aim of this study was the development of a powder ingredient, rich in umami compounds, from shiitake mushroom (*Lentinula edodes*) byproducts and evaluate its potential as a flavor enhancer in low-sodium food.

The specific aims were to:

- Extract the umami compounds from shiitake stipes and develop a new mushroom-based powder ingredient (Umami Ingredient), rich in umami compounds (Chapter 3);
- Evaluate the potential of the Umami Ingredient as flavor enhancer in low-sodium corn extruded snacks, assessing its effect, as well as the sodium reduction effect, on the physical and sensory characteristics of the products (Chapter 4);
- Evaluate the effect of sodium reduction and addition of Umami Ingredient on the physicochemical and texture properties of low-sodium beef burgers (Chapter 5).

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2. LITERATURE REVIEW

2.1. Mushrooms

Mushrooms are traditionally known by their use in the oriental culinary. Besides this, some species are applied for medicinal use by Asians thousands of years ago (Silva & Jorge, 2011). World production of edible mushrooms reached 34 billion tons in 2013 (Royse, Baars, & Tan, 2017). In Brazil, mushrooms' consumption is expanding, due to the popularization and growth of oriental restaurants in the country. Concomitantly, vegetarian and vegan diets have become increasingly common, and for these cases, mushrooms are great alternatives as a source of non-animal protein.

As it is a recent and very fragmented culture, Brazilian official data on fungiculture are scarce, which makes its valorization difficult. It is estimated that Brazil produces 12 thousand tons of mushrooms a year. According to the National Mushroom Producers Association (ANPC), fungiculture, which used to be concentrated in the state of São Paulo, is spreading to states in the South, Midwest and Northeast of the country. (ANPC, 2013). Among the species grown in São Paulo, the Paris mushroom (*Agaricus bisporus*) is in first place, being cultivated by 52.2% of producers. Then, the genus *Pleurotus* (known worldwide as oyster mushroom, however called shimeji in Brazil) is cultivated by 24.55% of the producers. The cultivation of shiitake (*Lentinula edodes*) represents 16.44%, being the mushroom with the most established value in the fresh produce market (Gomes et al., 2016). Worldwide, the genus *Lentinula* is the most cultivated, representing 22% of the total (Royse, Baars, & Tan, 2017).

Mushrooms can be structurally divided into two parts: pileus (cap) and stipe (stem that supports the cap). Among the macronutrients present in shiitake, most of them are present in the cap, nevertheless the stipe contains valuable quantity of nutrients. Carbohydrates stand out, representing 42% of the cap and 44% of the stipe. This mushroom is rich in proteins, 28% in the cap and 19% in the stipe; it presents low lipid content, around 2% (cap and stipe). Mineral content is around 6% for cap and 4% for stipe, containing zinc, potassium, iron, calcium and others (Li et al., 2018). However, at harvest, stipes (especially those from shiitake) are generally discarded because they do not have the sensory characteristics appropriate to consumers taste, which can be related to their high crude fiber content and poor palatability (Li et al., 2018). Mushroom production residues are estimated to account for 25 to 33 % of the mushrooms fresh weight (Chou, Sheih, & Fang, 2013). When considering the worldwide production of mushrooms, nearly 8.5 to 11.2 million tons of byproducts are generated, which are normally used for animal feed or composting, alternatives with low economic value. Therefore, in order to reduce the volume of organic material discarded inappropriately and also add value to this material, it is necessary to look for more profitable alternatives for the application of mushroom stipes.

2.2. Umami compounds

Discovered in 1908 by Kikunae Ikeda, the fifth basic taste, umami, is described as delicious or tasty (Yamaguchi & Ninomiya, 2000). The perception of umami is mainly related to the presence of monosodium glutamate (MSG), but it is also related to the presence of some free amino acids (FAA), 5’-nucleotides and peptides (Kong et al., 2019; Manninen, Rotola-Pukkila, Aisala, Hopia, & Laaksonen, 2018; Sun et al., 2020; Zhang, Venkitasamy, Pan, & Wang, 2013). The main free amino acids linked to umami taste are the glutamic acid and the aspartic acid, because they are chiral molecules, these amino acids must be in a levogyre form, that is, L-glutamic acid (L-Glu) and L-aspartic acid (L-Asp), to elicit umami taste, since their dextrogyre form does not elicit umami taste. In addition, the 5’-nucleotides 5’-IMP (5’- inosine monophosphate), 5’-GMP (5’-guanosine monophosphate), 5’-AMP (5’- adenosine monophosphate), 5’-XMP (5’-xantosine monophosphate), have a synergistic effect, improving the perception of umami taste (Dermiki, Phanphensophon, Mottram, & Methven, 2013; Mouritsen & Styrbæk, 2014; Yamaguchi, Yoshikawa, Ikeda, & Ninomiya, 1971). More recently, peptides like Arg-Leu (RL), Gly-Glu (GE) and Val-Phe (VF) have been reported as umami compounds (Dang et al., 2019; Kong et al., 2019). Dang et al. (2019) reported that some umami peptides, like Asp-Asp-Asp (DDD) and Glu-Ser-Val (ESV), have synergistic effect with MSG, which can increase the umaminess.

The umami compounds can be found in various food such as meat, poultry, fish, cheese, tomatoes, corn, seaweed and mushrooms (Mouritsen & Styrbæk, 2014; Ninomiya, 1998; Yoshida, 1998). The content of umami compounds found in mushrooms can vary due to several factors, such as species, maturity stage, part of the mushroom used, processing and also the storage time (Sun et al., 2020; Zhang et al., 2013). In general, the free amino acids related to umami are more abundant than the 5’-nucleotides in edible mushrooms. However, the presence of 5’-nucleotides is relevant due to the synergistic effect on umami taste (Dermiki, Phanphensophon, et al., 2013).

Regarding the maturity stage, each species has an optimal maturity stage that makes it possible to obtain a higher concentration of umami compounds, therefore, each case must be evaluated, because each species has a different behavior. Shiitake mushroom, for example, exhibit higher Equivalent Umami Concentration (EUC) values in stage 5 compared to the stage 6, at mature stage (Chen et al., 2015). Using the mushroom pileus or stipe can bring a different result, since the pileus contains a higher concentration of umami amino acids and 5'-nucleotides than the stipe (Cho, Choi, & Kim, 2010; Zhang et al., 2013). Processing applied to both increase shelf life and culinary preparations affects the umami compounds. Rotola-Pukkila, Yang, & Hopia (2019) evaluated the effect of cooking on umami compounds from wild mushrooms, they reported that FAA content decreased when cooking temperature increased and 5'-GMP was detected only in cooked samples. The drying methods, as well as the temperature, applied to increase mushrooms shelf life, can affect the umami compounds. Hot-air drying has shown the ability of increasing the content of FAA, as well as the EUC on dried shiitake (Xu et al., 2019; Yang et al., 2019). In addition, pre-drying treatment at high temperature have positive effect on dried shiitake quality, increasing the rehydration and decreasing shrinkage and browning (Xu et al., 2019).

Facing mushrooms potential as source of umami enhancing substances, there is an increasing interest in using them as natural umami ingredients in food formulations (Poojary, Orlien, Passamonti, & Olsen, 2017; Sun et al., 2020; Zhang et al., 2013).

2.3. Sodium reduction in food

Currently, consumers are concerned with the consumption of processed food, as many diseases are related to lifestyle and consumption of food high in fat, sodium and sugar. Faced with this situation, the demand for healthier products, but which does not neglect convenience, is growing (Asioli et al., 2017).

In this scenario there is a worldwide concern to reduce the sodium consumption of foods, since its excessive intake can lead to the development of cardiovascular problems (Aburto et al., 2013). WHO recommends that the daily sodium intake does not exceed 2 g, which is equivalent to 5 g of salt/day (WHO, 2014). However, Brazilians consume, on average, almost twice the amount recommended by WHO, consuming 9.34 g of salt/day (Mill et al., 2019). In Brazil, salt table contributes for 74,4% of the sodium intake, while processed food is estimated to provide 18,9% of sodium consumption (Sarno et al., 2013). Decreasing sodium intake in the diet is imperative, as it can reduce blood pressure and the risk of chronic non-communicable diseases (Aburto et al., 2013).

Brazilian industries are facing the challenge of reducing sodium in processed food. Data divulged by ABIA (Brazilian Association of Food Industries) show that the food industries have reduced approximately 17.2 thousand tons of sodium in their products since 2011 (ABIA, 2017). Anyway, sodium reduction is not an easy task, because it results in technological and sensory modifications (Dötsch et al., 2009) that can impact the consumers perception and acceptance regarding the low-sodium products (Liem, Miremadi, & Keast, 2011). Although there are some alternatives for sodium reduction in food that can help this process, such as reduction of salt content (reduction by stealth), use of salt substitutes, change size and shape of salt, and addition of flavor enhancers (Desmond, 2006; Dötsch et al., 2009; Inguglia, Zhang, Tiwari, Kerry, & Burgess, 2017).

The use of salt substitutes is the most common approach applied for sodium content reduction, for this purpose potassium chloride (KCl) and calcium chloride ($CaCl_2$) are employed. However, the use of these salts can result in bitter and metallic taste in the products (Mitchell, 2019), that is undesirable. Another alternative is the improvement of other tastes that enhance the perceived saltiness (Kremer, Mojet, & Shimojo, 2009). According to Yamaguchi and Takahashi (1984), there is an interaction between MSG and NaCl (salt). In that study, it was demonstrated that MSG is able to increase the perceived saltiness, thus making it possible to reduce the content of NaCl and, consequently, sodium, since MSG contains about 1/3 of the amount of sodium present in the NaCl. Mojet, Heidema and Christ-Hazelhof (2004) evaluated the interaction between the five basic tastes (salty, sweet, sour, bitter and umami) and again it was demonstrated that the presence of umami compounds (MSG and 5'-IMP) increases the perceived saltiness. In this way, acting on food umami taste can help sodium reduction, contributing to lessen the impairment on the sensory perception caused by salt reduction.

The presence of umami compounds in some food has enabled the development of new products with reduced sodium content, through the use of alternative sources of umami compounds, instead of using traditional additives, such as MSG. Radam et al. (2010) reported that consumers have a positive perception of "no added MSG" products, considering them healthier and safer. This is mainly because most consumers have heard or read about studies indicating health problems caused by MSG, which, nevertheless, are inconclusive, as several other scientific studies point to MSG as a safe ingredient for human consumption (Beyreuther et al., 2007; Henry-Unaeze, 2017). Based on indicators from recent years, consumers are more concerned about their health and part of them avoid foods with added chemical additives, fostering the search for foods prepared with natural additives (Carocho, Morales, & Ferreira, 2015). This is the current trend of clean label food, which can be defined as food that have a simple and short list of ingredients, not containing artificial or chemical-sounding ingredients, and only containing ingredients that are familiar to consumers (Asioli et al., 2017).

2.4. New products and ingredients to reduce sodium in foods

In order to use the umami compounds naturally present in food (like mushrooms) to develop new products with low-sodium content, several scientific studies have been carried out on different types of food matrices, in order to investigate the impact of the new formulations on the technological and sensory properties.

The incorporation of naturally brewed soy sauce made possible the reduction of sodium in salad dressing (50%), soups (17%) and stir-fried pork (29%) without impairing the intensity of flavor and acceptance of the products, because the ingredient is rich in umami compounds, which may have helped to maintain the flavor of the evaluated products (Kremer et al., 2009). In a study carried out with frankfurters, the use of a natural ingredient (composed of water, soy, wheat and alcohol) to enhance the flavor, enabled a reduction of 35% in the sodium content of this product, without affecting consumer acceptance. In addition to the incorporation of the natural ingredient, KCl was also used as a partial NaCl substitute. According to the authors, the natural ingredient showed the ability to act as an enhancer of salty taste and flavor, beyond attenuating the bitter taste due to the utilization of KCl (McGough, Sato, Rankin, & Sindelar, 2012).

According to Miller et al. (2014), mushrooms can be attenuating agents in sodium reduction in meat products, contributing to the overall flavor maintenance. Dermiki, Mounayar, et al. (2013) reported that the use of natural ingredients rich in umami compounds (yeast extract, shiitake extract, tomato puree, soy sauce and soybean paste) can increase the perception of salty taste in ground meat. In another study, aqueous extract of shiitake was used as flavor and aroma enhancer in meat formulations. The incorporation of shiitake extract increased the concentration of 5'-nucleotides, however, in this case, there was no significant difference in the perception of umami taste. The authors suggested the concentration of the shiitake extract to promote substantial changes in umami taste (Dermiki, Phanphensophon, et al., 2013). Wong et al. (2017) evaluated the inclusion of champignon mushrooms to reduce the sodium content in beef tacos. In the sensory evaluation, consumers preferred samples with less salt (45%) and containing 45% mushroom. This may be related to umami taste and also to the mushroom aroma that improved the tacos flavor, thus showing the potential of mushrooms in maintaining the quality and sensory acceptance of tacos with reduced salt content.

Some studies have evaluated the use of mushrooms or their extract in burgers. Patinho et al. (2019) evaluated the use of *A. bisporus* in beef burgers. The authors reported that the new formulations presented changes in color, water activity and texture (being softer than the control). Shiitake liquid extract was used as a natural ingredient to improve the sensory characteristics of low-sodium beef burgers. Sensory analysis revealed that the formulation with 50% of sodium reduction and addition of shiitake extract was well accepted as the control, despite presenting changes in physicochemical characteristics, such as pH, yield, shrinkage and color. The shiitake liquid extract showed potential as a flavor enhancer, supported the process of sodium reduction without compromising the sensory acceptance (Mattar et al., 2018). Tom, Alnoumani, & Were (2018) applied dried *A. bisporus* powder in beef patties and reported that patties with dried *A. bisporus* and 33% less salt were sensorially similar to the control. According to the authors, the use of mushroom powder can improve chemical stability during shelf life at the retail and compensate de NaCl reduction without affecting the consumers sensory acceptance.

As can be seen, the use of mushrooms as natural flavor enhancers in food has been investigated. Meanwhile, most of these studies were performed on meat-based products. Although these studies reported good results for mushrooms use in low-sodium meat products, more research is needed to evaluate their behavior in other food matrices. Some studies have evaluated the incorporation of mushrooms in cereal-based products, such as extruded snacks (Brennan, Derbyshire, Tiwari, & Brennan, 2012; Lu et al., 2020; Vallée et al., 2017) and pasta (Lu et al., 2018; Lu, Brennan, Serventi, Mason, & Brennan, 2016), with the purpose of enhancing their fiber content, antioxidant activity or to reduce the glycemic index, but they did not evaluate the influence of mushrooms on the products' flavor. Recently, Tepsongkroh et al. (2020) reported results suggesting that the incorporation of straw and phoenix mushrooms in the extrusion of rice snacks increased the saltiness expectation and that samples with mushroom added were perceived as saltier than the control. Nevertheless, the authors recommended more investigation to confirm this finding.

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3. UMAMI INGREDIENT: FLAVOR ENHANCER FROM SHIITAKE (*Lentinula edodes*) BYPRODUCTS[1]

Abstract

An alternative use of shiitake stipes, usually treated as waste, was proposed for the production of a powder ingredient, rich in umami compounds, aiming its application in food. The extraction of umami compounds was optimized through the Response Surface Methodology (RSM), in order to obtain an extract with high umami taste intensity. From the optimized condition, a comparative analysis of shiitake stipes dehydration method was performed. Stipes were dehydrated by hot air drying (HD) and freeze drying (FD), submitted to extraction and the umami compounds in the extracts were compared. The comparative analysis showed that the 5' - nucleotides are more sensitive to prolonged heating, while the release of free amino acids (FAA) was favored by hot air drying. The HD samples extract showed higher Equivalent Umami Concentration (EUC). The spray drying of the HD samples extract allowed the production of a newly powder ingredient rich in umami compounds (Umami Ingredient) that can be applied in diverse food matrices. Due to the presence of umami compounds, Umami Ingredient can be a potential alternative to help in the process of sodium reduction by enhancing food flavor.

Keywords: Free amino acids (FAA), 5' – nucleotides, umami taste, mushroom stipes, optimization, agro-industrial waste.

3.1. Introduction

World-famous, mushrooms are known for their unique flavor. This flavor comes from the mixture of various elements present in these fungi, including soluble sugars, free amino acids (FAA), 5'- nucleotides, peptides and organic acids, which contribute to taste perception, and volatile compounds that contribute to aroma (Chen et al., 2015; Dermiki, Phanphensophon,

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Mottram, & Methven, 2013; Kong et al., 2019). Among the taste components stand out those linked to umami taste, as edible mushrooms are considered good sources of umami compounds (Sun et al., 2020; Y. Zhang, Venkitasamy, Pan, & Wang, 2013).

Discovered by Kikunae Ikeda in 1908, umami (fifth basic taste) is described as delicious or savory (Yamaguchi & Ninomiya, 2000). The umami taste is mainly attributed to the presence of L - glutamic acid (L - Glu) and its salt, monosodium glutamate (MSG). Besides these, L - aspartic acid (L - Asp) and 5'- nucleotides, including 5'- guanosine monophosphate (5'- GMP), 5'- inosine monophosphate (5'- IMP), 5'- adenosine monophosphate (5'- AMP) and 5'- xanthosine monophosphate (5'- XMP) also provide umami taste. The 5'- nucleotides combined with L - Glu and L - Asp have synergistic effect on umami taste perception (Dermiki et al., 2013; Yamaguchi, 1991). More recently, some peptides like Gly-Cys-Gly (GCG), Glu-Pro-Glu (EPE) and Cys-Met (CM) have also been reported as umami-enhancing substances (Kong et al., 2019).

Mushrooms umami compounds have aroused the interest of the scientific community in recent years (Chen et al., 2015; Kong et al., 2019; Phat, Moon, & Lee, 2016; Rotola-Pukkila, Yang, & Hopia, 2019). Studies indicate the potential of using mushrooms as umami ingredients to enhance food flavor (Mattar et al., 2018; Wong et al., 2017; Y. Zhang et al., 2013). The shiitake (*Lentinula edodes*) is the main edible mushroom cultivated worldwide (Royse, Baars, & Tan, 2017), being known, mainly, for its typical flavor and aroma (Dermiki et al., 2013; Tian, Zhao, Huang, Zeng, & Zheng, 2016). Although shiitake is among the most studied edible species, many of these studies on mushrooms umami compounds evaluate only the pileus, and few studies evaluate mushroom byproducts (stipes) (Chen et al., 2015; Cho, Choi, & Kim, 2010). In general, stipes are not used for commercial purposes, probably because they do not have the sensory characteristics suited to taste of consumers (S. Li et al., 2018), which may be related to the firmer texture, due to the high fiber content (S. Li et al., 2018). Finding alternatives for the use of umami compounds (and/or nutrients) present in the mushroom stipes is an economic and environmental issue, since stipes represent 25 to 33% of mushrooms fresh weight, and are usually used for activities of low economic value, such as animal feed and composting (Chou, Sheih, & Fang, 2013).

Umami compounds can play an important role in the process of reducing sodium in foods, since they enhance the perception of salty taste (Mojet, Heidema, & Christ-Hazelhof, 2004; Yamaguchi & Takahashi, 1984), avoiding loss in sensory quality and consequent rejection of food products. MSG is the most widely used umami compound to enhance food flavor. However, consumers consider food without added MSG to be safer and healthier

(Radam, Yacob, Bee, & Selamat, 2010) - although this is only linked to consumer perception, and not to a proven fact, as MSG is scientifically recognized as safe (Beyreuther et al., 2007; Henry-Unaeze, 2017) – it can interfere with the positioning of added MSG products on the market. In view of this situation, the use of umami compounds naturally present in mushrooms as flavor enhancers is an alternative to satisfy the demand for savory and reduced sodium foods with fewer synthetic additives.

The aim of this study was to extract the umami compounds present in shiitake (*Lentinula edodes*) stipes to develop a newly mushroom based powder ingredient, rich in umami compounds. This new ingredient can be a potential alternative to be applied in reduced sodium foods, helping to maintain the products' flavor.

3.2. Material and methods

3.2.1. Shiitake stipes samples

The shiitake stipes were collected in an industry in the state of São Paulo (Brazil) in March 2019. The samples were frozen, transported in thermal boxes with ice, and kept in a freezer (-24 °C) until use. For processing, the stipes were unfrozen and sanitized in an aqueous solution of 200 ppm sodium hypochlorite for 10 min, followed by rinsing in flowing water. Immediately after, the samples were cut (width approximately 1 cm) and blanched using water steam (Philips Walita RI9120, Blumenau, Brazil) for 3 min (Maray, Mostafa, & El-Fakhrany, 2018). Then part of the stipes was dehydrated by: 1) hot air drying at 70 ± 2 °C for 12 h in an oven (Tecnal TE 394/1, Piracicaba, Brazil) with parallel air flow renewal at constant velocity of 2.0 m/s (HD); 2) freeze drying, where the samples were frozen and freeze-dried (Liotop L101, São Carlos, Brazil, -51 °C, 0.15-0.3 mmHg) for 96 h (FD). In both cases the dehydrated material was ground in a hammer mill (Marconi MA 090, Piracicaba, Brazil), sieved (40 mesh), packed in plastic bags (polyethylene) and stored in a dry, ventilated place away from light.

3.2.2. Optimization of umami compounds extraction using response surface methodology (RSM)

For optimization, HD stipes were submitted to umami compounds extraction following the Central Composite Rotatable Design (CCRD), in which the independent variables were the solute:solvent ratio (w:v) and the bath temperature (Table 1). The independent variables selection and ranges were defined considering the conclusions of previous studies (Dermiki et al., 2013; Poojary, Orlien, Passamonti, & Olsen, 2017) and a preliminary test (data not shown). The variable ‘extraction time’ was also evaluated in the preliminary test, but it did not show significant influence on the umami taste intensity. In addition, previous research showed that the recovery of MSG-like FAA and 5’-nucleotides is not significantly influenced by extraction time (Poojary et al., 2017). Thus, the independent variable, ‘extraction time’, was not applied to CCRD design.

Table 1. Central composite rotatable design (CCRD) applied to umami compounds extraction from shiitake stipes and dependent variable (umami taste sensory intensity).

Experimental assay	Run order	Independent variables				Dependent variable
		Coded value		Real value		
		Volume (V - mL)	Temperature (T - °C)	Volume (V - mL)	Temperature (T - °C)	Umami taste intensity
1	2	-1	-1	27	31	4.73
2	4	1	-1	63	31	1.22
3	7	-1	1	27	63	4.14
4	6	1	1	63	63	1.68
5	12	-1.41	0	20	47	5.13
6	1	1.41	0	70	47	1.03
7	9	0	-1.41	45	24	1.40
8	3	0	1.41	45	70	3.26
9	11	0	0	45	47	2.50
10	5	0	0	45	47	1.75
11	10	0	0	45	47	1.61
12	8	0	0	45	47	1.88

In order to apply the umami compounds of the shiitake stipes in food products, water was used as the extraction solvent. For the variable solute:solvent ratio, 1 g of solute was used with the different volumes that make up the design (Table 1). The extraction was conducted in a heated bath (Tecnal Dubnoff - NT 269, Piracicaba, Brazil) with temperature control (± 1 °C). All flasks were shaken (180 rpm) for 30 min (Poojary et al., 2017). The extracts were then filtered through a qualitative paper filter (Unifil – 501.018, pore size 4 – 12 µm) and frozen (-24 °C) until use. The experiments were conducted in random order to minimize the effects of uncontrolled factors.

The dependent variable was the umami taste intensity in aqueous extracts, evaluated by panelists. The sensory analysis was conducted following Stone & Sidel (2004) recommendations, with adaptations. To participate in the study, eighteen panelists were recruited, they performed a difference-from-control test with MSG solutions in different concentrations (0.2, 0.4 and 0.6% (control) w/v), in three repetitions. Fourteen panelists were pre-selected regarding their discriminative capacity (panelists' ability to discriminate different samples, $p \leq 0.30$) and reproducibility capacity (panelist's ability to repeat judgments in different sessions of analysis, $p > 0.05$).

In the next step, the pre-selected panelists defined the umami taste attribute as: 'Fifth basic taste, characteristic of a monosodium glutamate aqueous solution', as well as they defined the references of minimum (None: Water) and maximum (High: Monosodium glutamate aqueous solution 0.6%). From this, they were trained to evaluate the umami taste intensity in the samples and to use the unstructured linear intensity scale of 90 mm.

The training stage lasted seven sessions. At each session, the panelists evaluated five different samples of mushroom extract (run 5, 6, 7, 8 and 9 - Table 1), firstly three and after two samples, with 1 h break between each group in order to avoid sensory fatigue. Data resultant from the last three sessions was used for evaluation of the panelists regarding to their discriminative capacity ($p \leq 0.30$), reproducibility capacity ($p > 0.05$) and consensus with the sensory panel (Pearson's $r > 0.70$) (Damásio & Costell, 1991). For the final analysis, seven of the fourteen panelists were selected, who were again evaluated regarding their discriminative capacity ($p \leq 0.30$) and reproducibility capacity ($p > 0.05$), being necessary to exclude one more panelist. Thus, the final sensory panel consisted of six panelists, all female, aged between 19 and 32 years.

The final analysis of the extracts was conducted in individual booths, under white light and at a temperature of 23 °C, following the procedure performed during the training stage. The twelve samples of shiitake extract (15 mL) were presented in plastic cups, coded with three-digit random numbers and evaluated in two sessions of six samples each. This procedure was performed in three repetitions. The sample evaluation was performed in complete blocks, i.e., all the panelists evaluated all the samples. The sample presentation was monadic (one sample presented at a time) and in balanced order according to Macfie, Bratchell, Greenhoff, & Vallis (1989) to avoid bias by positional and carry over effects. The panelists received, along with the sample, a glass of water and a water biscuit to clean the entire oral cavity between the samples. This sensory test was approved by the Research Ethics Committee of ESALQ/USP (Decision 2.994.710).

From the optimized extraction condition, extracts were produced for the chemical determination (items 3.2.3 and 3.2.4) of umami compounds. In order to evaluate the effect of the dehydration method applied to the shiitake stipes prior to extraction, HD and FD samples (item 3.2.1) were submitted to the optimized extraction condition and their umami compounds were compared.

3.2.3. Free amino acids analysis

FAA were analyzed according to Wu & Meininger (2008) in a high performance liquid chromatograph (HPLC) with fluorescence detector (RF - 20 A) (HPLC-F) (Shimadzu, Kyoto, Japan), using as stationary phase a C18 column (Supelco LC-18, 250 mm x 4.6 mm, 5 µm). The mobile phase used was A: 100 mM sodium acetate (90.49%) + methanol (9%) + tetrahydrofuran (0.5%) + 6 N hydrochloric acid (0.0048%), and B: 100% methanol. The elution was conducted in gradient (LC - 10 AD pumps), as follows: 0 - 15 min, 14 % B; 15 - 20 min, 14 - 30% B; 20 - 24 min, 30 - 35% B; 24 - 26 min, 35 - 47% B; 26 - 34 min, 47 - 50% B, 34 - 38 min, 50 - 70% B; 38 - 40 min, 70 - 100% B; 40 - 50 min, 100% B; 50 - 51 min, 14% B; 51 - 56 min, 14% B. The flow rate used was 1 mL/min, and the column temperature was maintained at 40 °C (CTO oven - 10 AC). All samples were filtered (0.20 µm) before injection into the system.

For the derivatization of amino acids, an OPA-Borate solution + β - mercaptoethanol (OPA + MeOH + borate buffer + β - mercaptoethanol, filtered in 0.20 µm) was used. Fluorescence detector was used to detect the amino acids with excitation at 340 nm and emission at 455 nm. Amino acids were identified by comparison with the retention time of authentic standards (Sigma Aldrich, St Louis, USA). The concentrations of analytes in the samples were determined using their calibration curves and expressed as mg free amino acid per gram of dry sample (mg FAA/g DW).

3.2.4. 5'- nucleotides analysis

The extracts were centrifuged at 3500 rpm (Fanem Excelsa 2206, Guarulhos, Brazil) for 20 min, 1 mL of the supernatant was filtered in a 0.22 µm membrane and packed in vials. The method proposed by Poojary et al. (2017) with minor modifications was used. The analyses were performed on a HPLC (Shimadzu, Kyoto, Japan) with diode arrangement detector (DAD, model SPD-6AV). The stationary phase used was the Agilent eclipse XDB-C18 column (250 mm × 4.6 mm; 5 µm), maintained at 25°C. The mobile phase was composed by A: potassium phosphate buffer - KH_2PO_4, 50 mM, pH 4.8 and B: methanol, which was eluted in gradient, being: 0-5 min, 0% B; 14-22.5 min, 10% B and 23-30 min, 0% B. The flow was maintained at 0.5 mL/min. The analytes were detected and quantified at 254 nm. The identity of the analytes was confirmed by co-injection of the samples with the authentic nucleotide standards (all 5'-nucleotides standards were purchased from Sigma Aldrich, St Louis, USA, except 5'-XMP, that was purchased from Santa Cruz Biotechnology, Dallas, USA), as well as, by comparison of their UV-Vis scanning spectra. The analyte concentrations in the samples were determined with the aid of their calibration curves and expressed as mg of 5'-nucleotide per gram of dry sample (mg/g DW).

3.2.5. Equivalent umami concentration (EUC)

EUC is the concentration of MSG (g/100g) equivalent to the umami taste intensity provided by the mixture of the umami amino acids (Glu and Asp) and 5'- nucleotides, which is calculated according to equation (1) (Yamaguchi, Yoshikawa, Ikeda, & Ninomiya, 1971):

$$\boldsymbol{Y = \sum a_i b_i + 1218 \left(\sum a_i b_i\right) . \left(\sum a_j b_j\right)} \qquad (1)$$

Where Y is the EUC of the sample (g MSG/100g), $\boldsymbol{a_i}$ is the concentration (g/100g) of each umami amino acid (Glu or Asp); $\boldsymbol{a_j}$ is the concentration (g/100g) of each umami 5'-nucleotide (IMP, GMP, XMP or AMP); $\boldsymbol{b_i}$ is the relative umami concentration (RUC) for each umami amino acid in MSG (Glu, 1; Asp, 0.077); $\boldsymbol{b_j}$ is the RUC for each umami 5'- nucleotide in IMP (IMP, 1; GMP, 2.3; XMP, 0.61 and AMP, 0.18); and 1218 is the synergistic constant based on the concentration of g/100g used.

3.2.6. Umami ingredient

In order to facilitate the transport, storage and application of the shiitake extract in various food products, the liquid extract with higher EUC was spray dried to obtain a powder ingredient (Umami Ingredient).

3.2.6.1. Spray drying process: Umami Ingredient production

For powder extract (Umami Ingredient) production, some pre-tests were performed to evaluate the amount of carrier agent, maltodextrin (MD) (Ingredion DE-20), to be added to the liquid extract before drying. The concentrations of 10, 20, and 40% of solids of extract were tested, i.e. 10, 20, and 40 g of extract solids/100 g of total solids (data not shown). Each mixture was homogenized separately and dried in laboratory scale spray dryer (Mini Spray Dryer Buchi B-290, Flawil, Switzerland). The drying conditions were: inlet air temperature 170 ± 1 °C, outlet temperature 84 ± 2 °C, feed sample rate 9.4 mL/min, nozzle diameter 0.7 mm, aspiration

35 m³/h, atomization gas flow of 600 L/min equivalent to 0.75 bar of atomization pressure, room temperature 23 °C, room air humidity 35 - 45 %.

For the three concentrations assessed the process yield and the efficiency in the retention of L - Glu (main umami compound) was verified. The best result was obtained with 20% concentration, in which the drying process was facilitated, in addition to presenting higher retention of L - Glu and higher yield. The use of another carrier agent, modified starch (MS) (Capsul), was also evaluated in the proportion previously tested (20 g of extract solids/ 100 g of total solids). Comparatively, MD and MS showed very similar performance and process yields, as well as similar flavor (data not shown). Taking into account these results, MD was chosen as the carrier agent due to its lower cost.

3.2.6.2. Umami Ingredient physical properties

The drying yield was determined by the ratio of the mass of solids obtained at the end of the process to the mass of solids at the beginning of the process. The moisture was determined by Karl Fischer volumetric titration (Titrando 901, Methrom Pensalab, São Paulo, Brazil), using methanol: formamide (1:1, v/v) as a solvent.

The size distribution was determined by laser scattering using a LV 950-V2 equipment (Horiba, Kyoto, Japan). The samples were dispersed in absolute ethanol. The mean diameter was expressed as the average diameter of sphere of the same volume ($D_{4.3}$) and the polydispersity was given by span index, which was calculated according to Equation (2) (Alvim, Stein, Koury, Dantas, & Cruz, 2016).

$$SPAN = {(D_{90\%} - D_{10\%})}\big/{D_{50\%}} \qquad (2)$$

Where $D_{10\%}$, $D_{50\%}$ and $D_{90\%}$ correspond to the diameters for 10%, 50% and 90% of the cumulative size distribution.

The solubility was determined as described by Cano-Chauca, Stringheta, Ramos & Cal-Vidal (2005). In a blender (Philips, RI2160, 550W, Barueri, Brazil), 1.5 g (dry weight - DW) of sample were added in 150 mL of distilled water and shaken at high speed for 4 min. The solution was centrifuged at 3000 *g* for 5 min. Aliquots of 25 mL of the supernatant were transferred to previously weighed Petri dishes and dried at 105 °C for 5 h. Solubility (%) was calculated as the weight of dry solids in the supernatant represented as a percentage of the initial sample weight.

The water activity was determined in a water activity analyzer (Aqua Lab 4TE, Meter Group, Pullman, USA) at 25 ± 0.1 °C (Favaro-Trindade, Santana, Monterrey-Quintero, Trindade, & Netto, 2010).

To determine the hygroscopicity, the method described by Cai & Corke (2000), with modifications, was used. Approximately 1 g of sample was weighed and stored in a desiccator containing a saturated NaCl solution (75% RH) kept at 25 °C. After one week, the samples were weighed and the hygroscopicity was expressed as g of water absorbed by 100 g of dry solid (g H_2O/100 g DW).

The instrumental color was determined by colorimeter (Minolta Chroma Meter CR-400, Konica Minolta, NJ, USA), by reading the parameters L*, a* and b* of the CIELab system, with D65 light source.

3.2.6.3. Sodium content

The sodium content was determined according to the method described by AOAC (2010). The samples (1 g) were incinerated at 550 °C in muffle furnace until the ashes were obtained. These were diluted in nitric acid and ultrapure water for reading in a flame photometer (Micronal B262, São Paulo, Brazil). The equipment was calibrated with standard sodium solution.

3.2.6.4. Glutamic acid retention

To determine Glu retention (GR) in Umami Ingredient, this component was extracted from the powder sample (Glu quantified after processing), according to item 3.2.2. Glu concentration in Umami Ingredient and in liquid extract (Glu quantified before processing) was performed as described in 2.3. Equation (3) was used to calculate % GR:

$$\% \, GR = \frac{Glu \ quantified \ after \ processing}{Glu \ quantified \ before \ processing} * 100 \qquad (3)$$

3.3. Statistical analyses

The results of the experimental design were submitted to analysis of variance (ANOVA) and the significance of the experimental parameters was evaluated by the values of *F* and *P* ($p \leq 0.05$). The non-significant parameters were removed, and the mathematical model was readjusted, the lack of fit and the coefficient of determination (R^2) were verified. The response surfaces as well as the statistical analyses were performed in the Statistica 10.0 software (StatSoft Inc., USA). The other results were obtained using a completely randomized design (CRD) and the results were expressed as mean ± standard deviation. The data was submitted to exploratory analysis, checking the normality of the residues (Shapiro-Wilk test), homogeneity of variances (Brown and Forsythe's test) and the presence of outliers. The data that met the assumptions of the exploratory analysis was submitted to the *t* test ($p \leq 0.05$), the other data was analyzed using the non-parametric Wilcoxon-Mann-Whitney test ($p \leq 0.05$) to compare the results between the samples. The analyses were performed in SAS Studio software (SAS Institute, Cary, USA).

3.4. Results and discussion

3.4.1. Optimization of umami compounds extraction

There were a significant linear and quadratic effects ($p < 0.05$) only for the 'solute: solvent ratio (w:v)' variable. The adjusted quadratic model (Equation 4) exhibited a coefficient of determination (R^2) of 0.87 and no significant lack of fit ($p = 0.218$). Thus, the model obtained had a good fit and is considered predictive.

$$Umami\ taste\ intensity = 2.14 - 1.47 * V + 0.58 * V^2 \qquad (4)$$

Through the response surface (Figure 1) the contribution of each variable to umami taste intensity is evident. The highest intensity of umami taste (5.13) was reached when less water was used in the extraction, i.e., using the 1:20 ratio (solute:solvent ratio (w:v)), because in this condition, umami compounds were less diluted in water, i.e., they were more concentrated, reflecting in higher intensity of the umami taste. According to Poojary et al. (2017), that optimized the extraction of umami compounds from mushrooms, the optimized extraction condition employed a 1:50 ratio (solute:solvent ratio (w:v)), nevertheless the dependent variable used in that study was the chromatographic analysis of the umami compounds present in the extract, differing from what was done in our study. This difference in results is expected because the chromatographic evaluation of umami compounds shows high sensitivity to the presence of each compound individually, whereas in the sensory evaluation all umami compounds present in the sample are evaluated simultaneously, interacting with the umami taste receptors (T1R1/T1R3, brain and taste-mGluR1, brain and taste-mGluR4) and producing nerve signals in the brain, where the characteristic umami sensation is perceived (J. Zhang, Sun-Waterhouse, Su, & Zhao, 2019). Poojary et al. (2017) found different optimal extraction condition (70° C, 30 min, 50 mL of water). However, it should be remembered that those authors used whole mushrooms and not only stipes for extraction. Moreover, the response was based on the chemical determination of umami compounds (FAA and 5'-nucleotides) and not on a sensory evaluation.

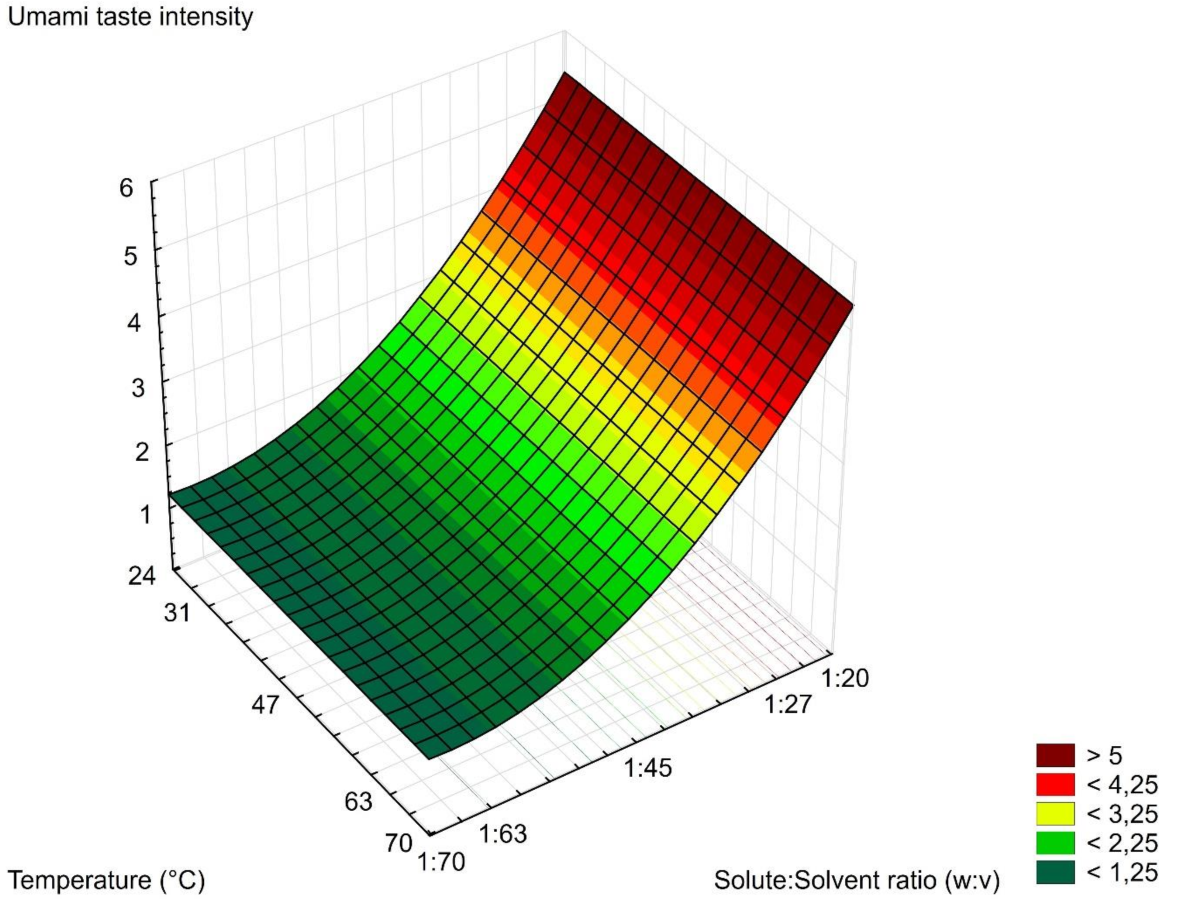

Figure 1. Response surface showing the effect of the independent variables (temperature and solute:solvent ratio (w/v)) on the umami taste intensity of shiitake stipes extract.

Thus, considering what was previously presented in the present study, to obtain an extract with high intensity of umami taste, 20 mL of water per gram of shiitake stipe (HD) during 30 min of extraction must be applied, regardless of the temperature.

3.4.2. Effect of dehydration method on umami compounds

Many factors influence the mushrooms taste compounds, such as the part used (stipe or pileus), the maturity stage (Chen et al., 2015; Cho et al., 2010), the conservation method (Liu et al., 2014), the type of cooking (Rotola-Pukkila et al., 2019), and the dehydration method applied (X. Yang et al., 2019). In the present study, it was possible to verify that the hot-air drying process made possible greater extraction of FAA (Table 2). Probably this is related to proteolysis promoted by the increase in temperature during hot-air drying, as pointed out by some authors (Xu et al., 2019; X. Yang et al., 2019).

Table 2. Free amino acids and 5'- nucleotides (mg/g DW) identified in hot-air dried (HD) and freeze-dried (FD) shiitake stipes.

	Hot-air dried (HD)	**Freeze dried** (FD)
Free amino acids		
L-Alanine	1.75 ± 0.12 (8.0)*[A]	0.02 ± 0.00 (3.0)[B]
L- Arginine	1.60 ± 0.06 (8.0)[A]	0.11 ± 0.00 (3.0)[B]
L- Asparagine	0.52 ± 0.02 (8.0)[A]	0.02 ± 0.00 (3.0)[B]
L- Aspartic	0.39 ± 0.02	TR
L-Citrulline	ND	ND
L- Glutamic	2.68 ± 0.09	TR
L- Glycine	0.44 ± 0.06	TR
L- Isoleucine	0.65 ± 0.03	TR
L- Leucine	0.99 ± 0.04 (8.0)[A]	0.01 ± 0.00 (3.0)[B]
L-Lysine	1.38 ± 0.25 (8.0)[A]	0.06 ± 0.00 (3.0)[B]
L-Methionine	0.10 ± 0.01	TR
L- Ornithine	5.16 ± 0.72	TR
L- Phenylalanine	0.78 ± 0.03 (8.0)[A]	0.01 ± 0.00 (3.0)[B]
L- Serine	0.41 ± 0.02 (8.0)[A]	0.06 ± 0.00 (3.0)[B]
L- Threonine	1.00 ± 0.04 (8.0)[A]	0.04 ± 0.00 (3.0)[B]
L- Tryptophan	0.24 ± 0.01	TR
L- Valine	1.05 ± 0.03 (8.0)[A]	0.01 ± 0.00 (3.0)[B]
MSG-like	3.07 ± 0.11 (8.0)[A]	0.00 ± 0.00 (3.0)[B]
5'- Nucleotides		
5'-AMP	1.46 ± 0.16 [a]	1.73 ± 0.24 [a]
5'-CMP	3.80 ± 0.44 [a]	3.92 ± 0.44 [a]
5'-GMP	0.96 ± 0.07 [b]	1.31 ± 0.12 [a]
5'-IMP	TR	TR
5'-UMP	0.69 ± 0.08 [b]	1.19 ± 0.16 [a]
5'-XMP	ND	ND
Umami nucleotides	2.42 ± 0.23 [b]	3.04 ± 0.35 [a]
Equivalent umami concentration (EUC)		
EUC (g MSG/ 100 g DW)	81.55 ± 6.25 (8.0)[A]	0.00 ± 0.00 (3.0)[B]

Each value is expressed as mean ± standard deviation (n = 5). Different capital letter following numbers within a row are significantly different by non-parametric Wilcoxon-Mann-Whitney test ($p \leq 0.05$). Different lowercase letter following numbers within a row are significantly different by test *t* ($p \leq 0.05$).
*Numbers in parentheses are mean scores used in non-parametric Wilcoxon-Mann-Whitney test.
TR: trace; ND: not detected. MSG-like = L- Asp + L- Glu; Umami nucleotides: 5'-GMP + 5'-IMP + 5'-XMP + 5'-AMP.

Some FAA can be classified according to their taste characteristics, being divided according to the basic taste they elicit: MSG-like, sweet, bitter, and tasteless FAA. The perception of basic tastes, meanwhile, also depends on hydrophobicity, size, charge, functional groups on the side chain and chirality of the alpha carbon. The MSG-like group includes L- Aspartic and L- Glutamic acid. The amino acid group linked to sweet taste includes L- Alanine, L- Glycine, L- Serine and L- Threonine which are small and hydrophilic molecules. The FAA linked to the bitter taste are larger molecules with hydrophobic behavior, such as L- Leucine, L- Isoleucine, L- Phenylalanine, L- Tryptophan. Other molecules with intermediate properties, such as L- Methionine, L- Valine, L- Histidine and L- Arginine, elicit bitter taste too (Kawai, Sekine-Hayakawa, Okiyama, & Ninomiya, 2012).

The characteristic taste of mushrooms, umami taste or palatable taste, is mainly due to the presence of MSG-like FAA, L- Aspartic e L- Glutamic (J.-H. Yang, Lin, & Mau, 2001), which were found in greater amount in the HD sample (3.07 mg/ g DW). According to J.-H. Yang et al. (2001), MSG-like FAA can be split into three ranges: low (< 5 mg/ g), middle (5 – 20 mg/g), and high range (> 20 mg/g). In this study, it was observed that HD sample fits in low range, a result close to that identified by Chen et al. (2015) for samples of *L. edodes* stipe in the first stage of maturity (3.56 mg/g DW). On the other hand, the amount of MSG-like FAA found in HD was higher than that reported by X. Yang et al. (2019) (0.9 – 1.3 mg/g DW) for dried shiitake samples.

Beyond MSG-like FAA, 5'- nucleotides also influence umami taste perception, since the presence of four of the six 5'- nucleotides (5'- AMP; 5'- IMP, 5'- GMP and 5'- XMP) exerts a synergistic effect on umami taste (Dashdorj, Amna, & Hwang, 2015; Poojary et al., 2017; Yamaguchi et al., 1971). In the assessed samples among the six 5'- nucleotides evaluated, 5' - XMP was not found and 5' - IMP was not found in any quantifiable amount (Table 2). The amount of umami nucleotides in the HD sample was lower than in the FD sample, indicating that nucleotides are sensitive to long processes at high temperature (X. Li et al., 2015). 5' - GMP and 5' - UMP contents were higher in FD sample, whereas, 5' - AMP and 5' - CMP content did not differ ($p > 0.05$). The average of umami 5' – nucleotides found in the samples was almost the same to the one found by S. Li et al. (2018) (3.78 mg/g DW) and higher than the amount found by Poojary et al. (2017) (1.54 mg/g DW), this difference may be related to the cultivation techniques used, part of the mushroom used, maturity stage and processing method (Y. Zhang et al., 2013).

The combination of MSG-like FAA and umami nucleotides has synergistic effect on umami taste, a fact which was demonstrated by Yamaguchi et al. (1971). This relation was studied through sensory analysis and expressed as an equation (Equation 1). By evaluating the EUC of the HD and FD samples (Table 2), the influence of the dehydration process on the umami compounds is noticeable, in which the conventional hot-air drying process allowed greater recovery of these compounds, contributing to the greater EUC. In the case of the FD sample, which showed only traces of MSG-like FAA, the presence of umami nucleotides was not sufficient for its EUC to be of significant value.

According to Mau (2005), EUC values can be arranged in four levels: (1) >1000 g MSG/ 100 g DW, (2) 100 - 1000 g MSG/ 100 g DW, (3) 10 -100 g MSG/ 100 g DW, (4) < 10 g MSG/ 100 g DW. Hence, the HD sample fits into the third level (81.55 g MSG/ 100 g DW), corroborating previous studies (Chen et al., 2015; Phat et al., 2016). This result indicates that drying the shiitake stipes by the conventional method (hot-air drying) allows greater extraction of umami compounds (in the optimized condition, item 3.4.1).

3.4.3. Umami ingredient process and properties

HD extract spray drying, using maltodextrin as a carrier agent, provided a yield of 60 ± 0.03 %, which is considered good for the scale used (laboratory). This result was superior to that reported by Francisco et al. (2018) and Ribeiro et al. (2015) for mushroom extract spray drying. Yield is affected by the amount of material deposited on the wall of the equipment, droplets and powder can stick on the walls and cyclone, reducing the amount of product collected at the end of the process (Wang & Langrish, 2009).

The Umami Ingredient showed low moisture and aw (Table 3), revealing the efficiency of the drying process and favoring good stability during future shelf life, since for the observed values of these properties, the microbial growth is not favored (Ross, 2007; Santana, Cano-Higuita, De Oliveira, & Telis, 2016). These results are in agreement to other studies in the literature, which used maltodextrin as a carrier agent in drying of berries extracts and mango pulp, with moisture ranging from 0.92 to 4.7 % (DW) and aw from <0.05 to 0.24 (Gagneten et al., 2019; Zotarelli, da Silva, Durigon, Hubinger, & Laurindo, 2017).

Another property linked to powder stability is the hygroscopicity. This property is closely related to the water concentration gradient between the product and the ambient air, as the higher the concentration gradient, the greater will be its hygroscopicity (Tonon, Brabet, & Hubinger, 2008). Umami Ingredient showed higher hygroscopicity than reported by Gagneten et al. (2019) in their study with berries extracts (11.8 - 13.14 %). However, its performance was similar to that of mango powder (18.8 - 25.4 %) studied by Zotarelli et al. (2017).

The product presented high solubility (Table 3) since maltodextrin has high water solubility (Cano-Chauca et al., 2005) and the umami compounds in the extract are hydrophilic. This characteristic is very important for an ingredient that has the function of enhancing food flavor, since umami compounds must be released in the mouth when in contact with saliva, allowing interaction with umami taste receptors. Studies with berries extracts (Gagneten et al., 2019) and mango juice (Cano-Chauca et al., 2005) also reported high solubility (> 90 %), using maltodextrin as a carrier agent.

Table 3. Physicochemical properties of Umami Ingredient.

Moisture (%)	2.76 ± 0.15
Solubility (%)	99.03 ± 0.01
Aw	0.159 ± 0.001
Hygroscopicity (%)	20.77 ± 0.16
Sodium (mg Na/100 g)	83.52 ± 1.07
Glu retention (%)	97 ± 0.01
Color	
*L**	90.56 ± 0.52
*a**	4.57 ± 0.03
*b**	17.97 ± 0.07
Particle size distribution	
D_{10} (µm)	4.24 ± 0.05
D_{50} (µm)	10.33 ± 0.28
D_{90} (µm)	17.80 ± 0.81
$D_{4.3}$ (µm)	10.87 ± 0.36
Span	1.31 ± 0.05

Each value is expressed as mean ± standard deviation (n = 3).

The coloring of an ingredient is another quality parameter that should be monitored as the color of food is related to consumer acceptance (Selani et al., 2016). Umami Ingredient exhibited a high value of L* (Table 3), indicating high luminosity, favored by the white coloration of maltodextrin. This is a positive result, as dark-colored ingredients have limited application in food (Toledo et al., 2019). The product under study presented low red color intensity (a* value) and higher yellow color intensity (b* value), which was expected since the extract of shiitake stipes has brownish coloring, which can lead to changes in yellow color (Mattar et al., 2018).

Particle size distribution is an important parameter of particulate system quality as it affects its transport, storage and physical and chemical properties, changing its performance (Tontul & Topuz, 2017). D_{10}, D_{50} and D_{90}, which represent, respectively, 10%, 50%, and 90% of the volumetric diameter of the accumulated particles, presented unimodal distribution

(Figure 2), with typical polydispersity of products obtained by spray drying (represented by the Span number, Table 3). Similar values were observed in other drying studies that used resembling equipment (Alvim et al., 2016; Fadini et al., 2018). The mean diameter of the particles, $D_{4.3}$, was close to that reported by Vardanega, Muzio, Silva, Prata, & Meireles (2019) (9.00 µm) which produced microparticles of Brazilian ginseng roots by spray drying. In accordance with the literature, the particle size is affected by process conditions, liquid viscosity and carrier agent concentration (Tonon et al., 2008).

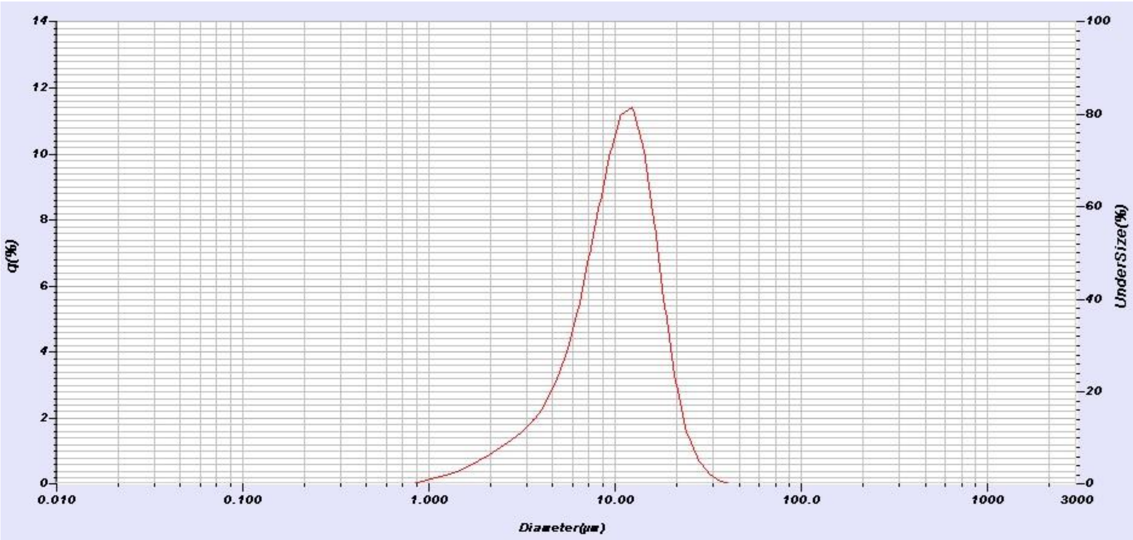

Figure 2. Particle size distribution of spray-dried shiitake stipe extract (Umami Ingredient).

The spray drying process using maltodextrin as an excipient, in addition to enable the drying of the liquid extract and convert it into powder, can help to protect the extract components during the process. To evaluate the efficiency in umami compounds retention, the main umami compound, L- Glu, was monitored. Umami Ingredient contained 1.81 mg L- Glu/g DW, representing 97% retention of L- Glu (Table 3) contained in the extract at the beginning of the spray drying process. This promising result shows the efficiency of the spray drying process in preserving L- Glu.

Considering the embedding of this ingredient in food products, the application of Umami Ingredient in salty foods with reduced sodium content is indicated, since the presence of umami compounds enhances the perception of salty taste (Mojet et al., 2004; Yamaguchi & Takahashi, 1984), assisting in the preservation of flavor. In addition to concerns about excessive sodium consumption and health risks (WHO, 2014), the inclusion of Umami Ingredient could enhance food flavor, adding little sodium (83.52 mg Na/100 g) to the product. It should also be emphasized that the umami compounds present in Umami Ingredient are those naturally present in the shiitake stipes. This supports the positive perception of consumers, who consider healthier and safer non-added MSG foods and, meets the growing demand for products without MSG added (Radam et al., 2010).

3.5. Conclusions

It was possible to obtain an extract with umami compounds from shiitake stipes after defining optimal extraction conditions (1:20 solute: solvent (w:v), i.e. 1 g of shiitake stipe (HD): 20 mL of water), 30 min of extraction, regardless of the temperature), using only water as a solvent. Hot-air drying favored the release of FAA and reduced the amount of some 5' - nucleotides (5' – GMP and 5' – UMP). Spray dried extract consisted in a high L - Glu (main umami compound) retention product, that presented high solubility, low moisture and sodium content, in addition to light color, all suitable characteristics for a new food ingredient. The development of Umami Ingredient indicates a possibility to add value to a food byproduct. The potential of this ingredient as a flavor enhancer for low sodium foods has been assessed and results will be shown in future publications.

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4. UMAMI INGREDIENT, FLAVOR ENHANCER FROM SHIITAKE BYPRODUCTS, IN LOW-SODIUM PRODUCTS: A STUDY CASE OF APPLICATION IN CORN EXTRUDED SNACKS[2]

Abstract

Although it is not an easy task, the sodium reduction in processed food is necessary, and in this scenario, the research for tasty food with reduced sodium content has an extremely relevant role. In this study, sodium reduction in corn extruded snacks was evaluated. In order to minimize sensory losses caused by sodium reduction, monosodium glutamate (MSG) and Umami Ingredient (a new flavor enhancer ingredient obtained from shiitake mushroom byproducts) were used as flavor enhancers. The physical (expansion ratio, density and cutting force) and sensory (acceptance and characterization by the Rate-all-that-apply method) characteristics of the snacks, resulting from sodium reduction and addition of Umami Ingredient, were investigated. Snacks with lower sodium content exhibited a lower acceptance compared to the control, but they were not rejected by the consumers. Umami Ingredient performed similarly to MSG on seasoning flavor, salty and umami tastes. From this result, Umami Ingredient can be considered a potential alternative as a substitute for MSG in low-sodium food, acting as a flavor enhancer and contributing to reduce the use of artificial additives in the products formulation.

Keywords: Sodium reduction; Umami taste; Mushroom; Sensory analysis; Rate-all-that-apply.

4.1. Introduction

Despite performing important functions in the body such as maintaining cellular functioning and osmotic pressure of extracellular fluids and the transmission of nerve impulses (Dötsch et al., 2009), sodium can also be a villain to health when consumed in excess. Scientific evidence points out that high sodium consumption can lead to the development of chronic non-communicable diseases, such as hypertension and increased risk of kidney and cardiovascular diseases (Gilbert & Heiser, 2005; WHO, 2012).

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In addition to these physiological functions, sodium elicits salty taste (Doyle & Glass, 2010), playing an important role in increasing palatability in foods, such as soups, breads, meats, sauces and snacks (Dötsch et al., 2009). However, its use in food should be cautious, since, to avoid the risk of chronic diseases, the World Health Organization (WHO) recommends the consumption of less than 5 g of salt/day, which corresponds to 2 g of sodium/day (WHO, 2014).

In several countries, sodium consumption is high (WHO, 2014). The Brazilian population consumes on average 9.34 g of salt/ day (Mill et al., 2019), which is approximately twofold the recommended amount. For this reason, actions have been implemented in Brazil to reduce the sodium content in several categories of processed foods, such as instant noodles and bakery products, dairy products, meat products and snack food (Brasil, 2018). Among these products, extruded snacks, traditionally, have high content of fat and salt, being regarded as highly energetic but nutritionally poor (Brennan, Derbyshire, Tiwari, & Brennan, 2013; Hess, Jonnalagadda, & Slavin, 2016).

Promoting significant changes in food sodium content is a challenge, as such changes are usually accompanied by technological and sensory modifications (Dötsch et al., 2009), and can reduce consumer acceptance of products (Liem, Miremadi, & Keast, 2011). One of the alternatives to minimize the sensory impacts of sodium reduction in foods is the replacement of sodium chloride (NaCl) with other types of salts, such as potassium chloride (KCl) and calcium chloride ($CaCl_2$). Notwithstanding that, such substitutions are not favorable, as potassium chloride and calcium chloride can also result in bitter and metallic taste in the products (Mitchell, 2019). Another alternative is the improvement of other tastes that enhance the perceived saltiness (Kremer, Mojet, & Shimojo, 2009), as is the case of umami taste (Mojet, Heidema, & Christ-Hazelhof, 2004).

Umami taste is mainly linked to the presence of glutamic acid and its salt, monosodium glutamate (MSG) (Dermiki, Phanphensophon, Mottram, & Methven, 2013; Yamaguchi, 1991), widely used by the food industry. However, there is a trend towards the use of natural flavor enhancers, since consumers have a negative perception about MSG-added products (Radam, Yacob, Bee, & Selamat, 2010). In this context, mushrooms stand out for being rich in umami compounds (Manninen, Rotola-Pukkila, Aisala, Hopia, & Laaksonen, 2018; Phat, Moon, & Lee, 2016; Poojary, Orlien, Passamonti, & Olsen, 2017). The use of mushroom extracts in food has shown promising results, as presented by Dermiki et al. (2013) and Mattar et al. (2018). Anyway, there is a lack of studies on the application of extracts obtained from shiitake mushroom byproducts as flavor enhancers in food.

Developed by Harada-Padermo et al. (2020), the Umami Ingredient consists of a new ingredient, rich in umami compounds, obtained from shiitake (*Lentinula edodes*) mushroom byproducts, which can assist in maintaining low-sodium food flavor. Therefore, this study aims to evaluate the physical and sensory characteristics of products with reduced sodium content added of Umami Ingredient (new flavor enhancer), having as study case the application on corn extruded snacks.

4.2. Materials and methods

4.2.1. Materials

Corn grits (Master SP Alimentos, Capela do Alto, Brazil) were used for extrusion. Sunflower oil (Soya, São Paulo, Brazil), salt (sodium chloride (NaCl), Cisne, Cabo Frio, Brazil), monosodium glutamate (Ajinomoto, Limeira, Brazil), cheese aroma (Duas Rodas, Jaraguá do Sul, Brazil) and Umami Ingredient were used for flavoring. The Umami Ingredient was obtained from shiitake (*Lentinula edodes*) byproducts (stipes) and all the information about it was described by Harada-Padermo et al. (2020).

4.2.2. Extrusion process and flavoring

Corn grits with 15% moisture (wet basis) were extruded in an RXPQ Labor 24 single screw extruder (INBRAMAQ, Ribeirão Preto, Brazil) with five independent heating zones. The extrusion conditions were: three helicoidally grooved barrel; screw with a large step of one exit with a compression ratio of 2.3:1 and length-to-diameter ratio of 15.5:1; pre-die extruder with holes of 3.01 mm; extruder die with a diameter of 2.93 mm (round hole); feed rate of 265 g/min; screw speed at 237 rpm; temperatures in zones 1 to 5: off (around 25 ºC), 70 ºC, 90 ºC, 140 ºC and 140 ºC respectively.

After extrusion, the snacks were cut into 50 mm length pieces, placed in plastic packaging and flavored with a mix of ingredients presented in Table 1. The sunflower oil was sprinkled on the snacks and the remaining ingredients were added. The concentration of the ingredients applied in this study (Table 1) was based on studies previously carried out (Menis-Henrique et al., 2019; Menis-Henrique, Janzantti, Monteiro, & Conti-Silva, 2020) and preliminar tests (data not shown).

The sodium concentration of the snacks was calculated as the sum of sodium from salt, monosodium glutamate (MSG) and Umami Ingredient (Harada-Padermo et al., 2020). The sodium content of the control is in line with that of commercial cheese-flavored snacks in

Brazil, whose content varies from 99 to 315 mg of sodium per portion of 25 g (survey of commercial samples on the market – data not shown).

Table 1. Flavoring ingredients concentration and snacks sodium content.

		Snack 1 (control)	Snack 2	Snack 3	Snack 4
Ingredients (g/100 g of extrudate)	Sunflower oil	6.0	6.0	6.0	6.0
	Cheese aroma	1.5	1.5	1.5	1.5
	Salt (NaCl)	2.8	0.8	0.8	0.8
	Monosodium glutamate (MSG)	0.6	0.6	0	0
	Umami Ingredient	0	0	1.0	1.5
Sodium content (mg/25 g of snacks)		263	89	72	71
Sodium reduction percentual (%)		0	66.2	72.6	73

4.2.3. Analyses of the physical properties of the snacks

For the physical properties' analyses, ten snack samples taken at random were used. The expansion ratio was determined by the ratio between the snack diameter and the extruder die diameter. Samples diameter and length were measured using a digital caliper (Digimess, São Paulo, Brazil), and they were also weighed on an analytical balance. Density (g/cm³) was calculated using the equation: $\rho = 4W/\pi D^2 L$, where W (g) is the weight, D (cm) is the diameter and L (cm) is the length of each snack (Chávez-Jáuregui, Silva, & Arêas, 2000).

The cutting force was determined using a TA.XT *plus* 50 (Stable Micro Systems, Godalming, UK) texture analyzer and the software *Exponent 32* (Stable Micro Systems, Godalming, UK). The samples were cut perpendicularly using a *Blade set* probe with guillotine (HDP/BS), using a pre-test speed of 5 mm/s, test speed of 1 mm/s and post-test speed of 10 mm/s. The maximum force (N) was considered the cutting force of the snack (Paula & Conti-Silva, 2014).

4.2.4. Sensory evaluation of the snacks

The sensory analyses were performed at the Sensory Analysis laboratory, of the Department of Food Technology and Engineering, Institute of Biosciences, Humanities and Exact Sciences, São Paulo State University. This study was approved by the Research Ethics

Committee from “Luiz de Queiroz” College of Agriculture, University of São Paulo (Decision 2.994.710), which contributed to this study. To participate in this study, ninety-nine consumers (from 18 to 62-year-old, 69% female) were recruited from students and employees of the institution. Among these, 92% reported ‘liking a lot or a little’ cheese-flavored snacks. In relation to consumption frequency, 19% reported consume cheese-flavored snacks fortnightly, while 64% consume once a month. In addition, 80% of consumers reported concern about sodium consumption from food in general and 70% said that they buy products with reduced sodium content. When asked about the decisive factors for purchasing low-sodium products (multiple-choice question, with the possibility to point how many answers were appropriate), 26% of consumers pointed concern of health, 25% price, 24% flavor and 21% quality and 4% brand.

The corn extruded snacks were evaluated regarding to: 1) sensory acceptance of the attributes odor, texture, salty taste, flavor and overall liking, using the nine-point structured hedonic scale (9 = like extremely, 5 = neither like nor dislike, 1 = dislike extremely); and 2) ideal intensity of salty taste and cheese flavor, using the Just-About-Right (JAR) scale of nine points (9 = extremely more intense than ideal, 5 = ideal intensity, 1 = extremely less intense than ideal) (Meilgaard, Civille, & Carr, 2016).

The samples were also evaluated using the descriptive method Rate-All-That-Apply (RATA) performed by consumers (Ares, Bruzzone, et al., 2014). The sensory attributes applied in this test were raised by experts after pre-tests and attributes from previous studies with corn extruded snacks were also used (Menis-Henrique et al., 2020). In RATA test, consumers selected the applicable attributes for each sample and indicated the intensity of the selected attributes, using a three-point scale (low, medium and high). Attributes that were not suitable for describing the samples were classified as ‘not applicable’. Thus, for data analysis, the scale was extended to 4 points and decoded into numerical values according to the recommendation of Meyners, Jaeger & Ares (2016).

The sensory analyses were performed in individual booths under white light, at 22 °C. The snacks were presented in napkins encoded with random three-digit numbers, in portions containing 3 pieces of 50 mm length each. Along with the samples, consumers received a glass of water to drink between the samples. The presentation was monadic and the order balanced in complete blocks (FIZZ Sensory Analysis Software, version 2.50) in both tests. RATA attributes were randomized in the evaluation forms for each sample and each consumer, following a Williams’ Latin Square design, as recommended by Ares, Etchemendy, et al. (2014).

4.3. Statistical analyses

Data from physical analyses was submitted to one-way ANOVA and the sensory data was submitted to two-way ANOVA, considering samples and consumers as factors. Then, for both cases, when difference between the samples was detected, the comparison of the variables' mean was performed using the Tukey test. The analyses were conducted at Statistica 10.0 (StatSoft Inc., Oklahoma, USA) at 5% significance level.

The PLS (Partial least square) analysis was applied to the data, considering the overall liking of the snacks as dependent variable and the sensory attributes from RATA as the explanatory (independent) variables. The analysis was performed using the XLSTAT statistical software for Microsoft Excel.

4.4. Results and discussion

4.4.1. Physical properties of the snacks

In this study, the extrusion conditions used for the snacks production were the same for all samples, so the density and cutting force (Table 2) did not differ significantly between the products. The expansion ratio differed between Snacks 2 and 3 (Table 2, $p \leq 0.05$), however, this is probably due to variations that occurred during the extrusion process and not to extrusion parameters modification. The dough degree of puffing after the extruder exit is described by the expansion ratio and the density. The expansion ratio considers the expansion in only one direction, perpendicular to extrudate flow, while the density considers the expansion in all directions (Falcone & Phillips, 1988). Therefore, expansion ratio measurement are more subject to variation than density measurements (Meng, Threinen, Hansen, & Driedger, 2010).

Expansion ratio and density measurements are useful to estimate the extrudates degree of expansion (Saldanha do Carmo et al., 2019), while the cutting force has a strong correlation with the sensory perception of texture attributes (hardness, fracturability and chewiness) (Paula & Conti-Silva, 2014). The snack samples presented low density and low cutting force, it occurs because high temperatures applied during extrusion (as applied here, 140°C) reduce the viscosity of the melt, allowing greater expansion and, consequently, less density, leading to a reduction in the cutting force (Menis-Henrique et al., 2020; Yuliani, Torley, D'Arcy, Nicholson, & Bhandari, 2006). According to Meng et al. (2010) desirable extruded snacks are characterized by high expansion ratio, low density and hardness, characteristics presented by the snack samples evaluated in the present study. Similar density, expansion ratio and cutting force results were found by Menis-Henrique et al. (2020) in their study about corn extruded snacks.

Table 2. Physical properties of the snacks.

Samples	Physical properties		
	Density (g/cm³)	Expansion ratio	Cutting force (N)
Snack 1 (control)	0.09 ± 0.01[ns*]	4.03 ± 0.28[ab]	19.47 ± 4.61[ns]
Snack 2	0.09 ± 0.01[ns]	3.85 ± 0.14[b]	21.92 ± 1.74[ns]
Snack 3	0.09 ± 0.02[ns]	4.26 ± 0.34[a]	21.34 ± 4.80[ns]
Snack 4	0.09 ± 0.01[ns]	4.12 ± 0.31[ab]	21.13 ± 3.22[ns]

Each value is expressed as mean ± standard deviation (n = 10). Different lowercase letters within a column are significantly different by Tukey test ($p \leq 0.05$). *ns: not significant.
Snack 1: 2.8% salt, 0.6% MSG, 0% Umami Ingredient; Snack 2: 0.8% salt, 0.6% MSG, 0% Umami Ingredient; Snack 3: 0.8% salt, 0% MSG, 1.0% Umami Ingredient; Snack 4; 0.8% salt, 0% MSG, 1.5% Umami Ingredient.

4.4.2. Sensory acceptance of the snacks

Sodium reduced snacks samples (Snacks 2, 3 and 4) had, in general, less sensory acceptance for odor, texture, salty taste, flavor and overall liking ($p \leq 0.05$) regarding to the control sample (Table 3). That is because reducing sodium can have adverse effects on salty taste and influence the entire flavor profile of a product, since flavor perception combines gustative perception of soluble and non-volatile compounds (basic tastes), volatile compounds through retronasal olfaction (aroma) and chemical sensations through the trigeminal nerve (Conti-Silva & Souza-Borges, 2019; Liem et al., 2011). Nevertheless, the snacks were not rejected by the consumers, since the sodium reduced samples had scores around 6 (like slightly) for overall liking (from 5.74 to 6.03). Besides this, the Umami Ingredient (Snacks 3 and 4) presented MSG-rated performance (Snack 2) ($p > 0.05$), exerting the function of flavor enhancer, showing statistically equal score for the attributes from the hedonic evaluation. This highlights the potential of the umami compounds present in the Umami Ingredient (Harada-Padermo et al., 2020) to enhance the salty taste, as reported in previous studies about the interaction of salty and umami tastes (Mojet et al., 2004; Yamaguchi & Takahashi, 1984).

The ideal salty taste intensity showed that Snack 1 presented salty taste intensity around 5 (4.96), which represents ideal intensity of salty taste. The other samples received lower scores ($p \leq 0.05$), between 3.38 and 3.95, which represents from slightly less intense to moderately less intense than ideal. The ideal cheese flavor intensity for sodium reduced snacks

(Snacks 2, 3 and 4) were lower than the control sample (Snack 1) ($p \leq 0.05$). The ideal intensity of certain sensory attributes goes beyond the sensory perception of each individual, it is also related to the preference and consumption habits of each person. Scientific evidence points out that salty taste preference is due to previous sensory experience (Antúnez, Giménez, Alcaire, Vidal, & Ares, 2019; Liem et al., 2011). In Brazil, for example, the population has the habit of consuming food with a high amount of salt (Mill et al., 2019), above the recommended by WHO (5 g of salt/day) (WHO, 2014). Thus, it is expected that, initially, reductions in food salt content cause drop in sensory acceptance by consumers. However, the preference for a specific level of saltiness can be modified by repeated exposures to products with less salt (Dötsch et al., 2009),and it is up to the food industry to increase the supply of tasty and low-sodium products.

Table 3. Sensory acceptance of the snacks.

	Samples			
	Snack 1 (control)	Snack 2	Snack 3	Snack 4
Hedonic scale				
Odor	6.74 ± 1.66^{a}	6.34 ± 1.49^{ab}	6.09 ± 1.78^{b}	6.08 ± 1.77^{b}
Texture	7.18 ± 1.45^{a}	6.64 ± 1.80^{b}	6.72 ± 1.81^{ab}	6.52 ± 1.76^{b}
Salty taste	7.06 ± 1.41^{a}	5.75 ± 1.80^{b}	5.55 ± 1.95^{b}	5.29 ± 2.08^{b}
Flavor	7.00 ± 1.55^{a}	5.79 ± 1.77^{b}	5.53 ± 2.09^{b}	5.47 ± 1.93^{b}
Overall liking	7.03 ± 1.34^{a}	6.03 ± 1.53^{b}	5.88 ± 1.80^{b}	5.74 ± 1.69^{b}
Just-about-right scale				
Ideal salty taste intensity	4.96 ± 1.01^{a}	3.95 ± 1.17^{b}	3.48 ± 1.25^{c}	3.38 ± 1.34^{c}
Ideal cheese flavor intensity	4.26 ± 1.07^{a}	3.70 ± 1.13^{b}	3.30 ± 1.22^{c}	3.19 ± 1.20^{c}

Each value is expressed as mean ± standard deviation (n = 99). Different lowercase letters within a row are significantly different by Tukey test ($p \leq 0.05$).
Snack 1: 2.8% salt, 0.6% MSG, 0% Umami Ingredient; Snack 2: 0.8% salt, 0.6% MSG, 0% Umami Ingredient; Snack 3: 0.8% salt, 0% MSG, 1.0% Umami Ingredient; Snack 4; 0.8% salt, 0% MSG, 1.5% Umami Ingredient.

4.4.3. Sensory profile of the snacks

There was no significant difference between the samples in relation to the yellow color (Table 4), showing that the application of the Umami Ingredient did not interfere in the snacks coloring. The sensory attributes related to the snacks texture, crispness and hardness, also did not differ among samples (Table 4) ($p > 0.05$), what was expected by the fact that all samples were obtained under the same process conditions (item 4.2.2).

Regarding to odor, the oil odor did not significantly differ among the samples, although, the cheese odor perception was reduced for Snack 4 compared to the other samples ($p \leq 0.05$). This sample contained greater concentration of Umami Ingredient (Table 1) and, this can have caused changes in the sample odor, since the dried shiitake, such as its extract, that are raw material used for Umami Ingredient production (Harada-Padermo et al., 2020), have a varied range of volatile compounds (Dermiki et al., 2013; Politowicz, Lech, Lipan, Figiel, & Carbonell-Barrachina, 2017) that may have altered the perception of the cheese odor.

Regarding the flavor attributes (Table 4), cereal flavor, oil flavor and garlic flavor did not differ among the samples ($p > 0.05$). The cheese flavor was more intense in the control sample (Snack 1) and less intense in the samples with less sodium, especially for Snack 4 ($p \leq 0.05$). In the same way, as previously explained for cheese odor, the volatile compounds of Umami Ingredient may have altered the cheese flavor perception (mainly in Snack 4), since the perception of flavor is due to the interaction of compounds connected to basic tastes together with volatile compounds perceived by retronasal olfaction (aroma) and chemical perceptions (Conti-Silva & Souza-Borges, 2019). For the onion flavor, only Snack 3 differed significantly from the control sample (Snack 1), probably it is due to the lower salt content and coupled with the lower concentration of Umami Ingredient. The seasoning flavor was perceived with greater intensity in the control sample than in the sodium reduced samples ($p \leq 0.05$). Altogether, the modifications on the snacks flavor perception regarding to the control sample is, mainly, due to the reduction in the salt content (sodium) of the samples (Snacks 2, 3 and 4), this because the reduction of perceived saltiness relates to multiple flavor effects. The perceived saltiness is also influenced by the food matrix nature (e. g. salty taste in aqueous solution is easier to perceive than in solid food matrix, at the same concentration), as well as, interactions with other taste components. Thereby, the general influence of sodium reduction is reduced saltiness, reduction of sweetness, higher bitterness, reduction on appetitive aromas associated with salty and sweet

Table 4. Sensory attribute scores obtained by Rate-all-that-apply (RATA) to the snacks.

Attributes	Samples			
	Snack 1 (control)	Snack 2	Snack 3	Snack 4
Yellow color	1.83 ± 0.73[ns*]	1.73 ± 0.71[ns]	1.77 ± 0.73[ns]	1.78 ± 0.69[ns]
Oil odor	0.67 ± 0.76[ns]	0.52 ± 0.75[ns]	0.60 ± 0.75[ns]	0.55 ± 0.66[ns]
Cheese odor	1.63 ± 0.80[a]	1.42 ± 0.76[a]	1.39 ± 0.79[ab]	1.17 ± 0.77[b]
Crispness	2.21 ± 0.66[ns]	2.05 ± 0.69[ns]	2.09 ± 0.77[ns]	2.06 ± 0.78[ns]
Hardness	1.38 ± 0.79[ns]	1.26 ± 0.80[ns]	1.41 ± 0.78[ns]	1.39 ± 0.73[ns]
Cereal flavor	0.93 ± 0.87[ns]	0.92 ± 0.87[ns]	1.07 ± 0.95[ns]	1.08 ± 0.91[ns]
Oil flavor	0.56 ± 0.73[ns]	0.51 ± 0.71[ns]	0.57 ± 0.74[ns]	0.57 ± 0.67[ns]
Cheese flavor	1.73 ± 0.75[a]	1.36 ± 0.61[b]	1.21 ± 0.66[bc]	1.13 ± 0.63[c]
Garlic flavor	0.34 ± 0.63[ns]	0.28 ± 0.55[ns]	0.22 ± 0.46[ns]	0.25 ± 0.52[ns]
Onion flavor	0.43 ± 0.64[a]	0.30 ± 0.60[ab]	0.24 ± 0.54[b]	0.32 ± 0.57[ab]
Seasoning flavor	1.53 ± 0.85[a]	1.01 ± 0.75[b]	0.86 ± 0.67[b]	0.96 ± 0.65[b]
Salty taste	1.95 ± 0.68[a]	1.23 ± 0.57[b]	1.28 ± 0.61[b]	1.20 ± 0.64[b]
Umami taste	0.92 ± 0.89[a]	0.89 ± 0.82[ab]	0.69 ± 0.75[b]	0.73 ± 0.81[ab]
Sweet taste	0.24 ± 0.55[b]	0.34 ± 0.64[ab]	0.34 ± 0.61[ab]	0.41 ± 0.73[a]

Each value is expressed as mean ± standard deviation (n = 99). Different lowercase letters within a row are significantly different by Tukey test ($p \leq 0.05$). *ns: not significant. Snack 1: 2.8% salt, 0.6% MSG, 0% Umami Ingredient; Snack 2: 0.8% salt, 0.6% MSG, 0% Umami Ingredient; Snack 3: 0.8% salt, 0% MSG, 1.0% Umami Ingredient; Snack 4; 0.8% salt, 0% MSG, 1.5% Umami Ingredient.

taste and higher of aversive aromas associated with bitter taste (Liem et al., 2011).

For the salty taste, it is remarkable that the reduction in salt content changed the perceived saltiness (Table 4), although, the Umami Ingredient performed similarly to the flavor enhancer MSG (umami taste standard) on the salty taste of the sodium reduced samples (Snack 2, 3 and 4) ($p > 0.05$). Regarding the umami taste, Snack 3 presented significantly less intense of this attribute than the control, possibly because it has lower concentration of the Umami Ingredient. For umami taste to reach similar intensity to that of the control sample, it would be necessary to increase the concentration of Umami Ingredient, as in the case of Snack 4. The sweet taste was perceived in low intensity for all samples, being higher in Snack 4 regarding to the control snack (Table 4) ($p \leq 0.05$), which may be related to the maltodextrin present in the Umami Ingredient. Despite playing an important role during the production (spray drying) of the Umami Ingredient (Harada-Padermo et al., 2020), maltodextrin may have low or no sweetness, which is related to its DE (dextrose equivalency), since as it increases, greater is the sweetness (BeMiller, 2019).

The PLS analysis (Figure 1) resulted in a cumulated R^2 of 67.5% for two components, and it shows the effects of the sensory attributes of the snacks on the overall liking. Snack 2 was not stand out by any attributes, which indicates that such sample showed intermediate intensities, of the attributes on the sensory profile. Snacks 3 and 4 were described by sweet taste, cereal flavor, hardness and oil flavor, while the control snack was described by all the others attributes. The attributes that described the control snack were responsible by the great overall liking for this sample. Researches have shown that today, although consumers are more concerned with their health and well-being, most of them still prioritize their preference and consume what they like or are used to, without taking into account the effects on health, prioritizing the sensory characteristics of the product (Carrillo, Varela, Salvador, & Fiszman, 2011; Conti-Silva & Souza-Borges, 2019; Hernández-Carrión, Varela, Hernando, Fiszman, & Quiles, 2015). However, there are some consumers that liked the low-sodium snacks, especially the Snacks 3 and 4, which shows that even with different sensory profiles, there are different market niches of consumers that search for low-sodium snacks and with good appreciation of such products. As shown by Nguyen & Wismer (2019), 52% of consumers perceived sodium reduced potato chips as *not salty enough*, while the regular version was perceived as *too salty* by 45% of consumers. They also showed that saltiness perception (as *too salty* or *not salty enough*) was different according to participants' consumption of dietary sodium sources, with higher liking of the reduced version by low dietary sodium sources participants. It ratifies that the current market presents different niches, due to consumers diversified preference, and it is a challenge to answer all consumers preferences and needs. Therefore, the utilization/application of the Umami Ingredient adds umami compounds naturally present in shiitake mushroom that can contribute to good consumer perception about the products to which it is added, known as ‘no added MSG’ (Radam et al., 2010), besides resulting in products with low-sodium content (Harada-Padermo et al. (2020) and Table 1), helping the development of new products that meet consumers’ desire.

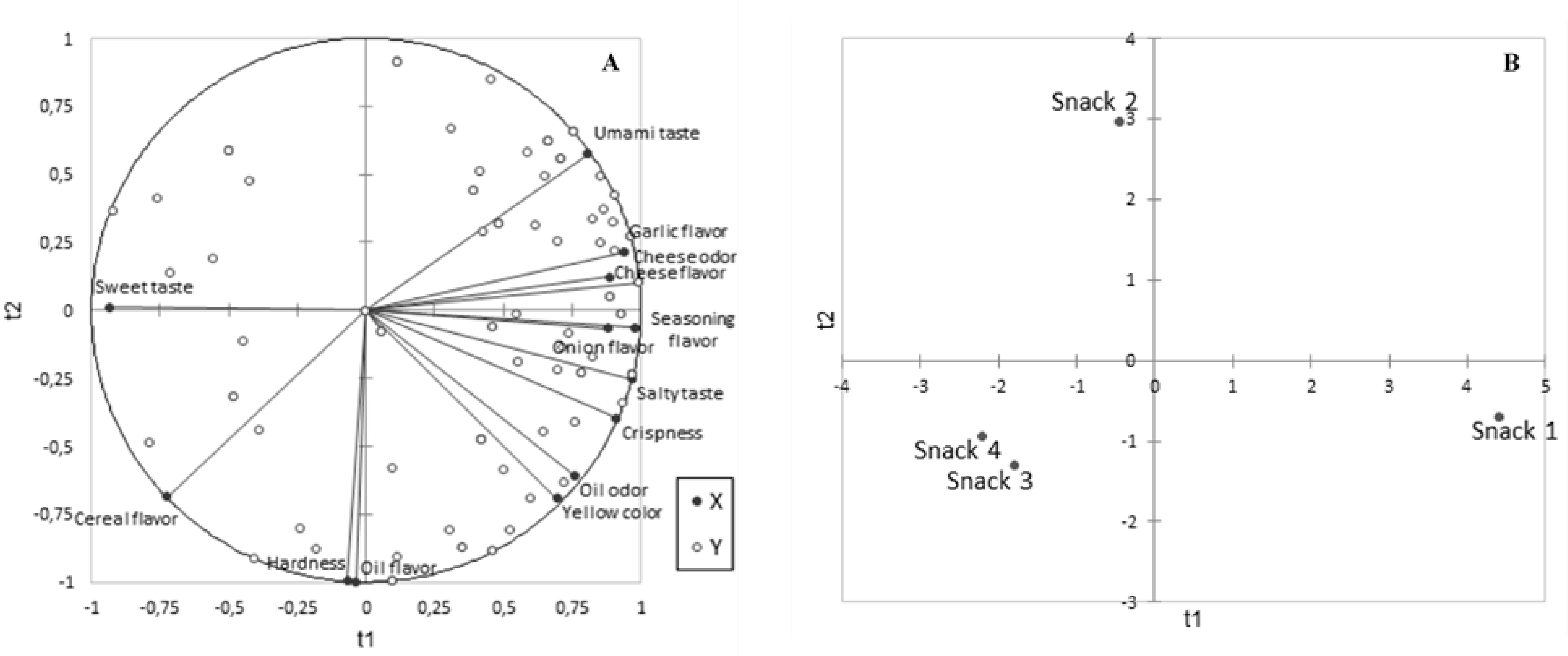

Figure 1. Results from PLS analysis for snacks. A - Projection of variables (X = explanatory variables - attributes; Y = dependent variables – consumers' overall liking). B - Projection of products. Snack 1: 2.8% salt, 0.6% MSG, 0% Umami Ingredient; Snack 2: 0.8% salt, 0.6% MSG, 0% Umami Ingredient; Snack 3: 0.8% salt, 0% MSG, 1.0% Umami Ingredient; Snack 4; 0.8% salt, 0% MSG, 1.5% Umami Ingredient.

4.5. Conclusions

The snacks presented low density and cutting force, in addition to high expansion ratio, all desirable characteristics for extruded snacks. The reduction of sodium in the snacks caused modifications in sensory profile and also in acceptance of the samples by consumers, especially in comparison to the control sample. Despite the decrease in acceptance, the sodium reduction did not result in product rejection, which may be related to the use of flavor enhancers. The Umami Ingredient proved to be efficient as a flavor enhancer, presenting similar results to MSG (standard umami compound) for attributes such as seasoning flavor, salty and umami tastes. This result is promising, since the Umami Ingredient can substitute the MSG, aggregating umami compounds obtained directly from shiitake byproducts, contributing for the products to have less additives added, especially in low-sodium food formulations. We emphasize here that its application and evaluation in other types of food is recommended to evaluate its potential in other food matrices. As it is a complex process, it is expected that, initially, the reduction in sodium content will cause a drop in the products acceptance by consumers, however, these actions are necessary for the population to habituate their sense of taste and be less exposed to the occurrence of non-communicable chronic diseases, such as hypertension and cardiovascular problems.

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5. UMAMI INGREDIENT IN LOW-SODIUM BEEF BURGERS: A PRELIMINARY STUDY OF THEIR PHYSICOCHEMICAL AND TEXTURE PROPERTIES[3]

Abstract

Lowering sodium in meat products is a challenge since salt plays important functions for technological and sensory characteristics. The development of low-sodium beef burgers added with Umami Ingredient and the impact on the physicochemical and texture properties were investigated. Among the evaluated treatments, the low-sodium samples showed significant differences for pH, color and aw. On the other hand, the treatments did not differ for the cooking properties (cooking loss and diameter reduction). For most of the textural properties (hardness, springiness and chewiness) the samples did not differ from the control sample. These results show that the evaluated treatments had minimal impact on the technological properties of the beef burgers, maintaining most of the physicochemical parameters similar to those of the control sample. This is a promising result, since in this study the sodium content was reduced by up to 70% and a mushroom byproduct-based flavor enhancer (Umami Ingredient) was applied. The sensory properties still need to be evaluated to understand the potential of the Umami Ingredient as a flavor enhancer in low-sodium beef burgers.

Keywords: Mushroom stipes; *Lentinula edodes*; Umami taste; Salt reduction; Meat products

5.1. Introduction

The sodium reduction in food is one of the most discussed subjects in the current days, since the excessive consumption of sodium increases the risk of occurrence of chronic non-communicable diseases, such as hypertension, kidney and cardiovascular problems (Aburto et al., 2013; Gilbert & Heiser, 2005). Aiming at reducing the occurrence of these diseases among the world population, the World Health Organization (WHO) recommends that the sodium consumption do not exceed 2 g per day, which is equivalent to approximately 5 g of salt/day (WHO, 2014).

[3] This chapter will be included in a manuscript intended to be submitted to Meat Science.

However, in food, salt performs functions that go beyond the contribution in the perceived saltiness. It has a relevant role for technological functions, such as assist in the texture development of pasta and meat products, as well as in the preservation and microbiological safety of food (Doyle & Glass, 2010). Because of this, reducing sodium in food is a challenge, as it can have multiple effects on the products, not only changing the physicochemical characteristics, but also affecting the products' acceptance by consumers (Liem, Miremadi, & Keast, 2011), depending on the level of reduction used.

In Brazil, the population consumes, on average, approximately twice as much salt as the WHO recommended amount, 9.34 g of salt/day (Mill et al., 2019). Thus, the Brazilian government and industries signed agreement to reduce sodium of several categories of industrialized food (e.g. as bakery products, meat products, dairy products and snacks) (Brasil, 2018), as a way to reduce the amount of sodium consumed by the population.

Among meat products, beef burger is one of the most consumed, both for its sensory characteristics and for its practicality. However, this product generally contains a high amount of sodium in its composition (Rios-Mera et al., 2019). Among the most used strategies to reduce sodium content in meat products are: the reduction of salt content by stealth, the use of salt substitutes, the change in the size and shape of the salt crystal and the use of flavor enhancers. However, such changes can trigger undesirable effects for both the physical (since salt plays an important role in the extraction of myofibrillar proteins) (Desmond, 2006; Inguglia, Zhang, Tiwari, Kerry, & Burgess, 2017), and sensory characteristics of the product (Almeida, Villanueva, Pinto, Saldaña, & Contreras-Castillo, 2016). Thus, the reduction of salt in meat products is a challenge that must be studied in detail, since the response to salt reduction appears to be highly product-specific (Inguglia et al., 2017).

In this context, the addition of flavor enhancers, linked to umami taste, can be an interesting alternative to improve the flavor of sodium reduced products, since the umami taste can enhance the perceived saltiness (Mojet, Heidema, & Christ-Hazelhof, 2004). The umami taste is mainly linked to the presence of glutamic acid and its salt, monosodium glutamate (MSG); but this taste can also be elicited or enhanced by other substances, like 5'-nucleotides and umami peptides (Dermiki, Phanphensophon, Mottram, & Methven, 2013; Kong et al., 2019; Yamaguchi, 1991).

However, there is a current trend for the utilization of natural ingredients in the food industry (Carocho, Morales, & Ferreira, 2015), and the umami substances can be naturally found in different kinds of food, such as mushrooms (Kong et al., 2019; Manninen, Rotola-Pukkila, Aisala, Hopia, & Laaksonen, 2018; Phat, Moon, & Lee, 2016; Poojary, Orlien, Passamonti, & Olsen, 2017). Besides, mushrooms have been highlighted as an alternative to be applied in food as natural umami ingredients (Poojary et al., 2017; Zhang, Venkitasamy, Pan, & Wang, 2013). This action can positively affect the consumers' perception regarding to the non-added MSG products (Radam, Yacob, Bee, & Selamat, 2010).

The use of mushroom or mushroom extract has been studied and valuable and positive results have been found for meat products, like beef burgers (Mattar et al., 2018; Patinho et al., 2019; Tom, Alnoumani, & Were, 2018; Wong, Corradini, Autio, & Kinchla, 2019) and beef taco blends (Miller et al., 2014; Wong et al., 2017), where salt (sodium) has been reduced. However, there is still a lack of studies aiming the utilization of mushroom byproducts extract as flavor enhancers in food. The Umami Ingredient (Chapter 3) can be a potential alternative as flavor enhancer to be applied in low-sodium beef burgers. Thus, the aim of this chapter is to evaluate the effect of sodium reduction and addition of Umami Ingredient (as a flavor enhancer) on the physicochemical and texture properties of low-sodium beef burgers[4].

5.2. Materials and methods

5.2.1. Materials

Fresh beef (*Quadriceps femoris*), beef fat and salt were purchased at the local market (Piracicaba, Brazil). The condiments and additives (black pepper, garlic powder, onion powder and sodium erythorbate) were provided by Ibrac (Rio Claro, Brazil). The Umami Ingredient was obtained from shiitake (*Lentinula edodes*) byproducts, all information about this ingredient was provided by Harada-Padermo et al. (2020).

5.2.2. Beef burgers formulations and manufacture

The beef burger formulations, as well as their salt content reductions, were previously tested in another study (to be published elsewhere). Salt reductions of 35% (R1), 52,5% (R2) and 70% (R3) were selected and the inclusion of Umami Ingredient as a flavor enhancer was evaluated. Thus, seven different formulations were performed, as presented in Table 1. The sodium content of the control sample is the average of commercial samples available in the market.

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[4] The sensory analysis of the beef burgers was not included in this chapter because this experiment was interrupted by the coronavirus pandemic and this data was not available until the thesis writing.

Fresh beef and fat were separately ground (Eccel, model MCIE-09, Brusque, Brazil) using a 0.5 cm plate and then the beef was divided into the treatments. After that, water and salt were added to the beef and mixed manually during 5 min. Then, the other ingredients (additives, spices, Umami Ingredient and beef fat) were added and mixed for 3 min. Portions of 100 g were shaped using a burger-maker (10 cm diameter and 1 cm thickness). Part of the analyses (pH, water activity and color) were performed in the raw samples. The cooking procedure was done in a hot plate (150 °C) (Compact 80, Caxias do Sul, Brazil), until the meat core temperature reach 75 °C.

Table 1: Beef burger formulations

Ingredients	Formulations (%)						
	C	**R1S1**	**R1S2**	**R2S1**	**R2S2**	**R3S1**	**R3S2**
Fresh beef	75	75	75	75	75	75	75
Beef fat	15	15	15	15	15	15	15
Water	7.40	7.06	6.06	7.39	6.39	7.73	6.73
Salt	1.90	1.24	1.24	0.91	0.91	0.57	0.57
Umami Ingredient	0	1	2	1	2	1	2
Black pepper	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Garlic powder	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Onion powder	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Sodium erythorbate	0.05	0.05	0.05	0.05	0.05	0.05	0.05

5.2.3. Physicochemical analysis

The pH of raw and cooked burgers was determined by a potentiometer (MS Tecnopon, model MPA-210, Piracicaba, Brazil) with automatic temperature compensation and glass penetration electrode. This analysis was performed on three raw and three cooked samples, with three readings in each sample.

The color of raw and cooked burgers was determined by a colorimeter (Minolta Chroma Meter CR-400, Konica Minolta, NJ, USA), by reading the parameters L* (lightness), a* (redness) and b* (yellowness) of the CIELab system, with D65 light source. This analysis was performed on three burgers of each treatment (raw and cooked), with three readings for each sample. For the cooked samples the inner color was measured, after they reach the room temperature (25°C).

The water activity (aw) was determined in a water activity analyzer (Aqua Lab 4TE, Meter Group, Pullman, USA) at 25 ± 0.1 °C, for the raw and cooked samples. This analysis was performed in triplicate.

5.2.4. Cooking properties

Cooking loss (CL) was expressed as percentage of the weight loss after cooking (Eq. 1) (Selani et al., 2016).

$$CL\ (\%) = \left(\frac{raw\ sample\ weight\ (g) - cooked\ sample\ weight\ (g)}{raw\ sample\ weight\ (g)}\right) * 100 \qquad (1)$$

Diameter reduction (DR) was calculated according to Eq. (2) (Sánchez-Zapata et al., 2010) and expressed as percentage.

$$DR\ (\%) = \left(\frac{raw\ diameter - cooked\ diameter}{raw\ diameter}\right) * 100 \qquad (2)$$

The cooking properties were measured in three burger samples of each treatment.

5.2.5. Texture profile analysis (TPA)

Prior to TPA, the samples were cooked as previously described (item 5.2.2) and cooled to room temperature (25 °C). Three burger samples were taken from each treatment, and three cylindrical samples (2.5 cm diameter) were taken from each beef burger for TPA.

TPA was performed in a TA-XT *plus* (Stable Micro Systems, Godalming, UK) texture analyzer, as described by Selani et al. (2016). The cylindrical samples were subjected to a two-cycle compression test, using a cylindrical probe of 3.6 cm diameter (P/36R, Stable Micro Systems, Godalming, UK), to 75% of their original height at a constant speed of 3,33 cm/min (pre-test speed and post-test speed: 6,66 cm/min). The parameters hardness (N), springiness, cohesiveness and chewiness were determined as described by Bourne (1978).

5.3. Statistical analyses

Data from the analyses was submitted to one-way ANOVA and when difference between samples was detected, Tukey test was performed to compare variables' mean. The statistical analyses were carried out in the R environment (R Core Team, 2017).

5.4. Results and discussion

5.4.1. Physicochemical parameters

Among the raw burgers, there is a tendency of decrease in the values of lightness (L*) and redness (a*) of samples with sodium reduction and addition of Umami Ingredient, which were significant only in the treatments R2S2 and R3S2 for lightness (L*) and in R3S2 for redness (a*) (Table 2, $p \leq 0.05$). The yellowness (b*) values were not affected ($p > 0.05$) in the raw product. Patinho et al. (2019) also did not found difference in yellowness for beef burgers added with fresh *Agaricus bisporus* regarding to the control sample. After cooking, there was a change in the color behavior of the samples with the Umami Ingredient, which did not significantly affect the redness (a*), but were lighter (L*) and more yellow (b*) ($p \leq 0.05$) Processes that occurred during cooking, such as protein denaturation, Maillard reaction, and loss of water and fat, may have promoted these changes, which, according to Sánchez-Zapata et al. (2010) can even mask undesirable changes resulting from the formulation.

Corroborating with this study, Mattar et al. (2018) also did not found difference in redness (a*) and reported higher yellowness (b*) for low-sodium beef burgers added with shiitake liquid extract. This change in the b* value can have been caused by the shiitake aqueous extract color, since it has a brownish color that can cause changes in yellow (Mattar et al., 2018).

The pH was significantly different among treatments for both raw and cooked burgers (Table 2), where a slight increase in values was observed in samples added with Umami Ingredient. According to Choe et al. (2018) the presence of basic amino acids like histidine, lysine and arginine in the mushroom (Kawai, Sekine-Hayakawa, Okiyama, & Ninomiya, 2012; Li et al., 2018; Poojary et al., 2017) can influence the pH of the meat batter. However, from a practical point of view, the pH variation is consistent with the literature, since the pH range of raw beef burgers (5.54 – 5,64) is within the normal meat pH, that is between 5.3 - 5.8 (Honikel, 2014) and the pH of cooked burgers was around 6 (5,98 - 6,02), that is reported to be the usual pH of cooked meat products (Hereu, Dalgaard, Garriga, Aymerich, & Bover-Cid, 2012). Similar pH results were found for raw beef burger by Mattar et al. (2018), who studied beef burgers added with liquid shiitake mushroom extract.

Table 2: Physicochemical properties of raw and cooked beef burgers

Treatments	Instrumental color			pH	Aw
	L*	a*	b*		
Raw burger					
C	47.80 ± 1.54[a]	15.74 ± 2.30[a]	14.77 ± 1.59[ns#]	5.54 ± 0.01[d]	0.980 ± 0.002[c]
R1S1	47.48 ± 0.58[ab]	15.19 ± 0.93[a]	15.43 ± 1.06[ns]	5.57 ± 0.00[c]	0.985 ± 0.002[ab]
R1S2	46.37 ± 1.79[ab]	14.65 ± 0.71[ab]	15.11 ± 0.35[ns]	5.59 ± 0.01[bc]	0.982 ± 0.002[bc]
R2S1	46.35 ± 1.71[ab]	14.68 ± 0.86[ab]	15.10 ± 0.32[ns]	5.60 ± 0.00[bc]	0.986 ± 0.000[ab]
R2S2	43.56 ± 1.01[b]	13.21 ± 0.57[ab]	13.65 ± 0.24[ns]	5.59 ± 0.01[bc]	0.986 ± 0.000[ab]
R3S1	44.44 ± 1.34[ab]	13.75 ± 0.78[ab]	13.69 ± 0.36[ns]	5.64 ± 0.03[a]	0.988 ± 0.001[a]
R3S2	43.68 ± 1.66[b]	11.74 ± 1.07[b]	13.16 ± 0.96[ns]	5.62 ± 0.01[ab]	0.987 ± 0.003[ab]
Cooked burger					
C	52.58 ± 0.35[c]	4.97 ± 0.12[ns]	13.60 ± 0.29[b]	5.99 ± 0.01[b]	0.962 ± 0.001[d]
R1S1	55.23 ± 1.07[ab]	4.98 ± 0.10[ns]	15.83 ± 0.21[a]	5.98 ± 0.01[b]	0.969 ± 0.001[c]
R1S2	54.20 ± 0.46[bc]	5.37 ± 0.73[ns]	15.45 ± 0.72[a]	6.00 ± 0.03[b]	0.975 ± 0.001[a]
R2S1	56.05 ± 0.26[ab]	4.75 ± 0.10[ns]	15.40 ± 0.13[a]	6.08 ± 0.03[a]	0.970 ± 0.001[bc]
R2S2	55.82 ± 1.24[ab]	4.93 ± 0.27[ns]	15.22 ± 0.27[a]	6.02 ± 0.02[b]	0.969 ± 0.002[c]
R3S1	56.89 ± 1.31[a]	5.22 ± 0.79[ns]	16.24 ± 0.23[a]	6.01 ± 0.01[b]	0.974 ± 0.001[ab]
R3S2	55.36 ± 0.36[ab]	5.02 ± 0.10[ns]	15.75 ± 0.58[a]	5.98 ± 0.02[b]	0.974 ± 0.003[ab]

Each value is expressed as mean ± standard deviation. Different lowercase letters within a column indicate significant difference by Tukey test ($p \leq 0.05$) between treatments in the respective form (raw or cooked). [#]ns: not significant.
R1S1: 1.24% salt, 1% Umami Ingredient; R1S2: 1.24% salt, 2% Umami Ingredient; R2S1: 0.9% salt, 1% Umami Ingredient; R2S2: 0.9% salt, 2% Umami Ingredient; R3S1: 0.57% salt, 1% Umami Ingredient; R3S2: 0.57% salt, 2% Umami Ingredient.

Other studies with meat products added with another species of mushroom, like *Agaricus bisporus* and *Flammulina velutipes*, reported an increase in pH of formulations with greater mushroom content (Choe et al., 2018; Jo, Lee, & Jung, 2018; Patinho et al., 2019). The pH of cooked samples is in agreement with the results found by Dermiki et al. (2013) for cooked minced meat with shiitake mushroom extract (5.81).

The aw was significantly different among treatments, being higher for the low-salt formulations. It happens because salt has the ability to reduce the aw, that is the amount of free water available. Salt promotes the diffusion process, in which water flows from the food to higher concentration zones (outside food), dissolving salt, that penetrates the food, consequently reducing the amount of free water, i.e. the aw (Albarracín, Sánchez, Grau, &

Barat, 2011; Inguglia et al., 2017). The aw results corroborates with those found by Patinho et al. (2019).

5.4.2. Cooking properties

The cooking properties were not affected by the salt reduction and addition of the Umami Ingredient (Table 3). Although it is known that salt increases the yield and reduces the cooking losses (Ockerman & Basu, 2014), the modifications performed throughout the treatments did not affect the solubilization of the myofibrillar proteins in the meat, which act binding water in the product, reducing the cooking losses (Desmond, 2006). The evaluated treatments do not seem to have changed the water holding capacity of the samples, which could have directly affected the cooking loss (CL). Another important feature is to maintain the product diameter, since beef burgers are generally used in burger buns, and fill properly the bun contributes to have an appealing product (Gujral, Kaur, Singh, & Sodhi, 2002). The performed treatments did not affect the diameter reduction (DR). The CL ranged from 34 to 40% among treatments and the DR from 23 to 26 %, which are in agreement with the previously reported by Rios-Mera et al. (2019).

Table 3: Cooking properties of beef burgers

Treatments	CL (%)	DR (%)
C	36.30 ± 1.20$^{ns\#}$	23.68 ± 2.22^{ns}
R1S1	35.12 ± 2.37^{ns}	25.78 ± 2.52^{ns}
R1S2	34.70 ± 2.86^{ns}	24.56 ± 1.21^{ns}
R2S1	39.38 ± 2.13^{ns}	26.45 ± 2.01^{ns}
R2S2	35.53 ± 1.37^{ns}	24.73 ± 1.96^{ns}
R3S1	37.66 ± 0.64^{ns}	23.91 ± 0.59^{ns}
R3S2	40.26 ± 2.49^{ns}	25.35 ± 1.50^{ns}

Each value is expressed as mean ± standard deviation. The results did not present significant difference ($p > 0.05$) by ANOVA $^{\#}$ns: not significant.
R1S1: 1.24% salt, 1% Umami Ingredient; R1S2: 1.24% salt, 2% Umami Ingredient; R2S1: 0.9% salt, 1% Umami Ingredient; R2S2: 0.9% salt, 2% Umami Ingredient; R3S1: 0.57% salt, 1% Umami Ingredient; R3S2: 0.57% salt, 2% Umami Ingredient.

5.4.3. Texture properties

The beef burgers did not present difference among the treatments for hardness, springiness and chewiness (Table 4, $p > 0.05$). The treatments R1S1 and R1S2 showed significant difference for cohesiveness, but it did not present a clear tendency to infer the possible cause of this variation. This shows that the addition of Umami Ingredient had minimal effect on beef burgers textural properties and that, even at low levels, the functional capacity of the salt to solubilize myofibrillar proteins and develop the texture was maintained. Mattar et al. (2018) also did not found significant difference for hardness (shear force) in beef burgers added with shiitake liquid extract. Patinho et al. (2019) reported no change in springiness of beef burgers with the addition of *A. bisporus*, but found alterations in hardness, cohesiveness and chewiness, differing from the present study possibly due to the amount (10 to 20%) and way of use of *A. bisporus* (cooked) in the burger formulation.

Table 4: Texture properties of cooked beef burgers

Treatments	Hardness (N)	Springiness (mm)	Cohesiveness	Chewiness (N*mm)
C	203.01 ± 16.31$^{ns\#}$	0.79 ± 0.01^{ns}	0.48 ± 0.01^{ab}	75.63 ± 4.88^{ns}
R1S1	196.24 ± 12.53^{ns}	0.76 ± 0.01^{ns}	0.50 ± 0.01^{a}	74.54 ± 4.38^{ns}
R1S2	162.57 ± 20.77^{ns}	0.74 ± 0.02^{ns}	0.45 ± 0.02^{b}	60.17 ± 9.33^{ns}
R2S1	187.27 ± 13.72^{ns}	0.77 ± 0.01^{ns}	0.46 ± 0.01^{ab}	67.47 ± 7.38^{ns}
R2S2	197.92 ± 26.21^{ns}	0.78 ± 0.01^{ns}	0.49 ± 0.01^{ab}	76.06 ± 12.40^{ns}
R3S1	176.77 ± 23.79^{ns}	0.76 ± 0.03^{ns}	0.48 ± 0.01^{ab}	65.30 ± 9.52^{ns}
R3S2	197.93 ± 19.79^{ns}	0.78 ± 0.00^{ns}	0.48 ± 0.01^{ab}	74.17 ± 8.52^{ns}

Each value is expressed as mean ± standard deviation. Different lowercase letters within a column are significantly different by Tukey test ($p \leq 0.05$). [#]ns: not significant.
R1S1: 1.24% salt, 1% Umami Ingredient; R1S2: 1.24% salt, 2% Umami Ingredient; R2S1: 0.9% salt, 1% Umami Ingredient; R2S2: 0.9% salt, 2% Umami Ingredient; R3S1: 0.57% salt, 1% Umami Ingredient; R3S2: 0.57% salt, 2% Umami Ingredient.

5.5. Conclusion

The levels of salt reduction and addition of the Umami Ingredient showed positive results for the physicochemical parameters of beef burgers. Slight changes in color, pH and aw were identified. For the cooking properties, there was no significant change between samples. Regarding textural properties, just two samples differed in terms of cohesiveness. However, for the other texture properties, the samples did not present difference. This is a promising result, since the treatments presented similar results compared to the control sample, indicating a minimum technological impact.

To understand the sensory impact, sensory analyses need to be performed and the results properly evaluated to infer the role of the Umami Ingredient as a flavor enhancer and its influence on sensory profile, as well as on the consumers acceptance.

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6. GENERAL CONCLUSIONS

Shiitake byproducts (stipes) can be used for the extraction of natural umami compounds, that can be further used for application in food as a flavor enhancer. This action meets the current demand for natural food additives, decreases the environmental impact, in addition to adding value to this material that is now treated as waste.

Umami compounds extraction was optimized using only water as solvent. Hot-air drying was the best dehydration method for higher recovery of umami compounds. The 5'-nucleotides were more sensitive to higher temperature than the FAA, and even so the hot-air dried sample provided an extract with higher EUC than the freeze-dried sample. The application of spray drying to produce the Umami Ingredient was successful, since the process provided a good yield (60%) and the powder ingredient presented light color, low moisture, aw and sodium content, high solubility and high retention of $_{L}$-Glu (97%), which are suitable characteristics for a new food ingredient.

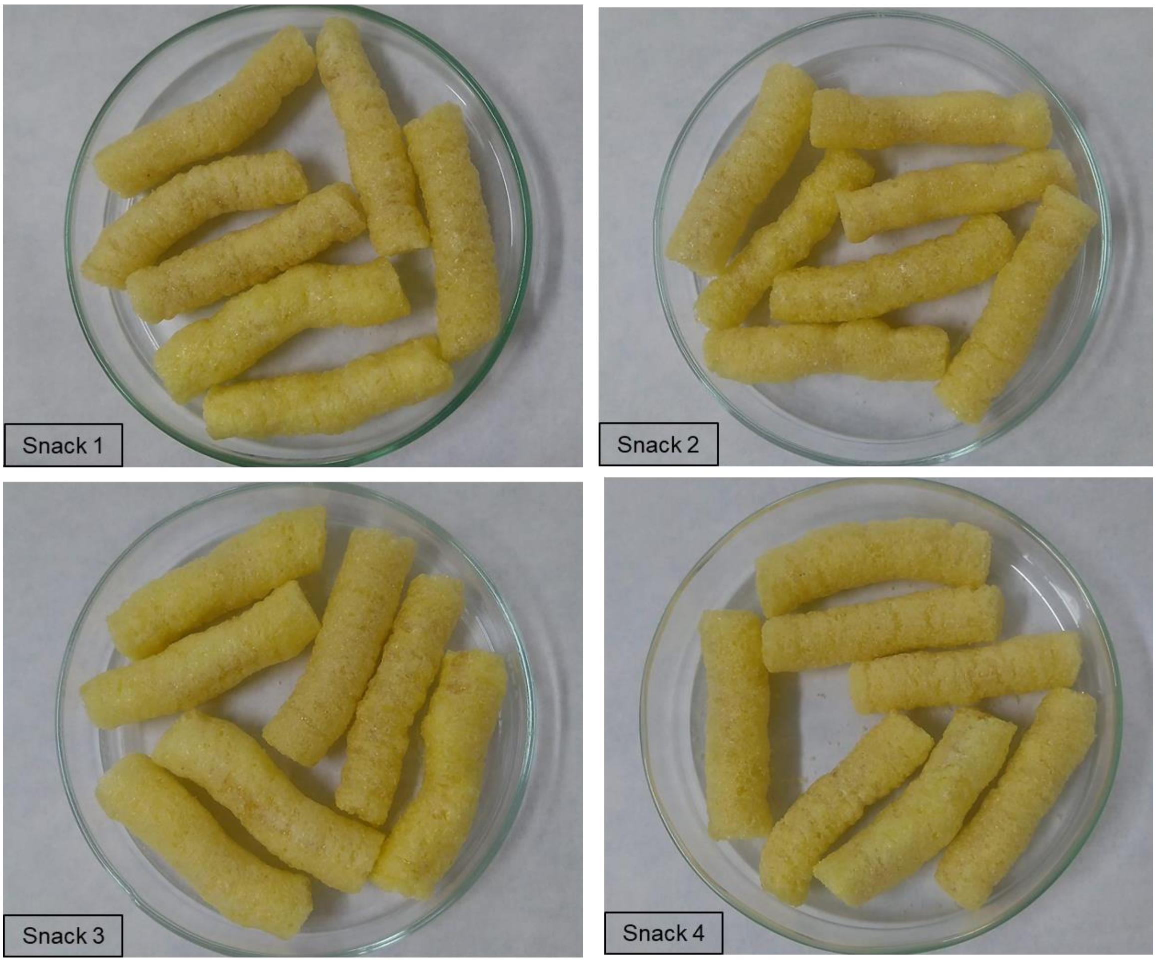
The application of the Umami Ingredient in low-sodium corn extruded snacks was evaluated and showed positive results. The snacks presented low density and cutting force, in addition to high expansion ratio. The reduction in sodium caused changes in the sensory profile, as well as in the sensory acceptance. The Umami Ingredient presented similar performance to the MSG for seasoning flavor, salty and umami taste, showing its potential as a flavor enhancer, being a candidate to substitute MSG.

In low-sodium beef burgers, the Umami Ingredient presented good results regarding the technological characteristics. Although the salt reduction and the addition of the Umami Ingredient caused minor changes in the physicochemical parameters (pH, color and aw), the cooking properties were not affected. For the textural properties just two samples presented difference for cohesiveness, being similar to the control for hardness, springiness and chewiness. The sensory influence of the Umami Ingredient needs to be further evaluated to understand its performance in low-sodium beef burgers.

This study shows the potential of the shiitake stipes as a source of umami compounds to be applied in food as a natural flavor enhancer. The new powder ingredient (Umami Ingredient) developed presented valuable results as a flavor enhancer in low-sodium corn extruded snacks (sensory and technological aspects) and beef burgers (technological aspects). Due to this, the results of this research are a valuable tool for further use of shiitake byproducts in the development of new flavor enhancers by the food industry, that can assist the sodium reduction process, helping the improvement of low-sodium products' flavor.

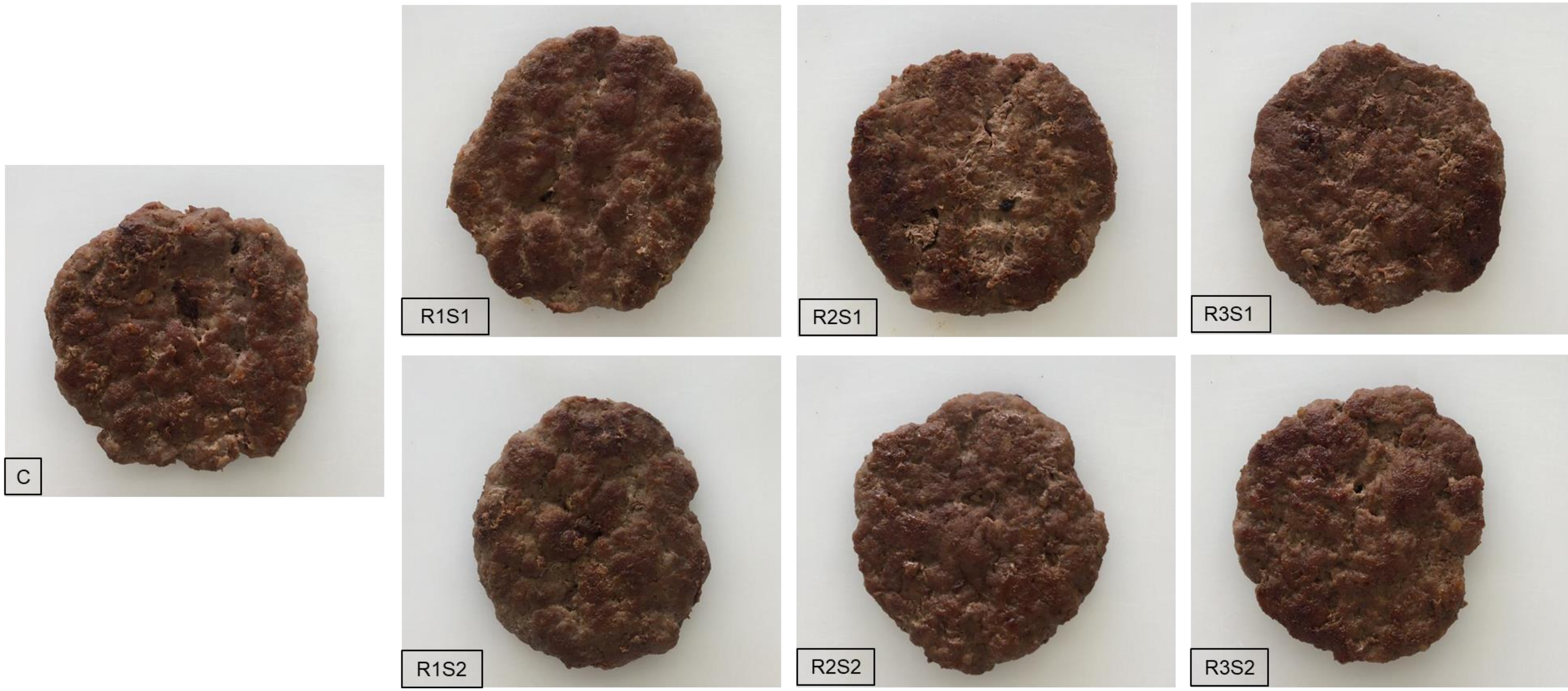
7. APPENDICES

7.1. Appendix 3 – Corn extruded snacks from Chapter 4



Snack 1: 2.8% salt, 0.6% MSG, 0% Umami Ingredient; Snack 2: 0.8% salt, 0.6% MSG, 0% Umami Ingredient; Snack 3: 0.8% salt, 0% MSG, 1.0% Umami Ingredient; Snack 4; 0.8% salt, 0% MSG, 1.5% Umami Ingredient.

7.2. Appendix 4 – Beef burgers from Chapter 5



R1S1: 1.24% salt, 1% Umami Ingredient; R1S2: 1.24% salt, 2% Umami Ingredient; R2S1: 0.9% salt, 1% Umami Ingredient; R2S2: 0.9% salt, 2% Umami Ingredient; R3S1: 0.57% salt, 1% Umami Ingredient; R3S2: 0.57% salt, 2% Umami Ingredient.

7.3. Appendix 4 - Informed Consent Form 1.

Termo de Consentimento Livre e Esclarecido – TCLE
(Conselho Nacional de Saúde, Resolução 466/2012 e Resolução 510/2016)

Você está sendo convidado a participar como voluntário do projeto de pesquisa "Desenvolvimento e aplicação de ingrediente realçador de sabor obtido de subprodutos de cogumelo", sob responsabilidade da pesquisadora Samara dos Santos Harada Padermo. Este projeto visa o aproveitamento dos subprodutos da produção de cogumelos (talos), visto que esse material geralmente é descartado pelas agroindústrias, tornando-se uma fonte de contaminação ambiental. Contudo, esse material contém compostos capazes de realçar o sabor dos alimentos, através do gosto umami. Sendo assim, este estudo justifica-se pelo seu papel relevante em buscar alternativas para o melhor aproveitamento dos subprodutos de cogumelo. O objetivo principal deste projeto é desenvolver um ingrediente realçador de sabor e posteriormente avaliar seu potencial no processo de redução de sódio em alimentos.

Nesta etapa da pesquisa, você analisará extratos aquosos produzidos com talos de cogumelo e os avaliará sensorialmente em relação ao gosto umami. No total, serão realizadas 10 sessões, incluindo sessões de treinamento e avaliação. Nas sessões de avaliação, você receberá 6 amostras diferentes de extrato, cada uma com 20 mL, estas serão codificadas com três dígitos aleatórios e apresentadas uma de cada vez. Cada uma das amostras deverá ser avaliada de acordo com a escala apresentada. Os riscos à sua saúde são mínimos, embora os produtos para elaboração dos extratos sejam de consumo comum e tenham sido manipulados utilizando-se as Boas Práticas de Fabricação. Serão excluídos da pesquisa quaisquer indivíduos que possuam alergias e/ou intolerâncias alimentares, além de outras possíveis patologias associadas aos alimentos testados. Você poderá consultar a pesquisadora responsável em qualquer época, pessoalmente ou pelo telefone da instituição, para esclarecimento de qualquer dúvida. Você está livre para, a qualquer momento, deixar de participar da pesquisa. Todas as informações por você fornecidas e os resultados obtidos serão mantidos em sigilo, e estes últimos apenas serão utilizados para divulgação em eventos e revistas científicas. Você será informado de todos os resultados obtidos, independentemente do fato destes poderem mudar seu consentimento em participar da pesquisa. Você não terá quaisquer benefícios ou direitos financeiros sobre os eventuais resultados decorrentes da pesquisa. Você não terá que arcar com nenhum ônus por participar desta pesquisa. No caso de eventual problema de saúde (efeito adverso) decorrente de sua participação nos testes sensoriais entrar em contato com o pesquisador responsável.

Diante das explicações, se você concorda em participar deste projeto, por favor, informe seus dados abaixo e assine este Termo.

Nome:____R.G.__________________

Endereço:____Fone:__________________

Data: ______/_______/_______

_____________________________________ _____________________________________

Participante ou representante legal | Pesquisador responsável

OBS.: Termo apresentado em duas vias, uma destinada ao participante ou seu representante legal, e a outra ao pesquisador.

Pesquisador responsável: Samara dos Santos Harada Padermo	Cargo/Função: Doutoranda
Instituição: Departamento de Agroindústria, Alimentos e Nutrição (LAN/ESALQ/USP)	
Endereço: Avenida Pádua Dias, 11 – CEP 13418-900 – Piracicaba/SP – Telefone: (19) 3429-4150	
Projeto submetido ao Comitê de Ética em Pesquisa com Seres Humanos da ESALQ/USP - Piracicaba/SP – Fone (19) 3429-4400	

7.4. Appendix 5 - Informed Consent Form 2.

Termo de Consentimento Livre e Esclarecido – TCLE
(Conselho Nacional de Saúde, Resolução 466/2012 e Resolução 510/2016)

Você está sendo convidado a participar como voluntário do projeto de pesquisa "Desenvolvimento e aplicação de ingrediente realçador de sabor obtido de subprodutos de cogumelo", sob responsabilidade da pesquisadora Samara dos Santos Harada Padermo. Este projeto visa o aproveitamento dos subprodutos da produção de cogumelos (talos), visto que esse material geralmente é descartado pelas agroindústrias, tornando-se uma fonte de contaminação ambiental. Contudo, esse material contém compostos capazes de realçar o sabor dos alimentos, através do gosto umami. Sendo assim, este estudo justifica-se pelo seu papel relevante em buscar alternativas para o melhor aproveitamento dos subprodutos de cogumelo. O objetivo principal deste projeto é desenvolver um ingrediente realçador de sabor e posteriormente avaliar seu potencial no processo de redução de sódio em snacks extrusados de milho.

Nesta etapa da pesquisa, você provará snacks extrusados de milho adicionados de um novo ingrediente realçador de sabor e os avaliará sensorialmente utilizando o teste de aceitação e o método Rate-All-That-Apply (RATA). Você receberá 4 amostras diferentes de snacks, codificadas com três dígitos aleatórios, uma de cada vez. Cada uma das amostras deverá ser avaliada de acordo com as escalas que lhe serão apresentadas. Os riscos à sua saúde são mínimos, embora os produtos para elaboração dos snacks de milho sejam de consumo comum e tenham sido manipulados utilizando-se as Boas Práticas de Fabricação. Serão excluídos da pesquisa quaisquer indivíduos que possuam alergias e/ou intolerâncias alimentares, além de outras possíveis patologias associadas aos alimentos testados. Você poderá consultar a pesquisadora responsável em qualquer época, pessoalmente ou pelo telefone da instituição, para esclarecimento de qualquer dúvida. Você está livre para, a qualquer momento, deixar de participar da pesquisa. Todas as informações por você fornecidas e os resultados obtidos serão mantidos em sigilo, e estes últimos apenas serão utilizados para divulgação em eventos e revistas científicas. Você será informado de todos os resultados obtidos, independentemente do fato destes poderem mudar seu consentimento em participar da pesquisa. Você não terá quaisquer benefícios ou direitos financeiros sobre os eventuais resultados decorrentes da pesquisa. Você não terá que arcar com nenhum ônus por participar desta pesquisa. No caso de eventual problema de saúde (efeito adverso) decorrente de sua participação nos testes sensoriais entrar em contato com o pesquisador responsável.

Diante das explicações, se você concorda em participar deste projeto, por favor, informe seus dados abaixo e assine este Termo.

Nome:____ R.G.________________

Endereço:____ Fone:________________

Data: ______/_______/_______

Participante ou representante legal	Pesquisador responsável

OBS.: Termo apresentado em duas vias, uma destinada ao participante ou seu representante legal, e a outra ao pesquisador.

Pesquisador responsável: Samara dos Santos Harada Padermo	Cargo/Função: Doutoranda
Instituição: Departamento de Agroindústria, Alimentos e Nutrição (LAN/ESALQ/USP)	
Endereço: Avenida Pádua Dias, 11 – CEP 13418-900 – Piracicaba/SP – Telefone: (19) 3429-4150	
Projeto submetido ao Comitê de Ética em Pesquisa com Seres Humanos da ESALQ/USP - Piracicaba/SP – Fone (19) 3429-4400	

8. ATTACHMENTS

8.1. Attachment 1 – Decision of the Ethics Committee for Human Research (ESALQ/USP)

USP - ESCOLA SUPERIOR DE AGRICULTURA "LUIZ DE QUEIROZ" DA UNIVERSIDADE

Plataforma Brasil

PARECER CONSUBSTANCIADO DO CEP

DADOS DA EMENDA

Título da Pesquisa: Desenvolvimento e aplicação de ingrediente realçador de sabor obtido de subprodutos de cogumelo

Pesquisador: SAMARA DOS SANTOS HARADA PADERMO

Área Temática:

Versão: 3

CAAE: 92722518.9.0000.5395

Instituição Proponente: "Escola Superior de Agricultura ""Luiz de Queiroz"" - ESALQ da Universidade

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 2.994.710

Apresentação do Projeto:

A redução de sódio em alimentos processados tem sido estimulada, devido ao consumo elevado de sódio estar relacionado ao desenvolvimento de doenças crônicas, como hipertensão e problemas cardiovasculares. Por outro lado, tem aumentado a demanda por produtos mais saudáveis e naturais, livres de aditivos. Frente a esta demanda, é necessário buscar por novos ingredientes de origem natural que tenham potencial para substituir ou reduzir o uso de aditivos sintéticos em alimentos. A utilização de cogumelos em formulações visando a redução de sódio já vem sendo pesquisada, contudo, estudos sobre o aproveitamento dos subprodutos da produção de cogumelos (estipes), com este mesmo objetivo, ainda são escassos. Frente a isto, o presente trabalho busca desenvolver um ingrediente rico em compostos umami a partir de subprodutos de cogumelo, em quatro etapas: 1) otimização do processo de extração dos compostos umami do estipe de shiitake com utilização de ultrassom, a fim de obter a condição otimizada de extração; 2) caracterização dos compostos umami presentes nos extratos de shiitake e champignon obtidos em condições otimizadas por meio de análises cromatográficas; 3) o extrato com maior EUC (Equivalent Umami Concentration) será atomizado, para intensificar o flavor e facilitar a aplicação e o armazenamento; 4) aplicação do ingrediente desenvolvido em snack extrusado de milho, visando avaliar, por meio de análises sensoriais com consumidores, seu potencial no processo de redução de sódio.

Endereço: Avenida Pádua Dias,11 Caixa Postal 9
Bairro: São Dimas **CEP:** 13.418-900
UF: SP **Município:** PIRACICABA
Telefone: (19)3429-4400 **E-mail:** cep.esalq@usp.br

Objetivo da Pesquisa:

Desenvolver um ingrediente rico em compostos umami a partir dos subprodutos da produção de cogumelos e avaliar o seu potencial como realçador de sabor, auxiliando na redução do conteúdo de sódio em alimentos.

Avaliação dos Riscos e Benefícios:

Os riscos relacionados ao consumo das amostras de estudo são mínimos, visto que serão seguidas as Boas Práticas de Fabricação para preparo das amostras. Todos os ingredientes a serem utilizados na formulação terão registro na ANVISA e estarão de acordo com os limites preconizados por ela. Todos os equipamentos e utensílios utilizados durante o processamento serão previamente higienizados, e os manipuladores utilizarão toucas, luvas e máscaras descartáveis, além de aventais durante todo o procedimento. Para atestar a segurança microbiológica das amostras a serem utilizadas neste estudo, as mesmas serão submetidas à análises microbiológicas (Coliformes a 45°C e Salmonella sp.) recomendadas pela RDC n°12,
de 12 de janeiro de 2001 da ANVISA.

Benefícios:

Não haverá benefício direto aos voluntários, porém as informações obtidas nestas avaliações serão de grande importância para o desenvolvimento do novo produto. Para os pesquisadores, o benefício será a obtenção de resultados práticos e reais que contribuirão para o desenvolvimento de um novo ingrediente realçador de sabor e também servirão para avaliar seu potencial no processo de redução de sódio dos alimentos, visando com isso promover o aproveitamento dos subprodutos da produção de cogumelos, além de contribuir para o avanço das pesquisas sobre redução de sódio em alimentos.

Comentários e Considerações sobre a Pesquisa:

A pesquisa é relevante e pode agregar ao campo acadêmico e consequentemente, trazer ganhos positivos à população que consome esse tipo de alimento.

Considerações sobre os Termos de apresentação obrigatória:

Os documentos obrigatórios estão presentes e adequados.

Recomendações:

Após a aprovação os pesquisadores devem atentar para a necessidade de envio de relatórios parciais (no mínimo um a cada 12 meses) ou final (ao término da pesquisa). Destaca-se que o parecer consubstanciado é o documento oficial de aprovação do sistema CEP/CONEP.

Intercorrências ou eventos adversos devem ser relatados ao CEP por meio da PB. Eventuais mudanças pretendidas no protocolo devem ser comunicadas como emendas ao CEP por meio da PB.

Conclusões ou Pendências e Lista de Inadequações:

As pendências foram atendidas.

Considerações Finais a critério do CEP:

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_1242361_E1.pdf	19/10/2018 14:47:02		Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_Samara_treinados_191018.pdf	19/10/2018 14:34:28	SAMARA DOS SANTOS HARADA PADERMO	Aceito
Projeto Detalhado / Brochura Investigador	cep_projetocogumelo_Samara_191018.pdf	19/10/2018 14:34:14	SAMARA DOS SANTOS HARADA PADERMO	Aceito
Outros	cep_cartaencaminhamento.pdf	10/09/2018 12:36:27	SAMARA DOS SANTOS HARADA PADERMO	Aceito
Outros	Samara_CVLattesresumo_140518.pdf	10/09/2018 12:33:10	SAMARA DOS SANTOS HARADA PADERMO	Aceito
Outros	sumula_curricular_AnaCarolina.pdf	10/09/2018 12:32:57	SAMARA DOS SANTOS HARADA PADERMO	Aceito
Outros	sumulacurricular_THAISVIEIRA2018.pdf	10/09/2018 12:32:41	SAMARA DOS SANTOS HARADA PADERMO	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_Samara_aceitacao_100918.pdf	10/09/2018 12:29:05	SAMARA DOS SANTOS HARADA PADERMO	Aceito
Outros	cepsujeito_pesquisa_Samara050918.pdf	10/09/2018 12:20:28	SAMARA DOS SANTOS HARADA PADERMO	Aceito
Folha de Rosto	FolhadeRosto_assinada_150618.pdf	15/06/2018 11:42:18	SAMARA DOS SANTOS HARADA PADERMO	Aceito
Orçamento	cep_orcamento.pdf	13/06/2018	SAMARA DOS	Aceito

Orçamento	cep_orcamento.pdf	11:37:34	SANTOS HARADA PADERMO	Aceito
Declaração de Pesquisadores	declaracaodepesquisadores.pdf	13/06/2018 11:37:04	SAMARA DOS SANTOS HARADA PADERMO	Aceito
Declaração de Instituição e Infraestrutura	cep_declaracao_infraestrutura_UNESP.pdf	13/06/2018 11:36:00	SAMARA DOS SANTOS HARADA PADERMO	Aceito
Declaração de Instituição e Infraestrutura	cep_declaracao_instituicao_UNESP.pdf	13/06/2018 11:35:48	SAMARA DOS SANTOS HARADA PADERMO	Aceito
Declaração de Instituição e Infraestrutura	declaracaodeinfraestrutura_ESALQ.pdf	13/06/2018 11:35:29	SAMARA DOS SANTOS HARADA PADERMO	Aceito
Declaração de Instituição e Infraestrutura	declaracaodainstituticao_ESALQ.pdf	13/06/2018 11:34:51	SAMARA DOS SANTOS HARADA PADERMO	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

PIRACICABA, 31 de Outubro de 2018

Assinado por:
Sandra Helena da Cruz
(Coordenador(a))