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Umami ingredient from shiitake (*Lentinula edodes*) by-products as a flavor enhancer in low-salt beef burgers: Effects on physicochemical and technological properties

Fabiana França [a], Samara dos Santos Harada-Padermo [b], Rafaela Alves Frasceto [a], Erick Saldaña [c], José Manuel Lorenzo [d,e], Thais Maria Ferreira de Souza Vieira [b], Miriam Mabel Selani [a,*]

[a] Centro de Ciências da Natureza, Campus Lagoa do Sino, Universidade Federal de São Carlos, Rod. Lauri Simões de Barros, Km 12, Buri, SP, Brazil
[b] Departamento de Agroindústria, Alimentos e Nutrição, Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Av. Pádua Dias 11, 13418-900, Piracicaba, SP, Brazil
[c] Escuela Profesional de Ingeniería Agroindustrial, Universidad Nacional de Moquegua, Prolongación calle Ancash s/n, Moquegua, 18001, Peru
[d] Centro Tecnológico de la Carne de Galicia, Adva. Galicia n° 4, Parque Tecnológico de Galicia, San Cibrao das Viñas, 32900, Ourense, Spain
[e] Área de Tecnología de los Alimentos, Facultad de Ciencias de Ourense, Universidad de Vigo, 32004, Ourense, Spain

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ABSTRACT

The impact of salt reduction and addition of Umami Ingredient, obtained from shiitake stipes, on the physicochemical and technological properties of beef burgers was evaluated. Seven treatments were performed: one control (regular salt content) and six low-salt formulations with different levels of salt and Umami Ingredient. Cooked burgers with salt reduction and Umami Ingredient addition showed a slight increase in lightness and yellowness, but no effect was found for redness, pH, and water activity. There was no decrease in cooking loss and diameter reduction in any of the formulations and only samples with 70% salt reduction were significantly less hard and chewy in comparison to the control. An increase in the free amino acids proline and phenylalanine was observed in treatments with Umami Ingredient. In general, the treatments did not affect amino acid profiles compared to the control and the most abundant amino acids were those related to the umami taste (glutamic and aspartic acids). The volatile profile of beef burgers showed a slight increase in compounds also found in the Umami Ingredient, mainly 3-methylbutanal, (S)-(+)-1,2-Propanediol and dimethyl sulfide. Based on physicochemical and technological parameters, the Umami Ingredient can be considered a promising natural flavor enhancer for low-salt beef burgers.

1. Introduction

The reduction of sodium in food is a highly discussed subject currently, given that excessive sodium consumption increases the risk of chronic non-communicable diseases such as hypertension and cardiovascular problems (Aburto et al., 2013; Gilbert & Heiser, 2005; He, Tan, Ma, & MacGregor, 2020). To reduce the occurrence of these diseases, the World Health Organization (WHO) recommends that sodium consumption should not exceed 2 g/day (~5 g of NaCl) (WHO, 2014). However, salt performs functions that go beyond the contribution to perceived saltiness. It plays a relevant role for technological properties, as it helps in the texture development of pasta and meat products and acts in the preservation and microbiological safety of food (Doyle & Glass, 2010; Pateiro, Munekata, Cittadini, Domínguez, & Lorenzo, 2021).

In many countries, including Brazil, sodium consumption is above the amount recommended by WHO (Mill et al., 2019; WHO, 2014). In industrialized countries, most of the total sodium consumed by the population comes from the intake of processed foods, especially meat products (Inguglia, Zhang, Tiwari, Kerry, & Burgess, 2017).

Among meat products, the beef burger is one of the most consumed, both for its sensory characteristics and convenience in preparation. However, it is also known for its high amount of sodium (Rios-Mera et al., 2019), which makes it a potential product that could be targeted

* Corresponding author.
E-mail address: miriamselani@ufscar.br (M.M. Selani).

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for NaCl reduction. The use of non-sodium salts, especially potassium chloride, has been widely evaluated for NaCl substitution, but most of these studies have reported the presence of a bitter flavor (Cittadini et al., 2020). Other commonly used strategies to reduce sodium in meat products are salt reduction by stealth, change in the size and shape of the salt crystal, and the use of salty tasting products (e.g., seaweeds) and flavor enhancers (da Silva et al., 2020; Gullón et al., 2021; Saldaña et al., 2021).

In this context, the addition of flavor enhancers associated with an umami taste can be an interesting alternative to improve the flavor of sodium-reduced products since it 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 it can also be elicited by other substances, such as 5′-nucleotides and umami peptides (Dermiki, Phanphensophon, Mottram, & Methven, 2013; Kong et al., 2019; Yamaguchi, 1991).

However, there is a current trend towards the use of natural ingredients in the food industry (Carocho, Morales, & Ferreira, 2015; Rios-Mera, Selani, Patinho, Saldaña, & Contreras-Castillo, 2021). Umami substances can be naturally found in different foods, such as tomatoes, cheese, soybeans, and mushrooms (Kurihara & Kashiwayanagi, 2000). The latter has been highlighted as a potential natural umami ingredient (Poojary, Orlien, Passamonti, & Olsen, 2017; Zhang, Venkitasamy, Pan, & Wang, 2013), whose umami compound content may vary according to mushroom species, maturity stage, part of the mushroom, quality grade, and storage time (Zhang, Venkitasamy, et al., 2013). Thus, its use as a flavor enhancer can positively affect consumers' perception regarding non-added MSG products (Radam, Yacob, Bee, & Selamat, 2010).

The use of mushroom or mushroom extract as a flavor enhancer has been studied and positive results were found in beef soup (Abd El-Aleem, Taher, Lotfy, El-Massry, & Fadel, 2017), beef burger (Mattar et al., 2018; Patinho et al., 2021), chicken sausage (Jo, Lee, & Jung, 2018), and frankfurter sausage (Cerón-Guevara et al., 2020). However, according to some studies, considerable amounts of umami compounds can also be found in the mushroom stipe (Chen et al., 2015; Harada-Padermo et al., 2020). Due to its unique flavor properties, which are strongly influenced by the presence of umami compounds, shiitake is highly accepted by the population (Hou et al., 2021) and is one of the most consumed mushrooms in the world (Rathore, Prasad, & Sharma, 2017). In this regard, Harada-Padermo et al. (2020) developed an Umami Ingredient (UI) from shiitake stipes and found that it has the potential to replace MSG in low-sodium extruded corn snacks (Harada-Padermo, Dias-Faceto, Selani, Conti-Silva, & Vieira, 2021). However, this ingredient has not yet been tested in another category of high-sodium products, such as meat products. Thus, this study aimed to evaluate the effect of UI addition on the physicochemical properties, texture profile, free amino acids, amino acid profile, and volatile compounds of low-salt beef burgers.

2. Materials and methods

2.1. Materials

Fresh beef (*Quadriceps femoris*), beef fat, and salt were purchased at a local market. Black pepper, garlic powder, onion powder, and sodium erythorbate were provided by the Ibrac (Rio Claro, Brazil). The UI was obtained from hot-air dried shiitake by-products (stipes), and all information about its obtention and characterization was described by Harada-Padermo et al. (2020). Reagents used in the free amino acid determination and amino acid profile were all HPLC grade. All other reagents were analytical grade.

2.2. Beef burgers

The beef burger formulations and their salt reductions were defined in a previous study (data not shown). Thus, salt reductions of 35% (S1), 52.5% (S2), and 70% (S3) were selected, and the inclusion of UI as a flavor enhancer was evaluated at two concentrations (U1 - 1% and U2 - 2%) (Table 1). The salt content of the control (1.9%) corresponded to the average content used in commercial beef burgers available in the Brazilian market.

Fresh beef and fat were grounded separately (Eccel, model MCIE-09, Brusque, Brazil) using a 5-mm plate and divided into 7 treatments. After that, water and salt were added to the beef and mixed for 5 min. The other ingredients were added and mixed for another 3 min. Portions of 90 g were manually shaped using a burger-maker (10 cm in diameter and 1 cm in thickness), placed in polyethylene packages, and stored at −18 °C. The cooking procedure was performed on a hot plate (150 °C) (Compact 80, Caxias do Sul, Brazil) until the internal temperature reached 75 °C. Samples were then cooled to room temperature (25 °C) for further analyses.

2.3. pH, color, and aw

The pH value was measured using a potentiometer (MS Tecnopon, model MPA-210, Piracicaba, Brazil) with a glass penetration electrode. Color was determined using a colorimeter (Delta Color, Colorium 7, São Leopoldo, Brazil) by reading the L* (lightness), a* (redness), and b* (yellowness) parameters, with a D65 light source. These analyses were performed on three burgers of each treatment (raw and cooked), with three readings for each sample.

Water activity (aw) was determined in triplicate for raw and cooked samples using an Aqualab apparatus (Aqua Lab 4 TE, Meter Group, Pullman, USA) at 25 °C.

2.4. Sodium content

The sodium content of cooked beef burgers was determined in triplicate as described by AOAC (1996). The readings were performed in a flame photometer (B462, Micronal, Series 40707, São Paulo, Brazil) and the results were expressed in mg/100 g.

2.5. Cooking properties

Cooking loss (CL) was expressed as the percentage of weight loss after cooking (Eq. (1))

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

Diameter reduction (DR) was calculated according to Eq. (2) and also expressed as a percentage.

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

Cooking properties were measured on three burgers of each treatment.

Table 1
Beef burger formulations.

Ingredients	Formulations (%)						
	C	S1U1	S1U2	S2U1	S2U2	S3U1	S3U2
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

2.6. Texture profile analysis (TPA)

Burgers were cooked as previously described (section 2.2). Three burgers of each treatment were used, and from each burger, three cylindrical samples (2.5 cm in diameter) were taken.

TPA was carried out in a TA-XT plus texture analyzer (Stable Micro Systems, Godalming, UK), 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 in diameter (P/36R, Stable Micro Systems), to 75% of their original height at a constant speed of 20 cm/min. The parameters of hardness (N), springiness (mm), cohesiveness, and chewiness (N*mm) were determined.

2.7. Free amino acids

The quantification of free amino acids was carried out for UI and for beef burgers. The extraction followed the protocol described by Lorenzo, Cittadini, Bermúdez, Munekata, and Domínguez (2015). The free amino acids were derivatized with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (Waters AccQ-Fluor reagent kit). Finally, the samples were analyzed according to the chromatographic conditions described by Domínguez, Borrajo, and Lorenzo (2015), using a high-performance liquid chromatograph (Alliance 2695 model, Waters, Milford, MA) and a scanning fluorescence detector (model 2475, Waters), equipped with a Waters AccQ-Tag column (3.9 mm × 150 mm, particle size of 4 μm). Amino acids were identified by retention times and quantified by the external standard technique using an amino acid standard (Amino Acid Standard H, Thermo Scientific) and the advanced software Empower 2 (Waters). Results were expressed in mg/100 g of dry matter.

2.8. Amino acid profile

Protein hydrolysis was carried out according to the procedures described by Domínguez et al. (2015). The derivatization and quantification of amino acids were determined as described in the free amino acids determination (section 2.7). Results were expressed in mg/100 g.

2.9. Volatile compounds profile

Volatile compounds profile was determined for UI and beef burgers. The extraction of volatile compounds was carried out by solid-phase microextraction (SPME), using a fused-silica fiber (10 mm length) coated with a layer of 50/30 mm in thickness of divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) (Supelco). The determination of the profiles was then obtained through gas chromatography (7890B model, Agilent Technologies) coupled to mass spectrometry (5977B model, Agilent Technologies), using a DB-624 capillary column (30 m, 0.25 mm i.d., 1.4 μm film thickness).

The extraction, conditioning, injection, and chromatographic conditions to determine the volatile compounds were conducted as described by Domínguez et al. (2019). The compounds were identified by comparing their mass spectra with those of the National Institute of Standards and Technology library (NIST14) (correspondence factor >85%) and/or by comparing their mass spectra and retention time with authentic standards (Supelco) and/or by calculating the retention index to a series of standard alkanes (C5–C14) (for calculating linear retention index, Supelco) and matching them with data described in the literature. The results were expressed in units of area × 10^4/g of the sample.

2.10. Statistical analyses

Burgers were prepared following a randomized block design (two independent processes). A mixed analysis of variance (ANOVA) was performed with treatment as a fixed factor and blocks as a random factor. A pairwise comparison was obtained applying Tukey's test ($p < 0.05$), using R software, version 3.5.1. A Principal Component Analysis (PCA) was carried out on the volatile compounds profile, free amino acids, and amino acid profile results using XLSTAT 2015 software (Addinsoft, New York, USA).

3. Results and discussion

3.1. pH, color and aw

The pH of raw burgers showed a slight but significant increase in treatments with UI addition and a reduction of 52.5% (S2U1 and S2U2) and 70% (S3U1 and S3U2) of salt content (Table 2). The presence of basic amino acids in mushrooms, such as histidine, lysine, and arginine (Choe et al., 2018) may have influenced this result. However, the pH of raw samples ranged from 5.60 to 5.68, which cannot be considered significant at a practical level since the normal pH of raw beef may vary between 5.5 and 5.7 (Matarneh, England, Scheffler, & Gerrard, 2017). An increase in pH was also observed in beef burgers with shiitake mushroom extract (Mattar et al., 2018) and in frankfurter sausages with different mushrooms (Cerón-Guevara et al., 2020; Pil-Nam et al., 2015). In contrast, there was no significant change in the pH of cooked burgers, which were within the value reported as the usual pH of cooked meat products (6.0) (Hereu, Dalgaard, Garriga, Aymerich, & Bover-Cid, 2012). A slightly higher pH (6.15) was found by Dermiki, Phanphensophon, et al. (2013) in cooked minced beef with shiitake extract.

The addition of UI at all levels evaluated did not significantly affect the lightness, redness, and yellowness of raw burgers (Table 2). Previous studies also found no change in the L* and b* values of raw burgers with *Agaricus bisporus* (Patinho et al., 2019) and L* and a* values of fermented sausages with shiitake by-product extract (Van Ba et al., 2016). After the cooking process, a significant increase in the lightness of treatments S3U1 and S3U2 was observed in comparison with the control, possibly caused by a greater salt reduction (70%) (Table 2). According to Baublits, Pohlman, Brown, Yancey, and Johnson (2006), an increase in L* values with the reduction of salt concentration may be due to lower ionic strength, causing less water retention by the myofibrillar proteins and, consequently, an increase in the reflectance of the light, resulting in a lighter appearance. However, according to Fig. 1, which shows the appearance of the beef burgers, there was no suppression of the natural color of the product. A similar result was reported in fermented meat products with a 75% salt reduction (Santos, Campagnol, Morgano, & Pollonio, 2014). Additionally, this change in lightness may have been caused by the addition of UI, since it has a light color (L* = 90.56 (Harada-Padermo et al., 2020)). Corroborating this fact, Akesowan (2016) reported an increase in the lightness of chicken nuggets formulated with shiitake mushroom powder.

Salt reduction and UI addition did not affect the a* value of cooked burgers, which is an important result since the red color of meat products is one of the main characteristics evaluated by the consumer for high sensory acceptance. However, concerning yellowness, a significant increase was observed in all treatments containing UI, which may be related to the color of the ingredient (b* = 19.97, Harada-Padermo et al. (2020)). Similar results were found in burgers with shiitake extract (Mattar et al., 2018; Pil-Nam et al., 2015) and sausages with shiitake powder (Cerón-Guevara et al., 2020).

The water activity of raw and cooked burgers was only slightly affected by salt reduction and UI addition (Table 2). Although a significant increase was found in the aw of raw samples with salt reduction, the variation between all treatments was minimal (0.01). After cooking, there was no effect from the treatments on water activity, although an incremental tendency among these values was observed. This slight variation may be due to the salt's ability to reduce aw. Salt promotes the diffusion process, in which water flows from the food to higher concentration zones (outside food), dissolving salt, which penetrates food and consequently reduces the amount of free water (Albarracín, Sánchez, Grau, & Barat, 2011; Inguglia et al., 2017). The aw results corroborate those found in beef burgers with salt reduction (Patinho

Table 2
Physicochemical properties of raw and cooked burgers.

Parameters	C	S1U1	S1U2	S2U1	S2U2	S3U1	S3U2	SEM
Raw burger								
Color								
L^*	45.5^{a}	46.2^{a}	44.83^{a}	44.54^{a}	43.98^{a}	45.15^{a}	44.42^{a}	0.43
a^*	13.46^{a}	12.89^{a}	12.76^{a}	12.64^{a}	12.08^{a}	11.85^{a}	11.11^{a}	0.54
b^*	14.06^{a}	13.47^{a}	13.56^{a}	13.80^{a}	12.85^{a}	13.12^{a}	12.74^{a}	0.35
pH	5.60^{b}	5.63^{ab}	5.64^{ab}	5.67^{a}	5.66^{a}	5.68^{a}	5.67^{a}	0.02
Aw	0.979^{c}	0.985^{ab}	0.982^{bc}	0.986^{ab}	0.984^{abc}	0.989^{a}	0.987^{ab}	0.001
Cooked burger								
Color								
L^*	49.22^{b}	51.57^{ab}	50.79^{ab}	52.96^{ab}	52.32^{ab}	54.65^{a}	53.41^{a}	0.97
a^*	5.04^{a}	5.35^{a}	5.48^{a}	5.06^{a}	5.12^{a}	5.30^{a}	5.05^{a}	0.07
b^*	13.10^{b}	14.89^{a}	14.48^{a}	14.95^{a}	14.63^{a}	15.24^{a}	15.33^{a}	0.27
pH	6.03^{a}	6.04^{a}	6.04^{a}	6.07^{a}	6.04^{a}	6.06^{a}	6.03^{a}	0.01
Aw	0.962^{a}	0.966^{a}	0.970^{a}	0.971^{a}	0.967^{a}	0.973^{a}	0.973^{a}	0.001
Sodium (mg/100g)	940.59^{a}	743.98^{b}	761.75^{b}	616.69^{c}	629.59^{c}	452.84^{d}	478.74^{d}	35.42

C: 1.9% NaCl + 0% Umami Ingredient; S1U1: 1.24% NaCl + 1% Umami Ingredient; S1U2:1.24% NaCl + 2% Umami Ingredient; S2U1: 0.91% NaCl + 1% Umami Ingredient; S2U2: 0.91% NaCl + 2% Umami Ingredient; S3U1: 0.57% NaCl + 1% Umami Ingredient; S3U2: 0.57% NaCl + 2% Umami Ingredient; SEM: standard error of the mean.
Means with different letters within a row are significantly different ($p < 0.05$, Tukey's test).

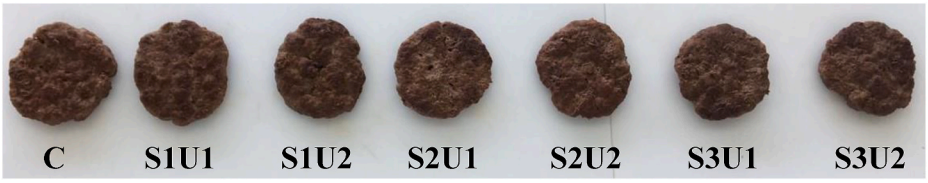

Fig. 1. Appearance of beef burgers.

et al., 2019).

As expected, differences were observed in the sodium content of cooked beef burgers (Table 2). The control showed significantly higher sodium content compared to all treatments with salt reduction. Among these treatments, those with the same level of NaCl were not significantly different.

3.2. Cooking properties and textural parameters

Cooking loss is an important parameter for the meat industry since salt reduction in these products decreases water holding capacity, leading to loss of yields (Fellendorf, Kerry, Hamill, & O'Sullivan, 2018). In this study, cooking loss ranged from 36.55% to 39.54% and was not affected by salt reduction and UI addition (Table 3). Diameter reduction after cooking is another important property, as the diameter of the product should be compatible with that of the burger bun to obtain a more attractive product for consumers (Gujral, Kaur, Singh, & Sodhi, 2002). Considering that cooking loss was not affected, it was expected that diameter reduction would not be affected either, since this cooking parameter is the result of the denaturation of meat proteins with the loss of water and fat (Besbes, Attia, Deroanne, Makni, & Blecker, 2008). The values obtained varied from 26.18% to 27.76% between the different formulations.

The results of the cooking parameters showed that salt reductions at the levels studied did not seem to have affected the extraction of myofibrillar proteins to the point of influencing the product's ability to bind water and affect the cooking yield. Similar results were found by Rosli et al. (2011) in chicken burgers containing *Pleurotus sajor-caju*, while Mattar et al. (2018) reported similar cooking loss (37–42%) in beef burgers with shiitake mushroom extract. Rios-Mera et al. (2019) found similar values for cooking loss (33.11%–41.16%) and diameter reduction (21.97%–27.61%) in low-sodium beef burgers.

Regarding texture parameters, treatments did not show significant differences in springiness and cohesiveness. There was a tendency to decrease hardness and chewiness as salt content was reduced, but only S3U1 and S3U2 were significantly affected (Table 3). The higher salt reduction (70%) in these treatments possibly influenced these results since salt promotes the solubilization of myofibrillar proteins, which increases their capacity to bind water and fat (Desmond, 2006), affecting hardness and juiciness. It also leads to the formation of a

Table 3
Cooking properties and textural parameters of cooked burgers.

Parameters	C	S1U1	S1U2	S2U1	S2U2	S3U1	S3U2	SEM
CL (%)	37.02^{a}	37.81^{a}	36.55^{a}	39.54^{a}	37.47^{a}	39.08^{a}	39.29^{a}	0.54
DR (%)	26.35^{a}	27.76^{a}	26.27^{a}	27.64^{a}	26.86^{a}	26.18^{a}	27.34^{a}	0.59
Hardness (N)	251.74^{a}	229.96^{ab}	226.43^{ab}	215.79^{ab}	210.17^{ab}	195.35^{b}	190.39^{b}	6.74
Springiness (mm)	0.78^{a}	0.76^{a}	0.75^{a}	0.76^{a}	0.78^{a}	0.77^{a}	0.76^{a}	0.01
Cohesiveness	0.52^{a}	0.53^{a}	0.50^{a}	0.50^{a}	0.52^{a}	0.51^{a}	0.50^{a}	0.01
Chewiness (N*mm)	105.33^{a}	89.63^{ab}	88.05^{ab}	82.96^{ab}	81.28^{ab}	72.97^{b}	72.12^{b}	3.55

CL: cooking loss; DR: diameter reduction; C: 1.9% NaCl + 0% Umami Ingredient; S1U1: 1.24% NaCl + 1% Umami Ingredient; S1U2:1.24% NaCl + 2% Umami Ingredient; S2U1: 0.91% NaCl + 1% Umami Ingredient; S2U2: 0.91% NaCl + 2% Umami Ingredient; S3U1: 0.57% NaCl + 1% Umami Ingredient; S3U2: 0.57% NaCl + 2% Umami Ingredient; SEM: standard error of the mean.
Means with different letters within a row are significantly different ($p < 0.05$, Tukey's test).

protein gel matrix when heated, resulting in a firmer product (Rios-Mera et al., 2020).

Chewiness is strongly influenced by the hardness of the product since it is calculated by multiplying the values of hardness, springiness, and cohesiveness. Because of this, the same trend found for hardness was also observed for chewiness, i.e., only treatments S3U1 and S3U2 showed significantly lower values compared to the control. Similarly, salt reduction and mushroom addition have been shown to decrease the hardness and chewiness of sausages (Cerón-Guevara et al., 2020) and beef burgers (Patinho et al., 2019).

3.3. Free amino acids

The quantification of free amino acids in UI and beef burgers is shown in Table 4. Seventeen amino acids were identified in UI, of which glutamic acid, proline, and alanine were the most abundant. According to Zhang, Venkitasamy, et al. (2013), the typical umami flavor of mushrooms is attributed to the presence of MSG-like amino acids, such as aspartic and glutamic acids. The umami-taste amino acid content of UI is the same as that reported in shiitake mushroom stipes in their last growth stage (1.58 mg/g of dry matter) (Chen et al., 2015). Similar values were observed in two varieties of shiitake mushrooms (1.71 and 1.93 mg/g of dry weight) (Yang, Lin, & Mau, 2001) and shiitake mushrooms dried at 70 °C (1.30 mg/g of dry weight) (Yang et al., 2019). According to Zhang, Venkitasamy, et al. (2013), the variation in mushrooms' free amino acid content is entirely related to geographic location, maturity stage, cultivation conditions, part of the mushroom used, and storage and drying conditions.

Regarding beef burgers, most free amino acids did not differ significantly between treatments, except for proline and phenylalanine. As shown in Table 4, proline is one of the amino acids found in greatest amount in UI. For this reason, treatments with the highest concentration of UI (2%) showed significantly higher proline content compared to the control. Regarding phenylalanine, the content of this amino acid in beef burgers was influenced by the addition of UI since all treatments to which this ingredient was added had an increase in phenylalanine content, which was significantly higher only for S1U2 and S3U2.

Although not significant, a trend towards an increase in the concentration of all amino acids in treatments with UI was observed, including those responsible for the umami taste - aspartic and glutamic acids. Similarly, Dermiki, Phanphensophon, et al. (2013) did not find significant differences in the content of umami-taste amino acids of cooked minced meat with shiitake extract but also reported an increase in values compared to the control. Nevertheless, in the sensory analysis, these authors reported significantly higher intensity of "salty" and "umami" taste in burgers with shiitake extract.

Fig. 2 shows the representation of free amino acids and treatments in the principal components PC1 and PC2, which accounted for 87.5% of total data variance. With the exception of S1U1, the first dimension separated the control from samples with UI addition and salt reduction. All free amino acids were located on the right side of PC1 and were associated with S1U2, S2U1, S2U2, S3U1, and S3U2. This fact can be

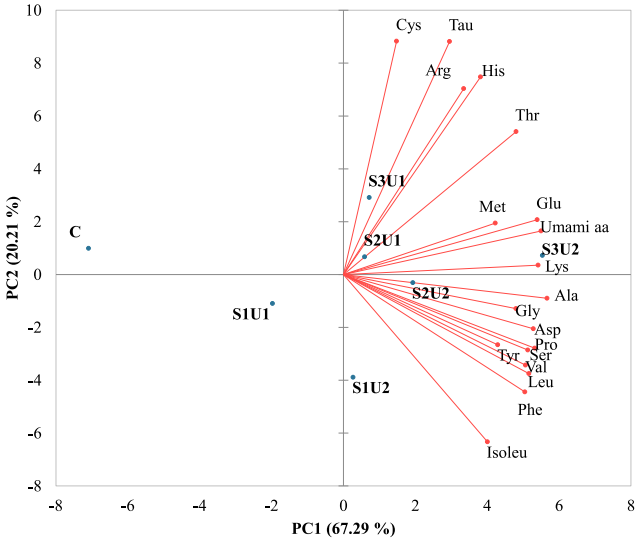

Fig. 2. Biplot of the first two dimensions of the PCA performed on the free amino acid data of beef burgers.

Table 4
Free amino acids (mg/100 g of dry matter) of Umami Ingredient (UI) and beef burgers.

Amino acid	UI (mean ± SEM)	Treatments							
		Control	S1U1	S1U2	S2U1	S2U2	S3U1	S3U2	SEM
Aspartic acid	20.19 ± 2.32	1.52 [a]	1.68 [a]	2.04 [a]	1.83 [a]	1.91 [a]	1.88 [a]	2.21 [a]	0.23
Serine	51.55 ± 5.60	13.47 [a]	15.94 [a]	16.41 [a]	15.05 [a]	16.24 [a]	15.56 [a]	17.85 [a]	1.82
Glutamic acid	137.55 ± 13.59	7.62 [a]	10.88 [a]	10.34 [a]	11.38 [a]	11.58 [a]	12.17 [a]	13.33 [a]	1.29
Glycine	28.43 ± 4.16	13.54 [a]	14.46 [a]	17.36 [a]	16.48 [a]	16.38 [a]	16.96 [a]	17.04 [a]	1.03
Histidine	89.01 ± 10.66	102.11 [a]	110.13 [a]	98.05 [a]	127.47 [a]	125.25 [a]	137.26 [a]	133.31 [a]	7.30
Taurine	–	32.27 [a]	32.49 [a]	30.16 [a]	34.80 [a]	34.55 [a]	37.72 [a]	36.26 [a]	4.35
Arginine	22.21 ± 1.85	99.67 [a]	93.32 [a]	95.68 [a]	105.91 [a]	99.34 [a]	121.71 [a]	125.65 [a]	10.76
Threonine	36.17 ± 2.94	11.61 [a]	12.31 [a]	12.07 [a]	13.71 [a]	14.24 [a]	14.47 [a]	15.04 [a]	1.44
Alanine	105.39 ± 11.69	53,95 [a]	59,33 [a]	62,74 [a]	61,62 [a]	63,98 [a]	62,17 [a]	67,18 [a]	0,35
Proline	137.53 ± 16.47	9.80 [b]	15.30 [ab]	17.56 [a]	15.17 [ab]	19.35 [a]	15.50 [ab]	20.03 [a]	1.06
Cysteine	18.15 ± 1.58	4.14 [a]	3.63 [a]	3.34 [a]	3.84 [a]	4.02 [a]	4.30 [a]	4.47 [a]	0.31
Tyrosine	8.39 ± 1.08	4.10 [a]	4.22 [a]	4.35 [a]	4.39 [a]	4.22 [a]	4.24 [a]	4.37 [a]	0.35
Valine	78.34 ± 9.93	8.01 [a]	9.61 [a]	10.16 [a]	9.17 [a]	10.58 [a]	9.22 [a]	11.15 [a]	0.70
Methionine	21.82 ± 2.75	4.12 [a]	4.22 [a]	4.17 [a]	4.43 [a]	4.39 [a]	4.21 [a]	4.41 [a]	0.34
Lysine	6.38 ± 0.32	6.56 [a]	7.58 [a]	7.60 [a]	8.20 [a]	7.99 [a]	7.72 [a]	8.50 [a]	0.62
Isoleucine	54.14 ± 6.99	6.11 [a]	7.04 [a]	7.71 [a]	6.69 [a]	7.22 [a]	6.19 [a]	8.01 [a]	0.54
Leucine	86.79 ± 11.53	9.42 [a]	11.20 [a]	12.98 [a]	11.44 [a]	13.18 [a]	11.46 [a]	13.59 [a]	0.86
Phenylalanine	54.87 ± 7.84	5.11 [b]	6.60 [ab]	8.04 [a]	7.21 [ab]	7.73 [ab]	6.78 [ab]	7.93 [a]	0.45
Total FFA	956.86 ± 111.26	393.102 [a]	419.925 [a]	337.73 [a]	458.79 [a]	462.130 [a]	489.50 [a]	510.30 [a]	47.34
Umami taste amino acids [a]	157.74 ± 15.21	9.14 [a]	12.56 [a]	12.38 [a]	13.22 [a]	13.49 [a]	14.05 [a]	15.54 [a]	0.31

C: 1.9% NaCl + 0% Umami Ingredient; S1U1: 1.24% NaCl + 1% Umami Ingredient; S1U2:1.24% NaCl + 2% Umami Ingredient; S2U1: 0.91% NaCl + 1% Umami Ingredient; S2U2: 0.91% NaCl + 2% Umami Ingredient; S3U1: 0.57% NaCl + 1% Umami Ingredient; S3U2: 0.57% NaCl + 2% Umami Ingredient; SEM: standard error of the mean.

Means with different letters within a row are significantly different ($p < 0.05$, Tukey's test).

[a] Umami taste amino acids = ∑ of glutamic acid and aspartic acid.

explained because the NaCl reduction leads to a more intense proteolytic activity of muscle enzymes and, consequently, to a greater release of free amino acids (Toldrá & Flores, 1998). It is interesting to note that the samples with the highest amount of UI (2%) were more related to glutamic (S3U2) and aspartic acids (S1U2 and S2U2) and that S3U2 was the closest to the sum of the umami-taste amino acids (umami aa).

3.4. Amino acid profile

The effect of UI on the amino acid profile of beef burgers is shown in Table 5. In general, salt reduction and UI addition did not modify the amino acid profile of beef burgers compared to the control, at the univariate level. The most abundant amino acids found in all samples were those related to the umami taste, i.e., the non-essential amino acids: glutamic (2.83–3.89 g/100 g) and aspartic acids (2.21–3.18 g/100 g), followed by the essential amino acids: lysine (2.05–2.88 g/100 g) and leucine (1.42–2.11 g/100 g). These amino acids, along with alanine, arginine, and valine, were also predominant in different cultivars of *Lentinula edodes* (Kim et al., 2017). Corroborating the present study, the addition of either *Agaricus bisporus* or *Pleurotus ostreatus* flour in frankfurter sausages did not affect the amino acid composition of the product, which also had as its major amino acids glutamic acid, aspartic acid, lysine, and leucine (Cerón-Guevara et al., 2020).

No significant differences were found between treatments and control regarding total, non-essential (NE), and essential (E) amino acids, which was reflected in similar E/NE ratios. This ratio for all treatments was higher than the reference value recommended by the Food and Agriculture Organization - FAO/WHO (1991) (>0.6), indicating that beef burgers are good sources of high-quality protein.

From a multivariate level, the PCA of the amino acid profile data accounted for 94.70% of the original information (Fig. 3). The first dimension separated the treatments with 70% salt reduction (S3U1 and S3U2) from those with intermediate salt reduction and the control and showed that S3 samples were closer to most of the amino acids. In the PCA map, it was not possible to observe a clear trend between samples and the amino acid profile. This can be explained by the results already presented (Table 5), which showed that, in general, the amino acid profile of the burgers was not affected by salt reduction and the use of UI. Cysteine and tyrosine were not related to any of the treatments, as they were found in low amounts and did not vary significantly between samples (Table 5).

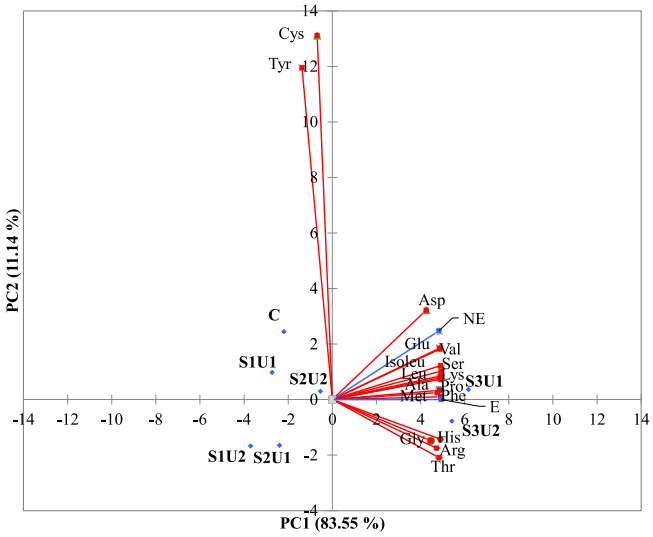

Fig. 3. Biplot of the first two dimensions of the PCA performed on the amino acid profile data of beef burgers.

3.5. Volatile compounds profile

Forty-two compounds were identified in UI (Table S1), which were grouped into the following chemical families: hydrocarbons (11), ketones (7), aldehydes (6), alcohols (4), esters (3), terpenes (3), amines (2), acids (2), sulfur compounds (2), ethers (1), and lactones (1).

Many volatile compounds are formed from reactions related to processing at elevated temperatures, such as Maillard reactions and Strecker degradation of free amino acids (Tian, Zhao, Huang, Zeng, & Zheng, 2016). Thus, the oven drying process (70 °C) that shiitake stipes underwent to produce UI certainly promoted changes in the volatile compounds profile of the sample.

Sulfur compounds are present in significant amounts in fresh

Table 5
Amino acid profile (g/100 g) of beef burgers.

Amino acid	Control	S1U1	S1U2	S2U1	S2U2	S3U1	S3U2	SEM
Aspartic acid	2.41 ab	2.32 ab	2.21 b	2.24 b	2.45 ab	3.18 a	2.56 ab	1.24
Serine	0.73 ab	0.70 ab	0.67 b	0.69 ab	0.754 ab	0.93 a	0.90 ab	1.02
Glutamic acid	3.13 a	2.95 a	2.83 a	2.83 a	3.08 a	3.89 a	3.83 a	1.02
Glycine	1.40 a	1.57 a	1.38 a	1.57 a	1.60 a	1.80 a	1.79 a	1.00
Histidine[a]	0.82 a	0.82 a	0.80 a	0.86 a	0.90 a	1.08 a	1.06 a	1.02
Arginine[a]	1.35 a	1.38 a	1.32 a	1.39 a	1.47 a	1.86 a	2.10 a	0.89
Threonine[a]	0.95 a	0.99 a	0.97 a	1.02 a	1.11 a	1.40 a	1.47 a	0.95
Alanine	1.24 ab	1.22 ab	1.14 b	1.22 ab	1.27 ab	1.69 a	1.58 ab	1.07
Proline	0.75 ab	0.74 ab	0.65 b	0.79 ab	0.81 ab	1.07 a	1.01 a	1.06
Cysteine	0.29 a	0.26 a	0.19 a	0.19 a	0.22 a	0.24 a	0.19 a	1.21
Tyrosine	0.59 a	0.52 a	0.39 a	0.41 a	0.52 a	0.41 a	0.44 a	0.93
Valine[a]	1.17 abc	1.06 bc	1.02 c	1.07 bc	1.20 a	1.53 a	1.43 ab	1.07
Methionine	0.68 a	0.61 a	0.60 a	0.62 a	0.71 a	0.85 a	0.93 a	0.91
Lysine[a]	2.23 a	2.16 a	2.09 a	2.05 a	2.21 a	2.87 a	2.88 a	1.00
Isoleucine[a]	1.03 ab	0.93 b	0.91 b	0.95 b	1.07 ab	1.37 a	1.31 a	1.04
Leucine[a]	1.57 abc	1.43 c	1.42 c	1.46 bc	1.66 abc	2.11 a	2.02 ab	1.05
Phenylalanine[a]	0.89 b	0.86 b	0.83 b	0.89 b	1.01 ab	1.22 a	1.18 a	1.03
Total	21.17 ab	20.49 ab	19.38 b	20.23 ab	22.00 ab	27.43 a	26.64 ab	1.03
Non-essential (NE)	10.52 ab	10.27 ab	9.45 b	9.92 ab	10.68 ab	13.18 a	12.29 ab	1.07
Essential (E)	10.65 a	10.22 a	9.93 a	10.30 a	11.32 a	14.26 a	14.35 a	0.99
E/NE	1.02 a	1.00 a	1.05 a	1.04 a	1.06 a	1.09 a	1.17 a	0.93

C: 1.9% NaCl + 0% Umami Ingredient; S1U1: 1.24% NaCl + 1% Umami Ingredient; S1U2:1.24% NaCl + 2% Umami Ingredient; S2U1: 0.91% NaCl + 1% Umami Ingredient; S2U2: 0.91% NaCl + 2% Umami Ingredient; S3U1: 0.57% NaCl + 1% Umami Ingredient; S3U2: 0.57% NaCl + 2% Umami Ingredient; SEM: standard error of the mean.
Means with different letters within a row are significantly different ($p < 0.05$, Tukey's test).
[a] Essential amino acid.

mushrooms, including straight-chain compounds, such as dimethyl disulfide and dimethyl trisulfide, as well as cyclic compounds, especially 1,2,4-trithiolane, 1,2,4,5- tetrathiane and lenthionine (Wu & Wang, 2000). None of these compounds were found in UI, which may be related to the use of high temperatures during its preparation, resulting in thermal degradation. A similar result was verified by Dermiki, Phanphensophon, et al. (2013), who reported that shiitake aqueous extract prepared at 70 °C had a lower content of sulfur compounds than that prepared at 22 °C. However, other sulfur compounds were identified, such as carbon disulfide and dimethyl sulfone, which were also detected in a previous study with dried shiitake (Zhang et al., 2020). The volatile compound lenthionine has been reported to be responsible for the aroma of shiitake mushrooms (Wu & Wang, 2000). According to Ito, Toyoda, Suzuki, and Iwaida (1978), when the dried mushroom is immersed in water, lenthionine is released and decomposed into carbon disulfide, which was found in considerable amounts in UI.

Eight-carbon-containing compounds comprise the main volatile compounds of fresh mushrooms, such as 1-octene-3-ol (Wu & Wang, 2000). However, UI did not present any C_8 compound, which may also be related to thermal decomposition during the oven drying of the sample. The decrease or disappearance of these compounds with an increase of temperature during the drying process of shiitake mushrooms was also reported in previous studies (Dermiki, Phanphensophon, et al., 2013; Tian et al., 2016; Xu et al., 2019).

According to Wu and Wang (2000), the aroma of dried shiitake mushrooms is more flavorful and meaty than that of fresh mushrooms, and therefore dried shiitake is more used in dishes with chicken, pork, or vegetables. Dermiki, Phanphensophon, et al. (2013) noted that compounds responsible for the typical shiitake flavor were present in greater quantity in extracts obtained at a lower temperature, while those prepared at higher temperatures had more umami-flavor compounds.

The effect of treatments on the volatile compounds profile of beef burgers is shown in Table S1. Forty-three volatile compounds were classified according to their chemical family: 11 hydrocarbons, 8 ketones, 5 aldehydes, 4 alcohols, 4 terpenes, 3 sulfur compounds, 3 esters, 2 amines, 1 ether, and 1 lactone.

The aldehydes are generated in meat products from lipid oxidation and Strecker degradation of amino acids (Olivares, Navarro, & Flores, 2009). Pentanal, hexanal, heptanal, and nonanal were found in similar concentrations in all treatments. These aldehydes are derived from the oxidation of fatty acids, mainly hexanal, which is related to an intense grass-like aroma and generally used as an indicator of lipid oxidation (Rodríguez-Carpena, Morcuende, & Estévez, 2012). Regarding the other linear saturated aldehydes, the literature has associated pentanal with "almond, malt, and pungent" notes, heptanal with "fat, citrus, and rancid" notes, and nonanal with "fat, citrus, and green" notes (Zhu, Wang, Xiao, & Niu, 2018). In turn, 2-methylbutanal and 3-methylbutanal are Strecker degradation products of isoleucine and leucine, respectively (Olivares et al., 2009). Burgers with UI showed a trend of increase in the contents of these two compounds, but a significantly higher concentration was observed only for 3-methybutanal in S2U2, S3U1, and S3U2. This result can be explained because the free amino acids isoleucine and leucine are the precursors of 2 and 3-methylbutanal, respectively, and showed numerically higher values in treatments with UI (Table 4). Moreover, these two aldehydes were found at considerable levels in UI (Table S1), which agrees with the high contents found in shiitake extract (Dermiki, Phanphensophon, et al., 2013). These volatile compounds have been reported to provide cocoa, almond (2-methylbutanal), and malt (3-methylbutanal) notes (Zhu et al., 2018).

Alcohols and ketones are also volatile compounds mainly generated from lipid oxidation in meat products (Soncin, Chiesa, Cantoni, & Biondi, 2007). In this study, 4 alcohols were identified in beef burgers. (S)-(+)-1,2-Propanediol was found at higher concentrations in treatments with UI, but its content was significantly higher compared to the control only in S3U2. This result may be due to the presence of 1,2-propanediol in UI (Table S1). 1-pentanol was another alcohol found in high concentrations, but there was no significant variation between treatments. According to Ramírez and Cava (2007), it is derived from the degradation of lipid hydroperoxides. Regarding the chemical class of ketones, 8 compounds were identified, but there was no significant change in their concentrations because of the treatments. Acetone is considered responsible for the buttery note in cooked meat (Barbieri et al., 1992) and 2,3-pentanedione and 2-heptanone are some of the ketones usually detected in meat products (Domínguez et al., 2019).

Three sulfur compounds were found in the volatile profile of beef burgers. These compounds are known to be part of the natural aroma of meat (Franke, Hilgarth, Vogel, Petermeier, & Langowski, 2019) and mushrooms (Wu & Wang, 2000). Although not significant, an increase in the content of dimethyl sulfone was found in treatments with UI, which can be explained given its high amount in this ingredient (Table S1). Dimethyl sulfide was not identified in UI, indicating that its presence in beef burgers is related to the degradation of sulfur-containing amino acids, such as methionine (Flores, 2017, pp. 383–417).

Among the hydrocarbons identified in the samples, lower concentrations of decane were observed in treatments with the highest UI content (2%) and with intermediate and low levels of salt (S2U2 and S3U2, respectively). According to Narváez-Rivas, Gallardo, and León-Camacho (2012), hydrocarbons are probably generated through the oxidation of lipids, but their presence does not cause a sensory impact (Bosse et al., 2017). 1H-Tetrazol-5-amine was the only amine that showed significant variation in content. Its concentration was low in the samples and a clear trend between treatments was not observed.

Regarding dimethyl ether, there was a significant variation in this compound's content among samples. The only treatment that differed from the control was S3U1, i.e., there was no clear trend between the formulations. This ether was also detected in roasted mutton and was reported to be generated from the pepper used in the formulations (Xi, Zhan, Tian, & Wang, 2019).

Only three esters and four terpenes were found in the burger samples, which were not significantly affected by the treatments. According to Flores (2018), esters and aldehydes contribute to green, fatty, and fruity odors, and terpenes impart a typical spicy aroma, usually derived from the spices used in manufacturing.

A PCA was also performed on the volatile compounds profile data and showed that the two first principal components explained 65.71% of the experimental data variation (Fig. 4). The first PC separated treatments according to salt content: samples with higher salt contents (C, S1U1, and S1U2) were placed on the right side, while those with lower salt contents (S2U1, S2U2, S3U1, and S3U2) were located on the left.

It is interesting to note that the right side of the first dimension presented the highest number of volatile compounds (30), which can be related to the high salt content of the samples. Meat aroma can be enhanced by the presence of NaCl, as it affects the osmotic pressure that reduces the solubility of volatile aroma compounds in the meat matrix, favoring their release (Bhat, Morton, Mason, & Bekhit, 2020). These compounds include the majority of hydrocarbons (10), aldehydes (4), and ketones (6), which were the main volatile compounds (in number and relative area) found in the beef burgers.

Pentanal (A3), hexanal (A4), heptanal (A5), and nonanal (A6) were the aldehydes correlated with the control and S1U1. These aldehydes are derived from the lipid oxidation of fatty acids (Rodríguez-Carpena et al., 2012). Considering that these two samples have a higher amount of NaCl, the prooxidant action of this salt may have favored the development of lipid oxidation, leading to the generation of these volatile compounds. On the other hand, 3-methylbutanal (A1) and 2-methylbutanal (A3) were located on the left side of the first dimension and were closer to treatments with higher salt reduction and UI addition. These two aldehydes are Strecker degradation products of isoleucine and leucine, respectively, which were found in considerable amounts in UI (Table S1).

Of the eight ketones identified in the samples, six (K1: Acetone; K2: 2,3-Pentanedione; K3: 2-Heptanone; K5: 3,6-Heptanedione; K6: 2(5H)-

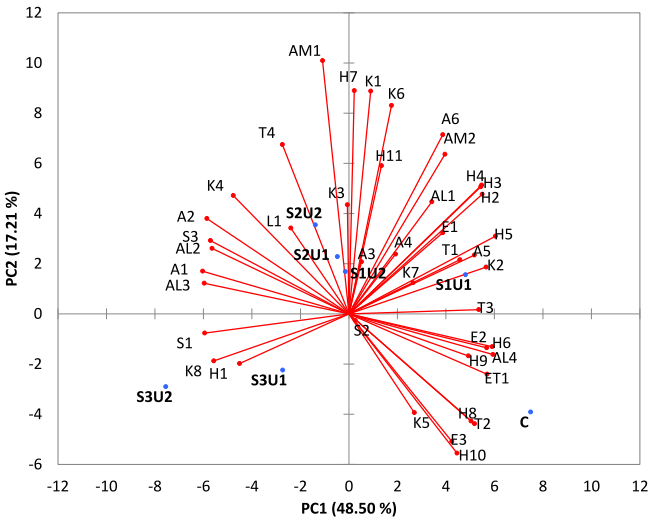

Fig. 4. Biplot of the first two dimensions of the PCA performed on volatile compounds data of beef burger with salt reduction and addition of Umami Ingredient.
Aldehydes: A1: Butanal, 3-methyl-; A2: Butanal, 2-methyl-; A3: Pentanal; A4: Hexanal; A5: Heptanal; A6: Nonanal; Alcohols: AL1: 1-Pentanol; AL2: 2,3-Butanediol; AL3: (S)-(+)-1,2-Propanediol; AL4: Ethanol, 2-phenoxy-; Ketones: K1: Acetone; K2: 2,3-Pentanedione; K3: 2-Heptanone; K4: Pyrolo[3,2-d]pyrimidin-2,4(1H,3H)-dione; K5: 3,6-Heptanedione; K6: 2(5H)-Furanone; K7: 2-Nonanone, 3-(hydroxymethyl)-; K8: 2H-Pyran-2-one, tetrahydro-; Hydrocarbons: H1: Pentane; H2: Methylene chloride; H3: n-Hexane; H4: Propane, 2-nitro-; H5: Methane, oxybis[dichloro-; H6: Butane, 2,2,3,3-tetramethyl-; H7: Heptane; H8: Heptane, 2,4-dimethyl-; H9: Nonane; H10: Decane; H11: Benzene, 1,3-bis(1,1-dimethylethyl)-; Esters: E1: Borinic acid, diethyl-, methyl ester; E2: Ethyl Acetate; E3: 5-Oxotetrahydrofuran-2-carboxylic acid, ethyl ester; Etheres: ET: Dimethyl ether; Terpenes: T1: Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-; T2: Bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(1-methylethyl)-; T3: D-Limonene; T4: Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1R*,4Z,9S*)]-; Amines: AM1: Methylamine, N,N-dimethyl-; AM2: 1H-Tetrazol-5-amine; Lactones: L1: Butyrolactone; Sulfur compounds: S1: Dimethyl sulfide; S2: Carbon disulfide; S3: Dimethyl sulfone.

Furanone; K7: 2-Nonanone, 3-(hydroxymethyl)-) were located on the right side of the first dimension, characterizing the control and S1U1. According to Flores (2018), in cooked meat products, such as those evaluated in this study, ketones originate from the thermal oxidation of fatty acids. Regarding hydrocarbons, approximately 91% of them were located on the right side of the map and, according to the literature, they are probably generated from the thermal oxidation of lipids, which is catalyzed by heme compounds, such as myoglobin and hemoglobin (Narváez-Rivas et al., 2012; Shahidi, Rubin, D'Souza, Teranishi, & Buttery, 1986). The results observed for ketones and hydrocarbons also show the possible lower oxidative stability of high-salt samples. Some of the possible mechanisms of NaCl prooxidant action are related to changes in the integrity of the cell membrane, allowing the access of oxidizing agents to lipids (Rhee, 1999) and facilitating the oxidation and liberation of Fe ions from iron-containing molecules (Kanner, Harel, & Jaffe, 1991). Another possibility for these results is the antioxidant potential of shiitake stipes (Zhang, Chen, Zhang, Ma, & Xu, 2013), which could have protected the samples with UI from lipid peroxidation.

All sulfur compounds were close to treatments with UI. Carbon disulfide (S2) was related to treatments S1U1 and S1U2, dimethyl sulfone (S3) to treatments S2U1 and S2U2, and dimethyl sulfide (S1) to treatments S3U1 and S3U2. Some of the major shiitake volatile compounds are straight-chain sulfur compounds (Wu & Wang, 2000), such as those identified in the burgers, showing the influence of shiitake stipes on the volatile profile of the samples.

4. Conclusion

Salt reduction and UI addition did not affect pH, water activity, and redness of cooked burgers, but increased lightness and yellowness. Even with the salt reduction, cooking loss and diameter reduction were not significantly affected, which is important for yield, especially in low-salt burgers. Positive results were also observed in terms of texture, as only samples with 70% salt reduction were significantly less hard and chewy than the control.

Regarding free amino acids from UI treatments, a significant increase was found only for proline and phenylalanine. In general, the treatments did not affect the amino acid profile of beef burgers compared to the control and the most abundant amino acids were those related to umami taste (glutamic and aspartic acids). The volatile profile of beef burgers showed a slight increase in compounds also found in UI, mainly 3-methylbutanal, (S)-(+)-1,2-Propanediol, and dimethyl sulfide.

This study revealed that shiitake by-products can be transformed into a flavor enhancer with the potential to aid the development of low-salt meat products, which translates into benefits related to sustainability and healthiness. However, further research should be carried out to study the economic feasibility of producing this ingredient and to evaluate the sensory profile and consumers' liking for a beef burger with UI addition.

CRediT authorship contribution statement

Fabiana França: Investigation, Writing – original draft. **Samara dos Santos Harada-Padermo:** Conceptualization, Writing – review & editing. **Rafaela Alves Frasceto:** Formal analysis. **Erick Saldaña:** Formal analysis, Writing – review & editing. **José Manuel Lorenzo:** Resources, Writing – review & editing. **Thais Maria Ferreira de Souza Vieira:** Conceptualization, Writing – review & editing. **Miriam Mabel Selani:** Supervision, Conceptualization, Resources, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2021.112724.

References

Abd El-Aleem, F. S., Taher, M. S., Lotfy, S. N., El-Massry, K. F., & Fadel, H. H. (2017). Influence of extracted 5-nucleotides on aroma compounds and flavour acceptability of real beef soup. *International Journal of Food Properties, 20*, S1182–S1194. https://doi.org/10.1080/10942912.2017.1286506

Aburto, N. J., Ziolkovska, A., Hooper, L., Elliott, P., Cappuccio, F. P., & Meerpohl, J. K. (2013). Effect of lower sodium intake on health: Systematic review and meta-analyses. *BMJ, 346*, Article f1326. https://doi.org/10.1136/bmj.f1326

Akesowan, A. (2016). Production and storage stability of formulated chicken nuggets using konjac flour and shiitake mushrooms. *Journal of Food Science & Technology, 53*(10), 3661–3674. https://doi.org/10.1007/s13197-016-2332-7

Albarracín, W., Sánchez, I. C., Grau, R., & Barat, J. M. (2011). Salt in food processing; usage and reduction: A review. *International Journal of Food Science and Technology, 46*, 1329–1336. https://doi.org/10.1111/j.1365-2621.2010.02492.x

AOAC. (1996). *Official methods of analysis of AOAC International - Official method 956.01* (16th ed.) Gaithersburg.

Barbieri, G., Bolzoni, L., Parolarí, G., Virgili, R., Buttini, R., Careri, M., et al. (1992). Flavor compounds of dry-cured ham. *Journal of Agricultural and Food Chemistry, 40*(12), 2389–2394. https://doi.org/10.1021/jf00024a013

Baublits, R. T., Pohlman, F. W., Brown, A. H., Yancey, E. J., & Johnson, Z. B. (2006). Impact of muscle type and sodium chloride concentration on the quality, sensory, and instrumental color characteristics of solution enhanced whole-muscle beef. *Meat Science, 72*(4), 704–712. https://doi.org/10.1016/j.meatsci.2005.09.023

Besbes, S., Attia, H., Deroanne, C., Makni, S., & Blecker, C. (2008). Partial replacement of meat by pea fiber and wheat fiber: Effect on the chemical composition, cooking characteristics and sensory properties of beef burgers. *Journal of Food Quality, 31*(4), 480–489. https://doi.org/10.1111/j.1745-4557.2008.00213.x

Bhat, Z. F., Morton, J. D., Mason, S. L., & Bekhit, A. E. D. A. (2020). The application of pulsed electric field as a sodium reducing strategy for meat products. *Food Chemistry, 306*, Article 125622. https://doi.org/10.1016/j.foodchem.2019.125622

Bosse, R., Wirth, M., Konstanz, A., Becker, T., Weiss, J., & Gibis, M. (2017). Determination of volatile marker compounds in raw ham using headspace-trap gas chromatography. *Food Chemistry, 219*, 249–259. https://doi.org/10.1016/j.foodchem.2016.09.094

Carocho, M., Morales, P., & Ferreira, I. C. F. R. (2015). Natural food additives: Quo vadis? *Trends in Food Science & Technology, 45*(2), 284–295. https://doi.org/10.1016/j.tifs.2015.06.007

Cerón-Guevara, M. I., Rangel-Vargas, E., Lorenzo, J. M., Bermúdez, R., Pateiro, M., Rodríguez, J. A., et al. (2020). Reduction of salt and fat in frankfurter sausages by addition of *Agaricus bisporus* and *Pleurotus ostreatus* flour. *Foods, 9*(6), 760. https://doi.org/10.3390/foods9060760

Chen, W., Li, W., Yang, Y., Yu, H., Zhou, S., Feng, J., et al. (2015). Analysis and evaluation of tasty components in the pileus and stipe of *Lentinula edodes* at different growth stages. *Journal of Agricultural and Food Chemistry, 63*(3), 795–801. https://doi.org/10.1021/jf505410a

Choe, J., Lee, J., Jo, K., Jo, C., Song, M., & Jung, S. (2018). Application of winter mushroom powder as an alternative to phosphates in emulsion-type sausages. *Meat Science, 143*, 114–118. https://doi.org/10.1016/j.meatsci.2018.04.038

Cittadini, A., Domínguez, R., Gómez, B., Pateiro, M., Pérez-Santaescolástica, C., López-Fernández, O., & Lorenzo, J. M. (2020). Effect of NaCl replacement by other chloride salts on physicochemical parameters, proteolysis and lipolysis of dry-cured foal "cecina". *Journal of Food Science and Technology, 57*(5), 1628–1635.

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

Dermiki, M., Phanphensophon, N., Mottram, D. S., & Methven, L. (2013). Contributions of non-volatile and volatile compounds to the umami taste and overall flavour of shiitake mushroom extracts and their application as flavour enhancers in cooked minced meat. *Food Chemistry, 141*, 77–83. https://doi.org/10.1016/j.foodchem.2013.03.018

Desmond, E. (2006). Reducing salt: A challenge for the meat industry. *Meat Science, 74*(1), 188–196. https://doi.org/10.1016/j.meatsci.2006.04.014

Domínguez, R., Borrajo, P., & Lorenzo, J. M. (2015). The effect of cooking methods on nutritional value of foal meat. *Journal of Food Composition and Analysis, 43*, 61–67. https://doi.org/10.1016/j.jfca.2015.04.007

Domínguez, R., Purriños, L., Pérez-Santaescolástica, C., Pateiro, M., Barba, F. J., Tomasevic, I., et al. (2019). Characterization of volatile compounds of dry-cured meat products using HS-SPME-GC/MS technique. *Food Analytical Methods, 12*(6), 1263–1284. https://doi.org/10.1007/s12161-019-01491-x

Doyle, M. E., & Glass, K. A. (2010). Reduction and its effect on food safety, food quality , and human health. *Comprehensive Reviews in Food Science and Food Safety, 9*, 44–56. https://doi.org/10.1111/j.1541-4337.2009.00096.x

FAO/WHO. (1991). *Protein Quality Evaluation: Report of Joint FAO/WHO Expert Consultation*. FAO Food and Nutrition Paper.

Fellendorf, S., Kerry, J. P., Hamill, R. M., & O'Sullivan, M. G. (2018). Impact on the physicochemical and sensory properties of salt reduced corned beef formulated with and without the use of salt replacers. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 92*, 584–592. https://doi.org/10.1016/j.lwt.2018.03.001

Flores, M. (2017). The eating quality of meat: III-flavor. *Lawrie's meat Science* (8th ed.). https://doi.org/10.1016/B978-0-08-100694-8.00013-3

Flores, M. (2018). Understanding the implications of current health trends on the aroma of wet and dry cured meat products. *Meat Science, 144*, 53–61. https://doi.org/10.1016/j.meatsci.2018.04.016

Franke, C., Hilgarth, M., Vogel, R. F., Petermeier, H., & Langowski, H. C. (2019). Characterization of the dynamics of volatile organic compounds released by lactic acid bacteria on modified atmosphere packed beef by PTR-MS. *Food Packaging and Shelf Life, 22*, Article 100400. https://doi.org/10.1016/j.fpsl.2019.100400

Gilbert, P. A., & Heiser, G. (2005). Salt and health: The CASH and BPA perspective. *Nutrition Bulletin, 30*(1), 62–69. https://doi.org/10.1111/j.1467-3010.2005.00484.x

Gujral, H. S., Kaur, A., Singh, N., & Sodhi, N. S. (2002). Effect of liquid whole egg, fat and textured soy protein on the textural and cooking properties of raw and baked patties from goat meat. *Journal of Food Engineering, 53*(4), 377–385. https://doi.org/10.1016/S0260-8774(01)00180-7

Gullón, P., Astray, G., Gullón, B., Franco, D., Campagnol, P. C. B., & Lorenzo, J. M. (2021). Inclusion of seaweeds as healthy approach to formulate new low-salt meat products. *Current Opinion in Food Science, 40*, 20–25. https://doi.org/10.1016/j.cofs.2020.05.005

Harada-Padermo, S., dos, S., Dias-Faceto, L. S., Selani, M. M., Alvim, I. D., Floh, E. I. S., et al. (2020). Umami Ingredient: Flavor enhancer from shiitake (*Lentinula edodes*) byproducts. *Food Research International, 137*, Article 109540. https://doi.org/10.1016/j.foodres.2020.109540

Harada-Padermo, S., dos, S., Dias-Faceto, L. S., Selani, M. M., Conti-Silva, A. C., & Vieira, T. M. F. de S. (2021). Umami ingredient, a newly developed flavor enhancer from shiitake byproducts, in low-sodium products: A study case of application in corn extruded snacks. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 138*, Article 110806. https://doi.org/10.1016/j.lwt.2020.110806

Hereu, A., Dalgaard, P., Garriga, M., Aymerich, T., & Bover-Cid, S. (2012). Modeling the high pressure inactivation kinetics of Listeria monocytogenes on RTE cooked meat products. *Innovative Food Science & Emerging Technologies, 16*, 305–315. https://doi.org/10.1016/j.ifset.2012.07.005

He, F. J., Tan, M., Ma, Y., & MacGregor, G. A. (2020). Salt reduction to prevent hypertension and cardiovascular disease: JACC state-of-the-art review. *Journal of the American College of Cardiology, 75*(6), 632–647. https://doi.org/10.1016/j.jacc.2019.11.055

Hou, H., Liu, C., Lu, X., Fang, D., Hu, Q., Zhang, Y., et al. (2021). Characterization of flavor frame in shiitake mushrooms (*Lentinula edodes*) detected by HS-GC-IMS coupled with electronic tongue and sensory analysis: Influence of drying techniques. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 146*, Article 111402. https://doi.org/10.1016/j.lwt.2021.111402

Inguglia, E. S., Zhang, Z., Tiwari, B. K., Kerry, J. P., & Burgess, C. M. (2017). Salt reduction strategies in processed meat products – a review. *Trends in Food Science & Technology, 59*, 70–78. https://doi.org/10.1016/j.tifs.2016.10.016

Ito, Y., Toyoda, M., Suzuki, H., & Iwaida, M. (1978). Gas-liquid chromatographic determination of lenthionine in Shiitake mushroom (*Lentinus edodes*) with special reference to the relation between carbon disulfide and lenthionine. *Journal of Food Science, 43*(4), 1287–1289. https://doi.org/10.1111/j.1365-2621.1978.tb15289.x

Jo, K., Lee, J., & Jung, S. (2018). Quality characteristics of low-salt chicken sausage supplemented with a winter mushroom powder. *Korean Journal for Food Science of Animal Resources, 38*(4), 768–779. https://doi.org/10.5851/kosfa.2018.e15

Kanner, J., Harel, S., & Jaffe, R. (1991). Lipid peroxidation of muscle food as affected by NaCl. *Journal of Agricultural and Food Chemistry, 39*(6), 1017–1021. https://doi.org/10.1021/jf00006a002

Kim, K.-J., Im, S.-B., Yun, K.-W., Je, H.-S., Ban, S.-E., Jin, S.-W., et al. (2017). Content of proximate compositions, free sugars, amino acids, and minerals in five *Lentinula edodes* cultivars collected in Korea. *Journal of Mushroom, 15*(4), 216–222. https://doi.org/10.14480/JM.2017.15.4.216

Kong, Y., Zhang, L. L., Zhao, J., Zhang, Y. Y., Sun, B. G., & Chen, H. T. (2019). Isolation and identification of the umami peptides from shiitake mushroom by consecutive chromatography and LC-Q-TOF-MS. *Food Research International, 121*, 463–470. https://doi.org/10.1016/j.foodres.2018.11.060

Kurihara, K., & Kashiwayanagi, M. (2000). Physiological studies on umami taste. *Journal of Nutrition, 130*(4), 931S–934S. https://doi.org/10.1093/jn/130.4.931S

Lorenzo, J. M., Cittadini, A., Bermúdez, R., Munekata, P. E., & Domínguez, R. (2015). Influence of partial replacement of NaCl with KCl, $CaCl_2$ and $MgCl_2$ on proteolysis, lipolysis and sensory properties during the manufacture of dry-cured lacón. *Food Control, 55*, 90–96. https://doi.org/10.1016/j.foodcont.2015.02.035

Matarneh, S. K., England, E. M., Scheffler, T. L., & Gerrard, D. E. (2017). The conversion of muscle to meat. In F. Toldrá (Ed.), *Lawrie's meat Science* (8th ed., pp. 159–185). Woodhead Publishing. https://doi.org/10.1016/B978-0-08-100694-8.00005-4.

Mattar, T. V., Gonçalves, C. S., Pereira, R. C., Faria, M. A., de Souza, V. R., & Carneiro, J. de D. S. (2018). A shiitake mushroom extract as a viable alternative to NaCl for a reduction in sodium in beef burgers: A sensory perspective. *British Food Journal, 120*(6), 1366–1380. https://doi.org/10.1108/BFJ-05-2017-0265

Mill, J. G., Malta, D. C., Machado, Í. E., Pate, A., Pereira, C. A., Jaime, P. C., et al. (2019). Estimation of salt intake in the Brazilian population: Results from the 2013 National health survey. *Revista Brasileira de Epidemiologia, 22*(suppl 2). https://doi.org/10.1590/1980-549720190009.supl.2

Mojet, J., Heidema, J., & Christ-Hazelhof, E. (2004). Effect of concentration on taste-taste interactions in foods for elderly and young subjects. *Chemical Senses, 29*(8), 671–681. https://doi.org/10.1093/chemse/bjh070

Narváez-Rivas, M., Gallardo, E., & León-Camacho, M. (2012). Analysis of volatile compounds from Iberian hams: A review. *Grasas Y Aceites, 63*(4), 432–454. https://doi.org/10.3989/gya.070112

Olivares, A., Navarro, J. L., & Flores, M. (2009). Distribution of volatile compounds in lean and subcutaneous fat tissues during processing of dry fermented sausages. *Food Research International, 42*(9), 1303–1308. https://doi.org/10.1016/j.foodres.2009.03.017

Pateiro, M., Munekata, P. E., Cittadini, A., Domínguez, R., & Lorenzo, J. M. (2021). Metallic-based salt substitutes to reduce sodium content in meat products. *Current Opinion in Food Science, 38*, 21–31. https://doi.org/10.1016/j.cofs.2020.10.029

Patinho, I., Saldaña, E., Selani, M. M., de Camargo, A. C., Merlo, T. C., Menegali, B. S., et al. (2019). Use of *Agaricus bisporus* mushroom in beef burgers: Antioxidant, flavor enhancer and fat replacing potential. *Food Production, Processing and Nutrition, 1*(1), 1–15. https://doi.org/10.1186/s43014-019-0006-3

Patinho, I., Selani, M. M., Saldaña, E., Bortoluzzi, A. C. T., Rios-Mera, J. D., da Silva, C. M., et al. (2021). *Agaricus bisporus* mushroom as partial fat replacer improves the sensory quality maintaining the instrumental characteristics of beef burger. *Meat Science, 172*, Article 108307. https://doi.org/10.1016/j.meatsci.2020.108307

Pil-Nam, S., Park, K. M., Kang, G. H., Cho, S. H., Park, B. Y., & Van-Ba, H. (2015). The impact of addition of shiitake on quality characteristics of frankfurter during refrigerated storage. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 62*(1), 62–68. https://doi.org/10.1016/j.lwt.2015.01.032

Poojary, M. M., Orlien, V., Passamonti, P., & Olsen, K. (2017). Improved extraction methods for simultaneous recovery of umami compounds from six different mushrooms. *Journal of Food Composition and Analysis, 63*, 171–183. https://doi.org/10.1016/j.jfca.2017.08.004

Radam, A., Yacob, M. R., Bee, T. S., & Selamat, J. (2010). Consumers ' perceptions, attitudes and Willingness to pay towards food products with "No added Msg" labeling. *International Journal of Marketing Studies, 2*(1), 65–77.

Ramírez, R., & Cava, R. (2007). Volatile profiles of dry-cured meat products from three different Iberian x Duroc genotypes. *Journal of Agricultural and Food Chemistry, 55*(5), 1923–1931. https://doi.org/10.1021/jf062810l

Rathore, H., Prasad, S., & Sharma, S. (2017). Mushroom nutraceuticals for improved nutrition and better human health: A review. *PharmaNutrition, 5*(2), 35–46. https://doi.org/10.1016/j.phanu.2017.02.001

Rhee, K. S. (1999). Storage stability of meat products as affected by organic and inorganic additives and functional ingredients. In Y. L. Xiong, C. Ho, & F. Shahidi (Eds.), *Quality Attributes of muscle foods* (pp. 99–113). New York: Kluwer Academic/Plenum Publishers.

Rios-Mera, J. D., Saldaña, E., Cruzado-Bravo, M. L. M., Martins, M. M., Patinho, I., Selani, M. M., et al. (2020). Impact of the content and size of NaCl on dynamic sensory profile and instrumental texture of beef burgers. *Meat Science, 161*, Article 107992. https://doi.org/10.1016/j.meatsci.2019.107992

Rios-Mera, J. D., Saldaña, E., Cruzado-Bravo, M. L. M., Patinho, I., Selani, M. M., Valentin, D., et al. (2019). Reducing the sodium content without modifying the quality of beef burgers by adding micronized salt. *Food Research International, 121*, 288–295. https://doi.org/10.1016/j.foodres.2019.03.044

Rios-Mera, J. D., Selani, M. M., Patinho, I., Saldaña, E., & Contreras-Castillo, C. J. (2021). Modification of NaCl structure as a sodium reduction strategy in meat products: An overview. *Meat Science, 174*, Article 108417. https://doi.org/10.1016/j.meatsci.2020.108417

Rodríguez-Carpena, J. G., Morcuende, D., & Estévez, M. (2012). Avocado, sunflower and olive oils as replacers of pork back-fat in burger patties: Effect on lipid composition, oxidative stability and quality traits. *Meat Science, 90*(1), 106–115. https://doi.org/10.1016/j.meatsci.2011.06.007

Rosli, W., Solihah, W. I., Aishah, M. A., Fakurudin, N., Mohsin, N. A., & Kelantan, K. (2011). Colour, textural properties, cooking characteristics and fibre content of chicken patty added with oyster mushroom *(Pleurotus sajor-caju)*. *International Food Research Journal, 18*, 621–627.

Saldaña, E., Merlo, T. C., Patinho, I., Rios-Mera, J. D., Contreras-Castillo, C. J., & Selani, M. M. (2021). Use of sensory science for the development of healthier processed meat products: A critical opinion. *Current Opinion in Food Science, 40*, 13–19. https://doi.org/10.1016/j.cofs.2020.04.012

Santos, B. A., Campagnol, P. C. B., Morgano, M. A., & Pollonio, M. A. R. (2014). Monosodium glutamate, disodium inosinate, disodium guanylate, lysine and taurine improve the sensory quality of fermented cooked sausages with 50% and 75% replacement of NaCl with KCl. *Meat Science, 96*, 509–513. https://doi.org/10.1016/j.meatsci.2013.08.024

Selani, M. M., Shirado, G. A. N., Margiotta, G. B., Rasera, M. L., Marabesi, A. C., Piedade, S. M. S., et al. (2016). Pineapple by-product and canola oil as partial fat replacers in low-fat beef burger: Effects on oxidative stability, cholesterol content and fatty acid profile. *Meat Science*. https://doi.org/10.1016/j.meatsci.2016.01.002

Shahidi, F., Rubin, L. J., D'Souza, L. A., Teranishi, R., & Buttery, R. G. (1986). Meat flavor volatiles: A review of the composition, techniques of analysis, and sensory evaluation. *Critical Reviews in Food Science and Nutrition, 24*(2), 141–243. https://doi.org/10.1080/10408398609527435

da Silva, S. L., Lorenzo, J. M., Machado, J. M., Manfio, M., Cichoski, A. J., Fries, L. L. M., et al. (2020). Application of arginine and histidine to improve the technological and sensory properties of low-fat and low-sodium bologna-type sausages produced with high levels of KCl. *Meat Science, 159*, Article 107939. https://doi.org/10.1016/j.meatsci.2019.107939

Soncin, S., Chiesa, L. M., Cantoni, C., & Biondi, P. A. (2007). Preliminary study of the volatile fraction in the raw meat of pork, duck and goose. *Journal of Food Composition and Analysis, 20*(5), 436–439. https://doi.org/10.1016/j.jfca.2006.09.001

Tian, Y., Zhao, Y., Huang, J., Zeng, H., & Zheng, B. (2016). Effects of different drying methods on the product quality and volatile compounds of whole shiitake mushrooms. *Food Chemistry, 197*, 714–722. https://doi.org/10.1016/j.foodchem.2015.11.029

Toldrá, F., & Flores, M. (1998). The role of muscle proteases and lipases in flavor development during the processing of dry-cured ham. *Critical Reviews in Food Science, 38*(4), 331–352. https://doi.org/10.1080/10408699891274237

Van Ba, H., Seo, H.-W., Cho, S.-H., Kim, Y.-S., Kim, J.-H., Ham, J.-S., et al. (2016). Antioxidant and anti-foodborne bacteria activities of shiitake by-product extract in fermented sausages. *Food Control, 70*, 201–209. https://doi.org/10.1016/J.FOODCONT.2016.05.053

WHO. (2014). *Global status report on noncommunicable diseases 2014*. Geneva: World Health Organization.

Wu, C.-M., & Wang, Z. (2000). Volatile compounds in fresh and processed shiitake mushrooms (*Lentinus edodes* sing.). *Food Science and Technology Research, 6*(3), 166–170. https://doi.org/10.3136/fstr.6.166

Xi, J., Zhan, P., Tian, H., & Wang, P. (2019). Effect of spices on the formation of VOCs in roasted mutton based on GC-MS and principal Component analysis. *Journal of Food Quality*. https://doi.org/10.1155/2019/8568920, 2019.

Xu, L., Fang, X., Wu, W., Chen, H., Mu, H., & Gao, H. (2019). Effects of high-temperature pre-drying on the quality of air-dried shiitake mushrooms (*Lentinula edodes*). *Food Chemistry, 285*, 406–413. https://doi.org/10.1016/j.foodchem.2019.01.179

Yamaguchi, S. (1991). Basic properties of umami and effects on humans. *Physicology & Behavior, 49*(2–3), 833–841. https://doi.org/10.1080/87559129809541156

Yang, J. H., Lin, H. C., & Mau, J. L. (2001). Non-volatile taste components of several commercial mushrooms. *Food Chemistry, 72*(4), 465–471. https://doi.org/10.1016/S0308-8146(00)00262-4

Yang, X., Zhang, Y., Kong, Y., Zhao, J., Sun, Y., & Huang, M. (2019). Comparative analysis of taste compounds in shiitake mushrooms processed by hot-air drying and freeze drying. *International Journal of Food Properties, 22*(1), 1100–1111. https://doi.org/10.1080/10942912.2019.1628777

Zhang, N., Chen, H., Zhang, Y., Ma, L., & Xu, X. (2013). Comparative studies on chemical parameters and antioxidant properties of stipes and caps of shiitake mushroom as affected by different drying methods. *Journal of the Science of Food and Agriculture, 93*(12), 3107–3113. https://doi.org/10.1002/jsfa.6151

Zhang, H., Peng, J., Zhang, Y. R., Liu, Q., Pan, L. Q., & Tu, K. (2020). Discrimination of volatiles of shiitakes (*Lentinula edodes*) produced during drying process by electronic Nose. *International Journal of Food Engineering, 16*(1–2), 1–13. https://doi.org/10.1515/ijfe-2019-0233

Zhang, Y., Venkitasamy, C., Pan, Z., & Wang, W. (2013). Recent developments on umami ingredients of edible mushrooms - a review. *Trends in Food Science & Technology, 33*, 78–92. https://doi.org/10.1016/j.tifs.2013.08.002

Zhu, J. C., Wang, L. Y., Xiao, Z. B., & Niu, Y. W. (2018). Characterization of the key aroma compounds in mulberry fruits by application of gas chromatography–olfactometry (GC-O), odor activity value (OAV), gas chromatography-mass spectrometry (GC–MS) and flame photometric detection (FPD). *Food Chemistry, 245*, 775–785. https://doi.org/10.1016/j.foodchem.2017.11.112