DOCTORAL THESES IN FOOD SCIENCES AT THE UNIVERSITY OF TURKU

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ABSTRACT

Umami, one of the five basic tastes, gives a special deliciousness, richness and meatiness to many vegetable and meat dishes. The special feature of umami compounds, mainly free glutamic acid and free 5′-nucleotides, is their synergistic interaction, which significantly enhances the taste sensation of umami when these compounds are simultaneously present in food. Even though umami compounds themselves do not taste particularly pleasant, the unique characteristic of umami is that it functions best when combined with other basic tastes. Although considerable research has been done on umami taste related compounds, there is less research on the effect of cooking on the concentration of these compounds and any possible changes in their concentration during heat treatment.

The aim of this thesis was to investigate the overall change in the concentrations of umami compounds in Nordic food induced by cooking. Multiple simultaneous reactions, both in the formation and loss of umami compounds are likely to occur during cooking. Changes in the content of the compounds may result from both enzymatic and chemical reactions, possibly accelerated or stabilized by the increase in temperature. The raw materials studied in this thesis, including meat, mushrooms and potatoes represent fauna, fungi and flora, differ from each other in terms of protein, carbohydrate and fat content. However, they all contain detectable concentrations of umami compounds, both free amino acids and nucleotides. The concentrations of the compounds in the examined raw materials, both fresh and cooked, were determined quantitatively by chromatographic techniques.

Cooking is essential for most food ingredients. This study showed cooking influenced the concentration of umami compounds in food raw materials, and the cooking temperature was more important variable than the cooking time. However, from the perspective of the synergy, heat treatment may have different effect on release and the content of certain umami compounds. Increase in temperature can accelerate inherent enzymatic activities in the raw material and result in changes in the content of compounds. In particular, the concentrations of nucleotides may increase or decrease until the temperature reaches the enzyme deactivation point. In addition, heat may promote the participation of free amino acids in chemical reactions with other compounds present in the raw material and lead to a decrease in the concentration of amino acids. The water-binding capacity of the raw material is affected by many factors. As sous vide cooking progresses, the denaturation of proteins and other structural changes caused by heat are important factors contributing to the weakening of the water-binding

capacity. As a result, umami compounds are released into the liquid. Thus, the cooking juice is also a valuable source of umami.

In pork, the concentration of the nucleotide 5′-IMP and the free amino acid glutamic acid remained almost at the same level at the three different sous vide cooking temperatures 60, 70 and 80 °C and three different cooking times 60, 120 and 180 min. The 5′-IMP content of the corresponding cooking juice released from meat also remained unchanged. However, the increase in the glutamic acid concentration in the cooking juice at the highest investigated temperature was statistically significant. In addition, the increase in the relative share of cooking juice from the total sample at the highest cooking temperature studied increases the role of juice as a source of umami.

The increase in 5′-nucleotide content due to the effect of sous vide cooking temperature was seen especially in the study of mushrooms. The concentrations of the umami nucleotide 5′-GMP was detectable only in samples cooked at a sufficiently high temperature 70, 80 or 90 °C for cooking time 10 minutes but was negligible at 60 °C and fresh mushrooms. Instead, the concentration of free amino acids both in the mushrooms and in the released cooking juice partially either decreased or increased during cooking. For different mushroom species, the amount and direction of the change varied as the cooking temperature increased. This indicates the presence of chemical reactions of free amino acids, as well as differences in concentrations between fungal species.

Growth conditions are known to have a major impact on potato quality. The results of our study showed that the concentration of umami FAA glutamic acid, as well as most of the other FAAs was significantly higher in short day (SD) conditions (8h light, 16h dark) developed tubers than long day (LD) tubers (15 h light, 9h dark).

This study focused on the umami compounds of Nordic food raw materials. The results of the research can be applied in the product development of delicious and tasty meals, for example, to increase the consumption of plant-based alternatives. The study also showed the potential of the cooking juice released from meat and mushroom as a source of umami. This result can be utilized in the efforts to use raw materials more efficiently and in the attempts to reduce food waste. In addition to cultivated mushrooms, the study showed the potential of forest mushrooms as a source of umami. Moreover, in the breeding of potatoes and in the selection of cultivars and the growing conditions used, more attention should be paid to the taste properties of the potato tubers and thereby to the possibilities of increasing potato consumption.

SUOMENKIELINEN ABSTRAKTI

Umami, yksi viidestä perusmausta, antaa erityistä herkullisuutta, täyteläisyyttä ja lihaisuutta moniin kasvis- ja liharuokiin. Umamiyhdisteiden, pääasiassa vapaan glutamiinihapon ja 5´-nukleotidien erityispiirteenä on niiden synergistinen vaikutus. Kun näitä yhdisteitä on ruoassa samanaikaisesti, lisää se merkittävästi umamimaun aistimista. Kuitenkaan sellaisenaan umamiyhdisteet eivät maistu erityisen miellyttävältä, vaan niiden ainutlaatuiset ominaisuudet tulevat parhaiten esiin yhdistettäessä muihin perusmakuihin. Vaikka umamiaistimusta aikaan saavia yhdisteitä on tutkittu paljon, tutkimusten määrä kypsennyksen vaikutuksesta näiden yhdisteiden pitoisuuteen ja pitoisuuksien mahdollisiin muutoksiin lämmön vaikutuksesta on vähäisempi.

Tämän väitöskirjan tavoitteena oli tutkia kypsennyksen aikaansaamaa kokonaismuutosta ruuan umamiyhdisteiden pitoisuudessa. Kypsennyksen aikana tapahtuu todennäköisesti useita samanaikaisia reaktioita, niin umamiyhdisteiden muodostumista kuin häviämistäkin. Tutkitut raaka-aineet, liha, sienet ja perunat edustavat sekä eläin- että kasvikuntaa ja eroavat toisistaan proteiini-, hiilihydraatti- ja rasvapitoisuuksiltaan. Ne kaikki sisältävät kuitenkin havaittavissa olevia pitoisuuksia umami-yhdisteitä, sekä vapaita aminohappoja että nukleotideja. Yhdisteiden pitoisuudet tutkituissa raaka-aineissa, sekä tuoreissa että kypsennetyissä, määritettiin kvantitatiivisesti kromatografisilla menetelmillä.

Kypsentäminen on välttämätöntä useimpien ruokaraaka-aineiden kohdalla. Tässä tutkimuksessa kypsennyksen todettiin vaikuttavan raaka-aineen umamiyhdisteiden pitoisuuksiin ja kypsennyslämpötilan todettiin olevan suurempi vaikutus kuin kypsennysajalla. Umamiyhdisteiden synergian näkökulmasta lämpökäsittely saattaa vaikuttaa eri tavoin tutkittujen umamiyhdisteiden vapautumiseen ja pitoisuuksiin. Lämpötilan nousu voi kiihdyttää raaka-aineessa esiintyvää luontaista entsyymiaktiivisuutta ja siten saada aikaan muutoksia yhdisteiden määrissä. Erityisesti nukleotidien pitoisuudet saattavat kasvaa tai laskea entsyymien deaktivoitumislämpötilaan saakka. Lisäksi lämpö saattaa edesauttaa vapaiden aminohappojen osallistumista kemiallisiin reaktioihin muiden raaka-aineessa esiintyvien yhdisteiden kanssa ja johtaa pitoisuuden laskuun. Raaka-aineen vedensidontakykyyn vaikuttavat monet tekijät. Kypsennyksen edetessä proteiinien denaturaatio ja muut lämmön aikaan saamat rakenteelliset muutokset ovat tärkeitä tekijöitä osaltaan heikentämään vedensidontakykyä, jonka seurauksena umamiyhdisteitä vapautuu nesteeseen. Tämän vuoksi myös liemi on arvokas umamin lähde.

Porsaanlihalla nukleotidi 5´-IMP:n ja vapaan aminohapon, glutamiinihapon pitoisuus lihassa pysyi lähes samalla tasolla kolmessa eri sous vide kypsennyslämpötilassa 60, 70 and 80 °C ja kolmella ei kypsennysajalla 60, 120 and 180 minuuttia. Myös lihasta vapautuneen liemen 5´-IMP pitoisuus pysyi muuttumattomana. Huomionarvoista kuitenkin on glutamiinihappopitoisuuden tilastollisesti merkittävä kasvu liemessä korkeimmassa tutkitussa lämpötilassa. Samanaikainen liemen suhteellisen osuuden kasvu kokonaisnäytteessä kasvattaa liemen roolia umamin lähteenä.

Nukleotidipitoisuuden kasvu lämpötilan vaikutuksesta oli nähtävissä erityisesti sienten kohdalla. Umami-nukleotidi 5´-GMP:n pitoisuudet olivat havaittavissa ainoastaan korkeammissa kypsennyslämpötiloissa 70, 80 ja 90 °C, 10 minuutin kypsennysajalla. kypsennetyissä näytteissä. Tuoreen ja 60 °C lämpötilassa kypsennettyjen sienien 5´-GMP:n pitoisuudet olivat määritysrajan alapuolella. Sen sijaan vapaiden aminohappojen pitoisuudet sekä sienissä ja että vapautuneessa liemessä osittain sekä laskivat että nousivat eri kypsennyslämpötiloissa. Eri sienilajeilla muutoksen määrä ja suunta vaihtelivat kypsennyslämpötilan kasvaessa. Tämä indikoi vapaiden aminohappojen mahdollisia kemiallisia reaktiota sekä sienilajien välisiä eroja pitoisuuksissa.

Kasvuolosuhteilla tiedetään olevan suuri vaikutus perunan laatuun. Tutkimuksemme tulokset osoittivat lyhyen päivänpituuden (SD) olosuhteissa (8h valoa, 16h pimeyttä) kasvaneiden perunoiden sisältävän korkeammat pitoisuudet vapaita aminohappoja, mukaan lukien umamimaulle tärkeää glutamiinihappoa, kuin pitkän päivän (LD) perunat (15h valoa, 9h pimeyttä).

Tämä tutkimus keskittyi pohjoismaisten ruokaraaka-aineiden umamiyhdisteisiin. Tutkimuksen tuloksia voidaan hyödyntää täyteläisten ja maukkaiden aterioiden tuotekehityksessä, esimerkiksi kasvispohjaisten vaihtoehtojen kulutuksen lisäämiseksi. Tutkimus osoittaa myös lihasta ja sienestä vapautuvan liemen mahdollisuudet umamin lähteenä. Tulosta voidaan hyödyntää pyrkimyksissä raaka-aineiden tehokkaampaan käyttöön ja ruokahävikin vähentämiseen. Tutkimus osoittaa metsäsienien mahdollisuudet umamin lähteenä viljeltyjen sienien lisäksi. Myös perunan jalostuksen lajikevalinnoissa ja kasvuolosuhteissa tulisi kiinnittää enemmän huomiota perunan makuun ja sitä kautta perunan kulutuksen lisäämiseen.

LIST OF ABBREVIATIONS

ADP	adenosine diphosphate
AMP	adenosine monophosphate
ATP	adenosine triphosphate
CMP	cytidine monophosphate
DLI	daily light integral
DNA	deoxyribonucleic acid
DW	dry weight
EUC	equivalent umami concentration
FAA	free amino acid
FD	fluorescence detector
FMOC	fluorenylmethyloxycarbonyl
FW	fresh weight
GMP	guanosine monophosphate
GPCR	G protein-coupled receptor
HPLC	high performance liquid chromatography
IMP	inosine monophosphate
LD	long day
MPA	mercaptopropionic acid
MSG	monosodium glutamate
OPA	o-phthaldehyde
PCA	principal component analysis
PRPP	5-Phosphoribosyl-1-pyrophosphate
RNA	ribonucleic acid
RI	refractive index
RUC	relative umami concentration
SD	short day
SV	sous vide
TMS	trimethylsilane
UMP	uridine monophosphate
UV	ultraviolet
VFTD	Venus Flytrap Domain
WHC	water holding capacity
XMP	xanthosine monophosphate

LIST OF ORIGINAL PUBLICATIONS

I. Rotola-Pukkila, M.; Pihlajaviita, S.; Kaimainen, M; Hopia, A. Concentration of Umami Compounds in Pork Meat and Cooking Juice with Different Cooking Times and Temperatures. *J. Food Sci*. **2015**, 80, C2711–C2716. doi:10.1111/1750-3841.13127

II. Rotola-Pukkila, M.; Yang, B; Hopia, A. The effect of cooking on umami compounds in wild and cultivated mushrooms. *Food Chem*. **2019**, 278**,** 56-66. doi.org/10.1016/j.foodchem.2018.11.044.

III. Rotola-Pukkila, M.; Välimaa, A-L.; Suomela, J-P.; Yang, B.; Karhu, S.; Hopia, A. Effect of daily light integral treatments on free amino acids and sugars contributing flavor and acrylamide formation in potato tubers of *Solanum tuberosum* L. *Agric. Food Sci*. **2021**, 30: 85–95. doi:10.23986/afsci.107898.

1 INTRODUCTION

Taste is one of the most important characteristics on which a consumer chooses a food product or food raw material. Furthermore, cooking has a significant impact on the quality and the sensory profile of food, and the pleasant taste of a meal is often created by complex, heat-induced reactions. Cooking causes chemical changes involving taste-active compounds and their precursors to react with other compounds present in the raw material. A rise in temperature may also enhance biochemical changes due to enzymatic reactions which have a high impact on the quality of the final product (Spanier et al., 1990). Heat treatment may also be required to remove harmful compounds, to ensure the food safety and to improve digestibility and bioavailability of nutrients (Bhat et al., 2021). Thermal processing also modifies the texture of the meal.

The taste sensation arises from the binding of chemical compounds to specific taste receptors, resulting in the perception of tastes such as umami, bitter and sweet. The umami taste, which is often described as a savory, brothy or meaty, gives many plant- and animal-based foods their particular delicious taste. Already in 1909, a Japanese chemist Kikunae Ikeda published an article on the isolation of monosodium glutamate (MSG) from seaweed (Ikeda, 2002). In the research article by Ikeda (2002), the ionic form of L-glutamic acid, glutamate was proposed as the substance responsible for the taste of umami. However, it took nearly a century before umami was universally accepted as the fifth basic taste, along with the other four basic tastes i.e. sweet, salty, sour and bitter (Zhang et al., 2017). Umami was officially recognized in 2002, along with the discovery of the receptors for free glutamate (Chaudlari et al., 2000; Nelson et al., 2002, Zhang et al., 2017). Before that, it was thought that all other tastes were combinations of the four other accepted basic tastes (Ninomiya, 2002).

Together with glutamate, aspartate, the ionic form of L-aspartic acid, is also known to contribute to the formation of the umami taste, although to a much lesser extent (Morris et al., 2007; Stapleton et al., 1999). The unique property of umami is the synergy between glutamate and nucleotides. When both free glutamate and free 5′-nucleotides are present, the intensity of the umami taste sensation is significantly enhanced by the synergistic effect of these compounds (Beauchamp, 2009). The most important nucleotides for an intensified sensation of the umami taste are inosine 5′-monophosphate (5′-IMP) and guanosine 5′-monophosphate (5′-GMP) (Ninomiya, 1998; Oruna-Concha et al., 2007). Adenosine 5′-monophosphate (5′-AMP), is also classified as an umami taste enhancing nucleotide, although its intensity is lower than that of IMP and GMP (Ninomiya, 1998; Oruna-Concha et al., 2007). In addition, there are indications of the contribution of small peptides to the umami sensation (Temussi, 2012).

Umami taste related compounds are found naturally in a wide variety of foods. Protein rich foods especially have considerable umami potential, as glutamic acid is one of the major amino acids in various plant and animal based protein sources (Davila et al., 2022; Ninomiya, 2002). Only the free forms of amino acids can produce a taste sensation, not the amino acids bound to proteins. Short peptides have also been proposed to exhibit an umami taste (Temussi, 2012; Zhang et al., 2017). By processing the food raw material in an appropriate way, free amino acids and free nucleotides can be released from their sources: proteins and nucleic acids. The use of naturally occurring taste and flavor potentiators and food preparation techniques resulting in the presence of umami compounds in food has been known for centuries in many cultures throughout the world (Maga & Yamaguchi, 1983; Maga, 1994). In particular, the production of fish and soy sauces which contain large amounts of free amino acids and peptides obtained through the breakdown of proteins, has a long history (Ninomiya, 2002). The characteristics of umami in the history of cooking are learned through experience, without any available scientific knowledge (Maga, 1994). Along with natural sources, umami can be added to food as commercially available flavoring substances. Today, monosodium glutamate (MSG) is a commonly used food additive and flavor enhancer with E number E621. Europe numbers or E numbers are classification numbers given to food additives designated for use within the European Union (EU). Disodium inosinate (E631) and disodium guanylate (E627), disodium salts of inosinic acid (IMP) and guanylic acid (GMP) or a mixture of them (E635), are used as a flavor enhancer in food naturally containing glutamate or added MSG.

Although extensive research has already been done on umami in the last century, interest has grown again in recent years. The current trend to the use more vegetables as part of the meal increases the need for tasty, plant-based meals and plant-based meat alternatives. To increase the consumption of these plant-based alternatives, it is important to understand the possibilities to increase savory taste and mouth-filling richness to meals, if needed. The special characteristics of umami, the prolonged aftertaste and the ability to intensify the overall taste of food can help to find the solutions. Plant-based raw materials naturally rich in umami compounds offer an alternative without using food additives or contributing to excess sodium. One challenge with plant-based raw materials is the bitter side taste characteristic of many plant-based ingredients. Examining the effect of processing on the concentrations of taste compounds provides information on the behavior of the compounds during cooking and on the optimization of cooking in terms of the preserving or increasing of desirable taste compounds.

Thermal and enzymatic treatments of the raw material intensify taste and flavor properties. The release of taste compounds from the raw food material as

well as their stability or decomposition and possible enzymatic and chemical reactions are interesting and important in terms of taste. To achieve a synergistic interaction of umami compounds in a meal, the challenge is to find such cooking conditions where the amount of both glutamic acid and 5´-nucleotides would be sufficient and in good balance with other tastes. To gain a better understanding of the significance of the umami taste and umami compounds in promoting a pleasant and healthy diet will be fundamental in future research.

The overall aim of the thesis was to study the concentration of umami compounds and their precursors in raw materials naturally containing umami and the effect of cooking or the effect of growth conditions on these concentrations. The complex changes taking place during thermal processing of food raw material are also discussed. The main focus is on free amino acids (FAAs) and 5´-nucleotides and their behavior during processing and under potential enzymatic activity. The literature review of this dissertation concentrates mainly on the possible enzymatic and chemical reactions of umami compounds in food and their perception in the oral cavity. Since umami is part of overall taste and flavor, the perception of other basic tastes in the oral cavity is discussed at a general level. In addition, umami peptides are discussed briefly in the theory section.

The experimental part of this thesis consisted of three studies, two of which focused on umami compounds and their precursor concentrations in pork meat and mushrooms and their cooking juice. The third study concentrated on the effect of daylight conditions on the content of taste compounds in potato tubers. The raw materials studied, pork loins, mushrooms and potatoes are all common food ingredients in the Finnish/Nordic diet representing the fauna, flora and fungi. They naturally contain compounds or precursors that create an umami taste. All the raw materials are such that they usually need heating, to ensure food safety and increase digestibility and palatability before being consumed as food. In this thesis, instrumental methods were applied to analyze the concentrations and changes in concentrations of known taste compounds, giving an indication of the most potential compound affecting the taste and flavor of a meal. In the first part, the aim was to analyze the taste compounds of pork loins and the changes in compound concentrations as a function of cooking temperature and cooking time. In the second part, the taste compounds of mushrooms were analyzed from raw and cooked mushrooms and corresponding cooking juice. Cultivated *A. bisporus* was used as a reference material for four forest mushrooms. In the third part the effect of growth (daylight) conditions on the taste compound concentrations of potato tubers was examined.

2 REVIEW OF THE LITERATURE

2.1 Chemistry of taste perception and taste receptors

Based on the received stimulus, human senses are divided into chemical and physical senses. The sense of taste and smell are chemical senses whose response occurs when a chemical compound meets a sensory organ. The sensation of taste is created by the compounds dissolved in substantial amounts in the food matrix. For a compound to produce a taste sensation, the molecule must be non-volatile, sufficiently small in size (<3000 Da), extractable and water soluble (Chen et al., 2021). The large molecules, such as carbohydrates and proteins, must be broken down into smaller constituents, mainly sugars and amino acids, in order to create the sensation of taste (Clausen et al., 2018). In addition, every taste molecule must have a receiving taste receptor, which provides a signal to the brain, where the taste characteristic in question is recognized. Each taste molecule individually stimulates its responsible taste systems to bring out relevant taste sensations. Although the compounds provide a specific taste stimuli, the concentration of the compound and its taste threshold value, as well as other compounds present in food matrix can enhance or suppress the effect of compound on taste.

The specialized chemoreceptor cells generating the signals of the perception of taste like sweet or umami are mainly located in the taste buds of the tongue and palate epithelium, and clustered in groups responding to chemical stimuli using diverse mechanisms (Chen et al., 2021; Lawless & Heymann, 2010; Roper and Chaudhari 2017). The taste compound either affects the function of the receptor cell of the sensory organ, or temporarily binds to the receptor site, generating a response (Tuorila & Appelbye, 2016). Each taste bud contains about 50-100 receptor cells (Roper & Chaudhari, 2017; Tuorila & Appelbye, 2016). The molecular recognition of tastants occurs at the chemosensitive apical tips of receptor cells, and finally resulting in sensory perceptions (Roper & Chaudhari, 2017).

There are several different types of taste receptors in the oral cavity. Of the five basic tastes, sweet, bitter and umami have taste receptors belonging to the G protein-coupled receptor (GPCR) family (Zhang et al., 2019). Sweet and umami taste receptors, T1Rs, are GPCRs consisting of about 850 amino acids and having a long extracellular N termini that contain a bilobed venus flytrap (VFT) domain (Lawless & Heyman, 2010; Roper & Chaudhari, 2017). Bitter-taste receptors, T2Rs, are also GPCRs, consisting of 300-330 amino acids, but they have short amino termini (Bachmanov & Beauchamp, 2007; Roper & Chaudlari, 2017). Furthermore, suggested taste receptors for salty (Na^+) and sour (H^+) tastes appear be more like ion channels rather than GPCRs (Bachmanov & Beauchamp,

2007; Lawless & Heymann, 2010). The receptors for the five basic tastes are all briefly reviewed in the following chapters.

2.1.1 Sweet, bitter, sour and salty taste receptors

Sugars like glucose, fructose and sucrose are the most common natural sweet taste stimuli (Bachmanov & Beauchamp, 2007). According to current knowledge, the most important receptor to detect sugars is the heterodimer of T1R2 and T1R3 responding to various natural and synthetic sweeteners like sugars, artificial sweeteners and free amino acids classified as sweet (Roper & Chaudhari, 2017). The umami taste receptor (T1R1/T1R3) and sweet taste receptor (T1R2/T1R3) are structurally close to each other by sharing a common subunit T1R3 (Zhang et al., 2008). Nevertheless, they are sensitive to different types of taste stimuli (Zhang et al., 2008).

Detecting a bitter taste is important as a sign of possible spoilage or toxicity of food or raw material. A large variety of compounds with diverse chemical structure can generate a bitter taste sensation. The compounds range from simple salts to large complex molecules, including free amino acids and small peptides. There are at least 25 taste receptors of type 2 (T2R) family members in humans, which comprise a large group of taste G protein-coupled receptors (GPCRs) that detect bitter compounds. (Roper & Chaudhari, 2017)

A stimulus for sour taste can be given by organic acids like acetic and citric acids (DeSimone & Lyall, 2006; Roper & Chaudhari, 2017). However, the sour taste intensity is not directly proportional to stimulus of pH and the concentration of hydrogen ions (H^+), as might be assumed (DeSimone & Lyall, 2006). Thus, organic, weak acids, like acetic acid is perceived as more sour than hydrochloric acid (HCl) when tested at a similar pH (DeSimone et al., 2001; Roper & Chaudhari, 2017). Over the years, numerous plasma membrane ion channels have been proposed as sour taste transducers (Bachmanov & Beauchamp, 2007; Roper & Chaudhari, 2017). However, their role in taste perception of sourness has not yet been clearly demonstrated. The recent studies suggest proton-conducting ion-channels Otopetrins (Tu et al., 2018), more precisely Otopetrin-1 (Zhang et al., 2019) as a candidate for sour receptor.

Sodium salts and some nonsodium salts also give a salty taste stimuli indicating the presence of minerals (Bachmanov & Beauchamp, 2007). In the case of the salty taste, the role of the selective, epithelial amilore-sensitive Na^+ channel ENaC has been studied extensively (Boughter & Gilbertson, 1999; DeSimone & Lyall, 2006; Roper & Chaudhari, 2017). However, the principal salt receptor in humans and the exact transduction mechanism for saltiness (NaCl) still remains unclear (Roper & Chaudhari, 2017).

2.1.2 Umami taste receptors

The taste of umami indicates the presence of proteins and amino acids in food (Bachmanov & Beauchamp 2007; Mouritsen & Styrback, 2014). Glutamic acid is one of the most common amino acids in proteins (Bachmanov & Beauchamp, 2007; Ninomiya 2002). The ionic form of free L-glutamic acid, glutamate, is the most known umami taste stimulus (Bachmanov & Beauchamp, 2007). Binding of the glutamate ion to a receptor of umami generates the taste sensation (Mouritsen & Khandelia, 2012). After the discovery of the first glutamate receptor, a metabotropic glutamate receptor *taste*-mGluR4 in year 2000 (Chaudhari et al., 2000), a substantial amount of research has been done to find out the details of umami receptor-molecule relationship. In addition to *taste*-mGluR4, two subsequently identified umami receptors, T1R1/T1R3 (Li et al. 2002; Nelson et al., 2002) and *taste*-mGluR1 (San Gabriel et al., 2005; San Gabriel et al., 2009), have received the most of attention (Mouritsen & Styrback, 2014; Roper & Chaudhari, 2017). In the T1R1/T1R3 umami receptor, two GPCRs called T1R1 and T1R3, form a heterodimeric complex (Zhang et al., 2008).

Taste-mGluR4 has been found to be selectively sensitive to free L-glutamate (Mouritsen & Khandelia, 2012). However, the heterodimeric receptor T1R1/T1R3 found in 2002 is, according to current knowledge, the only receptor known to respond to both L-glutamate and nucleotides (Clausen et al., 2018; Li et al., 2002; Mouritsen & Khandelia, 2012; Zhang et al. 2019) promoting the unique synergistic interaction between these compounds.

Both metabotropic glutamate receptors (mGluRs) and the monomer of T1R1 consist of an extracellular venus flytrap domain (VFTD) (Zhang et al., 2008), the proposed mechanism simulating the trapping mechanism of a carnivorous plant named Venus flytrap. The VFTD consists of two lobes, upper and lower lobe connected by a three-stranded flexible hinge, also containing an orthosteric ligand-binding site (Zhang et al., 2008; Zhang et al., 2010). When free glutamate binds close to the hinge region of VFTD, the upper and lower lobes will close (Kunishima et al., 2000; Zhang et al., 2008) (**Figure 1**). The presence of glutamate stabilizes the closed conformation and the closure of VFTD activates the signaling pathway, which therefore is the key event sensitizing the umami taste receptor (Mouritsen & Khandelia, 2012).

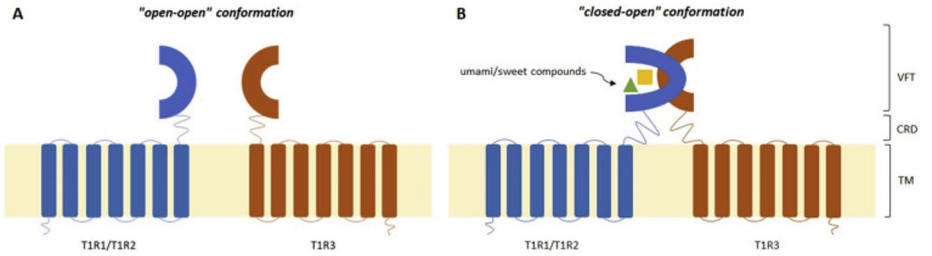

Figure 1. Structures of the T1R1/T1R3 umami taste receptor and T1R2/T1R3 sweet taste receptor. Schematic representation of the "non-active" ("open-open" conformation on the left) (A) and "active" ("closed-open" conformation on the right) (B). The T1R1 part of the umami taste receptor binds free glutamate close to region where the hinge will bend (the yellow square in B). In addition, a nucleotide can bind to the outer cleft (the green triangle), further stabilizing the closed conformation. TM = transmembrane α-helices, CRD = cysteine rich domain, VFT = venus fly trap (Spaggiari et al., 2020). Reprinted by permission from Elsevier.

The suggested molecular mechanism for the synergy of glutamate and nucleotides involves the T1R1 receptor responsible for binding free glutamate close to the hinge region of VFTD (Zhang et al., 2008) (**Figure 1**). When a ribonucleotide such as 5′-IMP or 5′-GMP binds to the outer cleft of the VFTD, it further stabilizes the closed conformation and enhances significantly the sensitivity of the umami receptor to glutamate (Mouritsen & Khandelia, 2012; Zhang et al., 2008).

According to a relatively new review by Zhang et al., (2019), there are indications that other potential receptors might be activated through different mechanisms and stimulated by various compounds and molecules responsible for the recognition of umami substances. However, potential receptors and their functions still need to be investigated more thoroughly to substantiate their response to taste molecules. The recent studies regarding peptides and their possible influence on the umami taste have focused on the T1R1/T1R3 receptor (Chang et al., 2023; Dang et al., 2019; Song et al., 2023). To show the binding and interaction of novel umami peptides with T1R1/T1R3 receptor, a computer simulation tool called molecular docking can be used to explore the umami mechanism. For example, in the study of Song et al., (2003), three potential umami peptides were found from extracts of the mushroom *Boletus edulis* by molecular docking, and they were further chemically synthesized for sensory evaluation.

2.2 Umami compounds and their synergy

Taste, palatability and acceptability of many foods is increased by the presence of umami compounds (Yamaguchi & Ninomiya, 2000). It is widely accepted that the umami taste is primarily contributed by the salts of L-glutamic acid and to a lesser extent by the salts of L-aspartic acid, aspartate (Stapleton et al., 1999). In neutral solutions, these FAAs exist in their ionic forms, glutamate and aspartate, and are able to stimulate the umami receptor (Mouritsen & Styrback, 2014). Certain free 5´-ribonucleotides such as inosinate (inosine-5´-monophosphate, 5´-IMP), guanylate (guanosine-5´-monophosphate, 5´-GMP) and adenylate (adenosine-5´-monophosphate, 5´-AMP), which are not known to contribute umami on their own, can enhance umami taste perception several-fold by interacting with glutamate (Maga., 1994; Mouritsen & Khandelia, 2012; Mouritsen & Styrback, 2014). This synergism of free glutamate and free 5´-nucleotides is a unique characteristic of the umami taste, and differentiates it from other tastes (Mouritsen & Khandelia, 2012; Zhang et al., 2008; Yamaguchi & Ninomiya, 2000). The mechanism of the synergy is explained in more detail in Chapter 2.1.2.

The synergistic interaction between umami amino acids and 5´-nucleotides can be evaluated by calculating equivalent umami concentration (EUC) using the equation derived from the sensory evaluation (Yamaguchi et al., 1971). EUC (grams of MSG per 100 g) is the concentration of MSG equivalent to the umami intensity given by a mixture of MSG and the 5′-nucleotide and is represented by the addition equation (1).

$$Y = \sum a_i b_i + 1218 \left(\sum a_i b_i \right) \left(\sum a_j b_j \right) \quad (1)$$

In the equation, Y is the EUC of the mixture, a_i is the concentration of each umami amino acid (Glu or Asp), a_j is the concentration of each umami 5′-nucleotide (5´-IMP, 5′-GMP or 5′-AMP), b_i is the relative umami concentration (RUC) for each umami amino acid to MSG (Glu = 1, Asp = 0.077), b_j is the RUC for each umami 5′-nucleotide to 5′-IMP (5′- GMP = 2.3, 5′-AMP = 0.18), and 1218 is a synergistic constant. All concentrations must be in grams per 100 g (Morris et al., 2007).

Another unique characteristic of umami is the more prolonged aftertaste it generates compared to other tastes (Yamaguchi & Ninomiya, 2000). Moreover, umami has the ability to intensify the overall taste of food, even though a pure umami compound like MSG in an aqueous solution is evaluated as neutral or even unpleasant (Nishimura et al., 2016; Yamaguchi & Ninomiya, 2000).

Related to this, one of the unique characteristics of umami compounds is their ability to intensify the salty taste in food. The intake levels of salt from food are nowadays higher than the recommendations of World Health Organization (WHO) for adults (Hayabuchi et al., 2020). The problem is especially the increase in the consumption of processed food, which contains excessive amounts of salt. In order to protect the population's health from chronic diseases, the intake levels of salt need to be reduced. The use of raw food ingredients naturally rich in umami could increase the acceptability of low-sodium foods by providing a salt-enhancing effect (Wang et al., 2020). The published research data have suggested partial replacement of salt (NaCl) with MSG (monosodium glutamate) (Hayabuchi et al., 2020; Jinap et al., 2016; Rosa et al., 2021) or with MSG, GMP and IMP (Roininen et al., 1996) as an alternative for sodium reduction in meals. The sodium content reduction could be done by adding glutamic acid in the form of natural ingredients rich in glutamate including parmesan cheese, mushrooms and tomatoes, as suggested in the research of Dos Santos et al., 2020.

There are also other substances including small peptides (Dang et al., 2019; Sun et al., 2020), L-amino acid theanine (Narukawa et al., 2011), and succinate, an ionic form of succinic acid (Narukawa et al., 2011; Zhang et al., 2019) which have also been reported to induce an umami taste sensation. However, this literature review mainly focuses on glutamate, aspartate and 5´-nucleotides.

2.2.1 Free amino acids

Amino acids are the main source of nitrogen in the organism and used as the building blocks for the synthesis of proteins and other nitrogenous compounds like hormones and enzymes (Blanco & Blanco, 2017). They are also involved in many biochemical reactions affecting e.g. to growth, metabolism and signaling functions (Häusler et al., 2014). The two major metabolic processes of amino acids, transamination and deamination, are an important part of a highly complex amino acid metabolism (Sun et al., 2020). Amino acids are also linked to nucleotides. Glycine, glutamic and aspartic acid act as raw materials in the synthesis of purine and pyrimidine nucleotides (Sun et al., 2020).

Protein molecules are too large to be detected by the taste receptors (Clausen et al., 2018). Only small compounds like the free forms of amino acids are able to stimulate the appropriate receptors and produce a taste sensation (Clausen et al., 2018). Of the free amino acids, glutamic acid has received the most attention due to its umami taste properties (Kawai et al., 2012), though amino acids have been found to elicit other basic tastes as well. Protein-rich foods such as meat, fish and milk as well as many vegetables contain high quantities of protein bound glutamic acid (Ninomiya, 1998; Ninomiya, 2002). In contrast, free forms of amino acids, including glutamic acid, occur in relatively low concentrations

compared to the content of protein bound amino acids (Ninomiya, 1998). However, in mushrooms, certain seaweeds and vegetables such as tomatoes where the total protein content and protein bound glutamate content are lower than in meat or milk, the free glutamic acid concentrations are relatively high (Ninomiya, 1998; Oruna-Concha et al., 2007).

The L-forms of FAAs, which are more common in nature, differ from their taste properties on the D-forms of FAAs (Belitz et al., 2009). In several publications (Beluhan & Ranogajec, 2011; Belitz et al. 2009; Kawai et al. 2012; Mau et al., 2001; Tsai et al., 2007; Tseng & Mau, 1999; Yang et al., 2001), the L-forms of FAAs have further been classified into groups according to their basic taste properties. However, due to the complexity of the taste imparted by amino acids, there are small differences between these classifications. This kind of classification based on basic tastes has been used, especially in mushroom-related publications (Beluhan & Ranogajec, 2011; Mau et al., 2001; Tsai et al., 2007; Tseng & Mau, 1999; Yang et al., 2001) and is represented in **Table 1**. Altogether, 16 L-form FAAs are used in this classification. The term MSG-like (monosodium glutamate-like) is used for umami FAAs, L-aspartic and L-glutamic acids. Four FAAs are classified as sweet, eight FAAs as bitter and two FAAs as tasteless. The classification in **Table 1** differs slightly from that of Belitz et al. (2009), where L-aspartic acid is classified as a neutral, L-tyrosine as a bitter and L-Lysine and L-Proline as contributing both sweet and bitter taste.

According to Kawai et al. (2012) there was more than just one taste property for many of the L-amino acids, depending on the concentration. In addition, the taste properties of free amino acids have been found to be dependent on their molecular configuration (chirality of the alpha carbon), hydrophobicity, size, charge and functional groups on the side chain (Kawai et al., 2012). The small-size, hydrophilic amino acids L-Alanine, Glycine and L-Serine were classified mainly as sweet but elicited a weak umami taste as well at high concentrations (Kawai et al., 2012). Small or medium size hydrophilic FAAs L-glutamic acid and L-asparagine were sour at low concentrations and elicited weak umami at higher concentrations (Kawai et al., 2012). In contrast, hydrophilic L-aspartic acid was not observed to have umami properties in the study of Kawai et al. 2012. However, the large size, hydrophobic amino acids including L-Isoleucine, L-Leucine, L-Phenylalanine and L-Tryptophan elicited only bitter taste (Kawai et al. 2012).

Table 1. Classification of 16 free amino acids into the four taste characteristic groups according to data used in mushroom studies (Beluhan & Ranogajec, 2011; Mau et al., 2001; Tsai et al., 2007; Tseng & Mau, 1999; Yang et al., 2001).

Umami (MSG-like)	Bitter	Sweet	Neutral
L-Aspartic acid	L-Arginine	L-Alanine	L-Lysine
L-Glutamic acid	L-Histidine	Glycine	L-Tyrosine
	L-Isoleucine	L-Serine	
	L-Leucine	L-Threonine	
	L-Methionine		
	L-Phenylalanine		
	L-Tryptophan		
	L-Valine		

2.2.2 Nucleotides and their degradation products

In a living organism, nucleotides are essential biomolecules providing readily releasable energy and serving as monomeric units of the DNA and RNA to store and transfer genetic information. A nucleotide molecule contains a five-carbon sugar, a nucleobase and at least one phosphate group (Baines & Brown, 2015). The sugar component of RNA is ribose, for DNA the sugar component is deoxyribose. The synthesis and degradation (metabolism/catabolism) of nucleotides in a living organism is briefly described in this literature review in order to better understand their importance and occurrence in food raw materials before and after harvest or slaughter.

The most common pathway for nucleotide synthesis in a living cell is the *de novo* pathway, which for purine nucleotides begins with the enzymatically assisted reaction of ribose 5-phosphate with adenosine triphosphate (ATP) to form 5-Phosphoribosyl-1-pyrophosphate (PRPP). In the first phase of the *de novo* synthesis, PRPP is converted to IMP through ten reaction steps and in the second phase AMP and GMP are generated from IMP through two subsequent steps, respectively. Another, less common pathway of nucleotide synthesis, a salvage pathway, reuses purine bases and nucleosides generated during the degradation of RNA and DNA. As a result, enzymes are catalyzing adenine to AMP as well as guanine and hypoxanthine to GMP and IMP, respectively. (Sun et al., 2020)

Several enzymes are also involved in nucleotide catabolism or degradation in postmortem or postharvest conditions. Each mononucleotide, AMP, IMP, GMP and XMP can be dephosphorylated to the corresponding nucleoside adenosine, inosine, guanosine and xanthosine (Baines & Brown, 2015; Sun et al. 2020) (**Figure 2)**. By further removal of ribose from nucleotides, free bases adenine, hypoxanthine, guanine and xanthine are generated. Finally, all purine nucleotides can be degraded to xanthine which is further oxidized to form uric

acid, the final circulation product (**Figure 2)**. The predominant nucleotide present differs between the food raw materials. The occurrence of ribonucleotides in food can be roughly divided into three different groups. In general, 5´-IMP is most abundant in animal sources, mainly meat and fish, while high contents of 5´-GMP is found in mushrooms and plant-based foods (Ninomiya, 1998). Moreover, fish and shellfish are rich in 5´-AMP (Ninomiya, 1998).

ATP, ADP (adenosine diphosphate) and AMP are important participants in energy processes in the living cell. In fresh meat and fish, 5´-IMP accumulates immediatley after rigor mortis following the rapid breakdown rate of ATP to ADP and AMP, until hydrolyzed at a slower rate to inosine and further degraded to hypoxanthine (Hernández-Cázares et al., 2011). The degradation of ATP to ADP and AMP is a very fast reaction and is therefore thought to be entirely associated with endogenous autolytic enzymes (Gram & Huss, 1996; Hernández-Cázares et al., 2011). However, the degradation of AMP to IMP, inosine and hypoxanthine or AMP to adenosine is a much slower reaction. Thus, there is time for microbes and bacteria together with enzymes to participate in the degradation of AMP, as was suggested in the study of Gram & Huss (1996) for fish and in the study of Seki et al. (2017) for shrimp muscle.

5´-GMP is a potential source of umami taste in potato tubers (Morris et al., 2007) and in mushrooms (Zhou et al., 2017) since 5´-GMP is present in all living organisms as a component of RNA (Ninomiya, 1998; Solms & Wyler, 1979). Concentration of 5´-GMP is suggested to be dependent on the activity of RNA-degrading nuclease enzymes in potato tubers (Buri et al., 1971; Morris et al., 2007; Solms & Wyler, 1979), and in mushrooms (Claudine et al., 2005; Zhou et al., 2017); heat treatment is often needed to enhance the degradation and release of compounds.

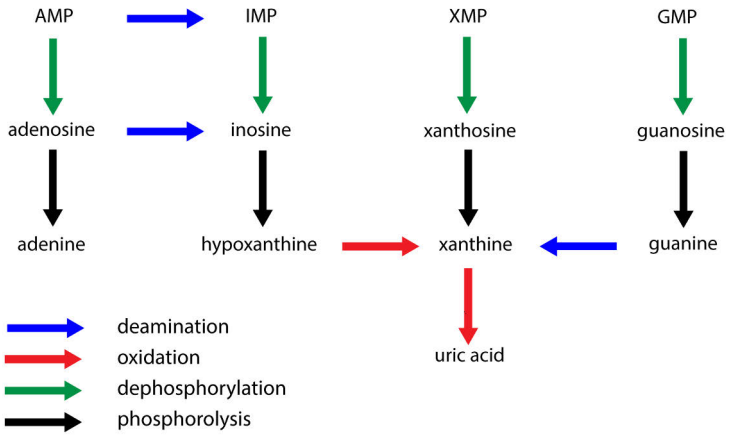

Figure 2. Postmortem or postharvest degradation pathways of purine nucleotides (adapted from Gill (1992), Hattula (1997), Sun et al. (2020) and Tikk et al. (2006)). Nucleotides AMP, IMP, XMP and GMP are dephosphorylated to corresponding nucleosides adenosine, inosine, xanthosine and guanosine (green arrows). Nucleosides are futher degraded to hypoxanthine, xanthine and guanine by purine nucleoside phosphorylase (black arrows). AMP is deaminated to IMP while adenosine and guanine are converted to inosine and xanthine by deaminases (blue arrows). Hypoxanthine is converted to xanthine and xanthine is converted to uric acid by xanthine oxidase (red arrow).

Nucleic acids (DNA and RNA) are not known to be crucial for human nutrition, but their breakdown products, nucleotides play a central role in the enhancement of an umami taste in foods. The taste intensity of nucleotides by themselves is weak (Yamaguchi & Ninomiya, 2000), but their effect on the umami taste sensation is based on the synergy of nucleotides with glutamate (see Chapter 2.2.). The nucleotides with the best enhancement capacity of the umami taste are reported to be 5′-IMP and 5′-GMP, the molecules where the ribose moiety is phosphorylated on the 5′ position (Baines & Brown, 2015). If the phosphorylation is on the 2′ or 3′ positions, the compounds umami enhancement capacity is negligible (Baines & Brown, 2015). A purine moiety as a nucleobase with a hydroxyl function on the 6-carbon of the purine ring is also required (Baines & Brown, 2015). Although 5′-AMP has an amino group (NH2) on the 6th carbon instead of an OH group, 5′-AMP is still classified as an umami taste enhancing nucleotide, although its intensity is lower than that of IMP and GMP (Ninomiya, 1998; Oruna-Concha et al. 2007). In the molecules of cytidine 5′-monophosphate (5′-CMP) and uridine 5′-monophosphate (5′-UMP), where the nucleobase is represented by pyrimidine, no umami taste impartment has been

detected. However, hypoxanthine, the breakdown product of IMP and inosine, has been found to cause a bitter taste (Tikk et al., 2006).

2.2.3 Short peptides

In addition to the most known umami compounds such as glutamic and aspartic acids and nucleotides, several short peptides have also been proposed to exhibit an umami taste (Zhang et al., 2017). In this literature review, the taste properties of peptides were examined mainly in terms of umami, although peptides are also known to impart sweet and bitter tastes (Temussi, 2012).

Most of the identified umami peptides are short linear peptides having a molecular weight distribution of less than 5000 Da (Zhang et al., 2017; Zhang et al., 2019). According to a recent review article of Zhang et al. (2019) about 60% of the identified 98 umami peptides are di- or tripeptides. In most of these short peptides, glutamic acid, aspartic acid and other hydrophilic amino acids were the dominant amino acids (Zhang et al., 2019). However, in the long chain peptides the spatial configuration together with amino acid sequence also affected the umami properties of peptides (Zhang et al., 2019).

Umami peptides have been studied since the 1970s (Chen et al., 2021; Zhang et al., 2019), but they have received significant attention in recent years. The studies related to the isolation of umami peptides have been recently carried out from hydrolysates of Sanhuang chicken (Chen et al., 2021), water extract of clams (Li et al., 2020) peanut protein hydrolysate (Zhang et al., 2019) and mushrooms (Chang et al., 2023; Kong et al., 2019; Song et al., 2023; Xu et al., 2019). Improved filtration, separation, purification, characterization and isolation techniques of compounds together with new technologies such as molecular docking and electronic tongue, have allowed a more precise study of the response of the taste receptors to umami compounds. According to Wang et al. (2020) the specificity of the umami receptor (T1R1/T1R3) is relatively low due to the multiple ligand binding sites on it. Thus, it can react to several chemically diverse molecules, including peptides (Wang et al., 2020). Recent studies have focused on examining this receptor (Chen et al., 2021; Chang et al., 2023; Song et al., 2023). In addition to the umami taste properties of peptides, there are also indications that peptides might show a synergistic response with glutamate (Dang et al., 2019). In the future, more detailed research results about the proposed contribution of peptides to the umami taste will likely be obtained with the help of new technologies.

2.3 Effect of cooking on the content of umami compounds in different food raw materials

Cooking has been found to have a significant effect on the concentrations of umami compounds in different plant and animal based raw materials. Simultaneous changes that either increase or decrease the content of umami compounds may occur during heat treatment. However, cooking cannot be viewed merely as an effect of increasing temperature and the associated physical and chemical changes, but also the enzymes naturally present in the raw material and their activity and inactivation with rising temperature must be taken into account. Cooking may even accelerate the activity of enzymes, especially when cooking takes place at low temperatures where enzymes are potentially more active for a longer period before inactivation (Ishiwatari et al., 2013). In other words, the rate of heating and the endpoint temperature are factors having an influence on taste by affecting the chemical and enzymatic reactions creating the taste and aroma of cooked food, such as meat (Ishiwatari et al., 2013; Spanier et al., 1990).

The structural behavior of the proteins when heated is one of the most important factors leading to alterations in texture and water binding capacity of the raw material. When proteins are denatured by heat, structural changes weaken their water-holding capacity (Tornberg, 2005). This leads to an increasing amount of released cooking juice (Wilkinson et al., 2014) and enhances the movement of water-soluble compounds, including umami FAAs and 5′-nucleotides, from food raw material like meat or mushrooms into the juice (Cambero et al. 2000b).

2.3.1 Changes in free amino acid concentrations during cooking

Food raw materials naturally contain a specific amount of free amino acids, which are able to be released due to structural changes such as protein denaturation during cooking. However, to increase the umami taste contributing free amino acids in food, the proteins of the raw material need to be hydrolyzed into smaller compounds such as short peptides and single amino acids, either enzymatically or by thermal processing techniques. In the process, under mild temperature conditions such as fermentation or aging of meat, the proteolytic enzymes actively break down the proteins into smaller peptides and even single amino acids (Zhao et al., 2016). Thus the probability of releasing a greater amount of taste compounds including umami-taste amino acids and peptides increases.

During cooking, both temperature and time are likely to affect the thermal degradation of proteins and peptides by enhancing the hydrolytic bond cleavage of amide bonds yielding shorter peptides and even free amino acids (Zhu et al.,

2019). Bikaki et al. (2021) studied the thermal degradation of different proteins and peptides using temperatures between 150-250 °C, and identified shorter peptides formed through thermal proteolysis for the majority of proteins. However, at low enough temperature, like temperatures used in sous vide cooking (<100 °C), the increase in temperature may even accelerate the activity of enzymes breaking down proteins. Some proteolytic enzymes in beef were discovered to be most active at a temperature of 68 °C and increased concentration of peptides and amino acids were reported with increasing end-point cooking temperature in the range of 51.7-76.7 °C (Spanier et al., 1990).

Several studies indicate that the content of free amino acids can also decrease during the cooking of food. The decrease in FAA concentrations including umami amino acids during cooking of food compared to fresh raw material, might be due to the reaction of FAAs with other molecules, such as reducing sugars, proteins or other biological molecules present in raw materials (Poojary et al., 2017). This finding was supported by the observation that pure FAA solution seemed to be thermally stable when heated, while in food matrix FAA concentrations tended to decrease. According to Poojary et al. (2017), no significant decrease in the concentration of umami amino acids and only a slight decrease in total FAA content was found when a pure FAA standard mixture was heated at temperatures 50 °C and 70 °C. In contrast, about a 50% decrease in the concentration FAAs was observed during heating of mushroom samples, supporting the idea of degradation or association reactions of amino acids with other molecules present (Poojary et al., 2017).

Free amino acids are known to participate in a Maillard reaction, an array of complicated, parallel chemical reactions occurring between carbonyl groups of reducing sugars and amino groups of amino acids, peptides or proteins in food during thermal treatment (Starowicz & Zieliński, 2019). Although this nonenzymatic browning reaction is mostly associated with higher cooking temperatures i.e. close to 120 °C and more giving flavor and color to food, it is also known to take place at mild temperatures when the cooking time is long enough. As an example, the Maillard reaction has been discovered to contribute even to the flavor of dry cured meat products processed for long periods at mild temperatures (Li et al., 2021). It is therefore difficult to assess whether the reduction of FAAs occurring at temperatures below 100 °C is directly related to the Maillard reaction or some other reactions.

2.3.2 Changes in nucleotide concentrations during cooking

The predominant umami-enhancing 5′-nucleotide and its increasing or decreasing concentration during cooking depends on whether the raw material is of animal or vegetable origin. In general, 5′-IMP is a dominant nucleotide in

meat (Maga, 1983). In plant-based raw materials such as potatoes, 5′-GMP together with 5′-AMP are often the dominant umami-enhancing nucleotides, but cooking is often required to achieve quantified concentrations (Morris et al., 2007; Solms & Wyler, 1979). In mushrooms, the predominant nucleotide seems to vary between mushroom species and between studies, 5′-GMP together with 5′-AMP and 5′-XMP are the most often reported (Tseng & Mau, 1999) (**Table 3**). Heating is observed to increase the concentrations of 5′-AMP and 5′-GMP in mushrooms as well (Li et al., 2011; Zhou et al., 2017). Generally, the changes in 5′-ribonucleotide concentrations are assumed mainly to be due to enzymatic degradation process, which is known to be pH- and temperature-dependent (Ninomiya, 1998; Solms & Wyler, 1979; Zhao & Fleet, 2005). Especially at the beginning of the heating process, when the temperature is not sufficiently high to deactivate the enzymes, the concentration of certain nucleotides may increase or decrease. Nucleotide 5′-GMP is present in all living organisms as a component of RNA (Ninomiya, 1998; Solms & Wyler, 1979). The reason for elevated concentrations of 5′-nucleotides, especially 5′-GMP, during cooking has been suggested to be the activity of RNA-degrading nuclease enzymes in potato tubers (Buri et al., 1971; Morris et al. 2007; Solms & Wyler 1979), and in mushrooms (Zhou et al. (2017).

The quantity and quality of nucleotides, nucleosides and nucleobases produced depends on the types enzymes present in the food matrix that actively degrade both RNA and its degradation products (Deoda & Singhal, 2003; Dermiki et al., 2013; Zhao & Fleet, 2005). As the temperature of the food raw material increases during cooking, activity of the enzymes naturally present increases to around 65-75 °C (Dermiki et al., 2013; Solms & Wyler, 1979). If the temperature of the raw material increases sufficiently slowly from 40 to 60 °C, high nuclease activity may significantly degrade RNA resulting in the accumulation of 5′-nucleotides (Solms & Wyler, 1979). Cooking is also needed to damage the cell structure to enable the release of molecules within the cells (Poojary et al., 2017).

The increase in 5′-AMP concentration, especially in the beginning of the cooking process, has been reported both in animal and plant based raw materials as well as in mushrooms (Arya & Parihar, 1979; Li et al., 2011; Madruga et al., 2010; Morris et al. 2007). The reason for the increase of AMP content (especially) at the beginning of the heating process is thought to be due to the presence of AMP precursors ATP and ADP in the food raw material and their hydrolysis as a result of the increase in temperature (Arya & Parihar, 1979; Macy et al., 1970) with the activity of enzymes probably contributing. Immediately after slaughter, rapid degradation of ATP to AMP and further accumulation of IMP in meat makes IMP likely to be the dominant nucleotide in fresh meat already before the cooking process. It seems that cooking either stabilizes the IMP concentration or

slightly decreases it, depending on the effectiveness of heat transfer and the end point temperature (Ishiwatari et al., 2013).

In meat, enzymes are expected to affect the degradation of 5′-IMP in particular (Ishiwatari et al., 2013). A sufficiently low cooking temperature, like those used in long term sous vide cooking (<100 °C), does not necessarily stop the 5′-IMP degrading enzyme activity in the meat immediately, but can even enhance it to certain point (Ishiwatari et al., 2013). This might lead to a decrease of 5′-IMP and a further increase of inosine and hypoxanthine concentrations. According to Ishiwatari et al. (2013), the decomposition rate of 5′-IMP was strongest at a cooking temperature of 40 °C for beef cooked for 60 min by the sous vide method. However, when the cooking temperature was increased to 65 or 70 °C, the 5′-IMP concentration was relatively constant during 60 min of cooking (Ishiwatari et al., 2013), indicating the inactivation of the degrading enzymes. According to Ishiwatari et al. (2013), the temperature for the complete deactivation of IMP decomposing enzyme, calculated with a kinetic model, was 64.1 °C.

2.4 Umami compounds in raw and cooked meat

Raw meat has a characteristic metallic taste and a serum like odor (Idolo Imafidon & Spanier, 1994; Mottram, 1998; Wasserman, 1972). The savory taste of meat is created during the heating process by a complex combination of numerous taste and aroma compounds and a series of chemical and enzymatic reactions including taste precursors, intermediate reaction compounds and degradation products (Aaslyng & Meinert, 2017; Mottram, 1998). As a result of increasing the temperature, the taste and flavor of meat changes significantly. Umami compounds and their precursors in meat are an important part of the taste and flavor development of cooked meat.

The main sources of the umami taste in meat are free glutamic acid and 5′-IMP (Aaslyng & Meinert, 2017) but short peptides have also been suggested to have an umami taste (Temussi, 2012). Free glutamic acid have been reported to be one of the most abundant free amino acids e.g. in pork (Jiménez-Martín et al., 2012; Triki et al., 2018), in goat meat (Madruga et al., 2010) and in beef (Koutsidis et al., 2008), whereas the concentration of free aspartic acid was relatively low in these meat studies (**Table 2**). Moreover, protein bound glutamic acid is one of the major amino acids in meat, representing approximately 20% of total amino acid content in various animal protein sources (Maga, 1998). To release glutamic acid or short peptides, proteins must be hydrolyzed at least partially, before free amino acids and short peptides bound to proteins could contribute to the umami taste of meat (Zhao et al., 2016; Zhang et al., 2017, Zhang et al., 2019).

Table 2. Free L-amino acids, 5´-nucleotides and nucleosides in meat (µmol/g, fresh weight).

Species	Asp[a]	Glu	5´AMP	5´GMP	5´IMP	Ino[b]	Hypox[b]	Treatment	Reference
Beef	0.02	0.43	N/A	0.11	6.27	1.14	0.84	Day 1 raw (4 °C)	Koutsidis et al. (2008)
	0.01	0.39	N/A	0.10	5.79	1.36	1.13	Day 3 raw (4 °C)	
	0.01	0.49	N/A	0.09	4.61	1.47	1.52	Day 7 raw (4 °C)	
	0.02	0.79	N/A	0.08	3.70	1.87	2.09	Day 14 raw (4 °C)	
	0.04	0.97	N/A	0.06	2.63	1.91	2.66	Day 21 raw (4 °C)	
Beef[c]	0.18	0.30	N/A	N/A	N/A	N/A	N/A	Day 0 raw (2 °C)	Triki et al. (2018)
	0.39	0.60	N/A	N/A	N/A	N/A	N/A	Day 3 raw (2 °C)	
	0.30	1.02	N/A	N/A	N/A	N/A	N/A	Day 6 raw (2 °C)	
	0.37	4.45	N/A	N/A	N/A	N/A	N/A	Day 10 raw (2 °C)	
Pork[c]	0.22	0.55	N/A	N/A	N/A	N/A	N/A	Day 0 raw (2 °C)	Triki et al. (2018)
	0.51	1.14	N/A	N/A	N/A	N/A	N/A	Day 3 raw (2 °C)	
	0.71	3.31	N/A	N/A	N/A	N/A	N/A	Day 6 raw (2 °C)	
	0.37	1.97	N/A	N/A	N/A	N/A	N/A	Day 10 raw (2 °C)	
Pork	N/A	N/A	N/A	N/A	3.76	1.75	0.52	Day 2 raw (2 °C)	Tikk et al. (2006)
	N/A	N/A	N/A	N/A	2.39	3.34	1.15	Day 15 raw (2 °C)	
	N/A	N/A	N/A	N/A	1.86	4.24	1.24	Day 21 raw (2 °C)	

Pork	N/A	0.80 (0.00) / 0.80	N/A	N/A	2.87 (0.00) / 2.87	N/A	N/A	Cooked 95 °C (0 min)	Sasaki et al. (2007)
muscle (juice) / total	N/A	0.54 (0.27) / 0.80	N/A	N/A	2.23 (1.09) / 3.32	N/A	N/A	Cooked 95 °C (10 min)	
	N/A	0.37 (0.47) / 0.85	N/A	N/A	1.62 (1.77) / 3.39	N/A	N/A	Cooked 95 °C (30 min)	
	N/A	0.30 (0.54) / 0.84	N/A	N/A	1.38 (2.01) / 3.39	N/A	N/A	Cooked 95 °C (60 min)	
	N/A	0.22 (0.53)/ 0.76	N/A	N/A	1.18 (2.16) / 3.34	N/A	N/A	Cooked 95 °C (180 min)	
Goat	N/A	N/A	0.20	0.15	2.58	3.41	0.36	Raw	Arya & Parihar (1979)
	N/A	N/A	0.29	0.05	0.82	4.91	0.82	Cooked 60 °C (30 min)	
	N/A	N/A	0.31	0.07	1.49	4.36	0.69	Cooked 80 °C (30 min)	
	N/A	N/A	0.32	0.07	2.03	3.92	0.62	Cooked 100 °C (30 min)	
	N/A	N/A	0.30	0.07	1.91	4.07	0.69	Cooked 120 °C (30 min)	
Sheep	N/A	N/A	0.20	0.15	3.10	3.29	0.33	Raw	Arya & Parihar (1979)
	N/A	N/A	0.31	0.07	0.91	5.21	0.77	Cooked 60 °C (30 min)	
	N/A	N/A	0.32	0.07	1.63	4.60	0.68	Cooked 80 °C (30 min)	
	N/A	N/A	0.32	0.08	2.32	4.01	0.63	Cooked 100 °C (30 min)	
	N/A	N/A	0.29	0.08	2.10	4.24	0.72	Cooked 120 °C (30 min)	
Pork	N/A	0.44	N/A	N/A	3.94	N/A	N/A	Raw	Chikuni et al. (2002)
	N/A	0.36	N/A	N/A	3.35	N/A	N/A	Cooked 70 °C (60 min)	

Goat[c]	0.09	0.75	0.01	0.07	3.01	5.67	N/A	Raw	Madruga et al. (2010)
	0.09	0.43	2.51	0.04	1.99	4.21	N/A	Grilled 80 °C (5-9 min)	
Chicken[c]	N/A	1.68	N/A	N/A	6.42	N/A	N/A	Raw	Jayasena et al. (2014)
	N/A	1.04	N/A	N/A	2.79	N/A	N/A	Cooked >72 °C (40 min)	

[a]Amino acid abbreviations: Asp: Aspartic acid. Glu: Glutamic acid

[b]Other abbreviations: Ino: Inosine, Hypox: Hypoxanthine

[c]Recalculated from the original concentration of mg/100g fresh weight

N/A = not available

The major 5′-nucleotide in postmortem muscle is 5′-IMP (Maga, 1983), which for example, together with its corresponding nucleoside, inosine, represented together 97% of the analyzed nucleotides and nucleosides in goat meat (Madruga et al., 2010). Therefore, the research on 5′-nucleotides of meat often focuses on 5′-IMP and its reactions (Ishiwatari et al., 2013; Sasaki et al., 2007; Tikk et al., 2006). In addition, 5′-AMP and 5′-GMP contents have been reported in the studies of (Cambero et al., 2000; Flores et al. 1999; Koutsidis et al., 2008; Madruga et al., 2010).

Post mortem metabolism and associated enzymatic reactions, which occur after slaughter, convert the muscle into meat (Hernández-Cázares et al., 2011) and also affect potential taste precursors (Wasserman, 1972). Due to microbial and enzymatic changes occurring in tissues, the concentration of free amino acids and peptides might increase through proteolysis and 5′-nucleotides and their breakdown products are generated via ATP degradation (Hernández-Cázares et al., 2011; Koutsidis et al., 2008; Lawrie & Leward, 2006). For beef the concentration of glutamic acid was observed to increase throughout the whole conditioning period of 21 days from 0.43 to 0.97 μmol/g (Koutsidis et al., 2008) and for 10 days from 0.30 to 4.45 μmol/g (Triki et al., 2018) (**Table 2**). With pork, the highest concentrations of glutamic acid 3.31 μmol/g, was measured after 6 days of the 10 days storage (Triki et al., 2018). In fresh meat, 5′-IMP accumulates following the rapid breakdown rate of ATP to AMP, until hydrolyzed with a slower rate to inosine and further degraded to hypoxanthine (Hernández-Cázares et al., 2011). The degradation of 5′-IMP and simultaneous accumulation of inosine, hypoxanthine and ribose was measured right from the beginning of the 21 days of conditioning the pork (Tikk et al., 2006) and beef (Koutsidis et al., 2008)(**Table 2**).

The effect of cooking on umami compounds varied between studies. The content of glutamic acid and 5′-IMP in meat has been reported to decrease in meat during cooking experiments (Arya and Parihar, 1979; Chikuni et al. 2002; Jayasena et al. 2014; Madruga et al. 2010). It should be noted that the concentrations of broth or cooking juice were not analyzed in these studies. A significant decrease in glutamic acid and 5′-IMP contents was observed during cooking pork (Chikuni et al., 2002), goat (Madruga et al., 2010) and chicken meat (Jayasena et al., 2014) (**Table 2**). Moreover, in the study of Arya & Parihar (1979), 5′-IMP concentration in cooked goat and sheep meat was lower than in raw meat, even though the content varied at different cooking temperatures (**Table 2**). In addition, a simultaneous increase in the concentrations of inosine and hypoxanthine were observed (Arya & Parihar, 1979), though Madruga et al. (2010) reported a significant decrease of inosine in goat meat. However, in the study of Sasaki et al. (2007), where both meat and broth were analyzed separately, the total content of glutamic acid did not change during moist heat cooking of

pork meat. The content of glutamic acid in meat decreased from 0.80 to 0.22 µmol/g and at the same time increased in broth from 0 to 0.53 µmol/g, indicating the release of compounds from meat to broth (**Table 2**). Moreover, the total content of 5′-IMP increased from 2.87 to 3.32 µmol/g in the early stages of moist heat cooking at 95 °C, while after 10 min the concentrations remained unchanged (Sasaki et al., 2007). The 5′-IMP content in meat was observed to decrese from 2.87 to 1.18 µmol/g and simultaneously to increase in broth from 0.0 to 2.16 µmol/g (fw). The broth in the study of Sasaki et al. (2007) was a water extraction of meat, not cooking juice.

2.5 Umami compounds in mushrooms

Mushrooms are considered to contain substantial amounts of umami substances (Mau et al., 2005) promoting the palatable umami taste of edible mushrooms (Dermiki et al., 2013; Li et al., 2014). The content of FAAs and 5′-nucleotides varies based on the mushroom species, both in cultivated and wild edible species (**Table 3**). In addition to genotype and pre-harvest conditions, several postharvest factors influence the chemical composition of edible fungi like maturity stage (Tsai et al., 2007), storage time (Tseng & Mau, 1999) and processing conditions (Li et al., 2011). These factors are discussed more in detail in the review articles of Zhang et al. (2013) and Samarashi & Chen (2022). Based on the results of Tsai et al. (2007), maturation increased the content of glutamic acid and decreased the total content of umami 5′-nucleotides in *A. bisporus* mushroom harvested at different maturity stages. According to Tseng and Mau (1999) the content of glutamic acid in *A. bisporus* mushrooms increased during 12 days of storage from 16.05 to 41.26 mg/g (dw) while the contents of 5′-nucleotides 5′-AMP, 5′-GMP and 5′-IMP were highest at days 3 and 6 (**Table 3**).

The effect of cooking on FAA content varied between mushroom studies that used different cooking times and temperatures (**Table 3**). According to Zhou et al. (2017), a short 15 minutes boiling of the fruiting body of *Tricholoma lobayense* did not affect to the content of glutamic acid (**Table 3**). However, a significant 20% decrease in the level of glutamic acid in boiled *A. bisporus* mushroom soup cooked for a relatively long time of 92 min at 60 °C was observed by Li et al. (2011), compared to the unheated control material (**Table 3**). There are indications that the effect of cooking varies between different nucleotides. There was a statistically significant increase in the concentrations of 5′-AMP, 5′-CMP and 5′-GMP in boiled (15 min) *Tricholoma lobayense* (Zhou et al., 2017) and in the boiled *A. bisporus* mushroom soup (60 minutes at 92 °C) (Li et al., 2011) compared to control mushrooms (**Table 3**). At the same time, concentration of 5′-XMP decreased significantly in boiled *A. bisporus* soup (Li et al., 2011).

Table 3. Free L-amino acids and 5′-nucleotides in selected cultivated and wild mushrooms (mg/g dry matter).

Species (binomial)	Asp[a]	Glu[a]	5′-AMP	5′-GMP	5′-IMP	5′-XMP	Treatment	Reference
Agaricus bisporus	6.62	16.05	2.04	1.52	0.44	2.23	Day 0, storage, freeze dried	(Tseng & Mau 1999)
Agaricus bisporus	6.02	19.55	2.76	3.36	0.48	2.46	Day 3 storage, freeze dried	(Tseng & Mau 1999)
Agaricus bisporus	6.04	28.59	2.22	2.47	0.60	2.80	Day 6 storage, freeze dried	(Tseng & Mau 1999)
Agaricus bisporus	6.02	31.02	1.46	1.96	0.39	2.87	Day 9 storage, freeze dried	(Tseng & Mau 1999)
Agaricus bisporus	5.86	41.26	1.60	2.29	0.25	2.96	Day 12 storage, freeze dried	(Tseng & Mau 1999)
Agaricus bisporus	0.36	1.39	0.41	0.21	0.03	0.14	Dried in shade	Ranogajec et al. 2010)
Boletus edulis	0.65	0.59	0.09	0.04	0.06	1.91	Air dried	(Tsai et al. 2008)
Boletus edulis	0.33	39.1	1.65	0.64	0.28	0.71	Freeze dried	(Beluhan & Ranogajec 2011)
Cantharellus cibarius	0.06	30.0	0.41	0.21	0.03	0.14	Freeze dried	(Beluhan & Ranogajec 2011)
Cantharelus cibarius	0.29	1.50	0.30	0.13	0.04	0.17	Dried in shade	(Ranogajec et al. 2010)
Lentinus edodes	N/A	N/A	0.85	1.70	0.21	N/A	Raw. not dried	(Li et al. 2011)
Lentinus edodes (271)	0.41	1.30	N/A	N/A	2.78	8.80	Air dried 60 °C	(Yang et al. 2001)
Pleurotus ostreatus	0.13	0.71	4.37	0.57	N/A	5.52	Air dried 60 °C	(Yang et al. 2001)

Agaricus bisporus. white	2.29	14.1	N/A	N/A	N/A	N/A	24 °C/180min	(Poojary et al. 2017)
Agaricus bisporus. white	2.19	17.1	0.18	0.35	N/A	N/A	70 °C/30min	(Poojary et al. 2017)
Agaricus bisporus. brown	0.97	21.1	N/A	N/A	N/A	N/A	24 °C/180min	(Poojary et al. 2017)
Agaricus bisporus. brown	2.67	13.0	0.51	0.02	0.12	0.66	70 °C/30min	(Poojary et al. 2017)
Lentinula edodes	1.20	9.07	N/A	N/A	N/A	N/A	24 °C/180min	(Poojary et al. 2017)
Lentinula edodes	0.98	5.88	0.80	0.74	N/A	N/A	70 °C/30min	(Poojary et al. 2017)
Agaricus bisporus L.	3.95	21.2	0.17	0.11	N/A	5.39	25 °C/2h control	(Li et al. 2011)
Agaricus bisporus L.	1.42	17.0	0.35	0.35	N/A	1.18	Boiling 92C/60min	(Li et al. 2011)
Tricholoma lobayense	0.21	1.78	0.13	0.55	0.15	N/A	Control	(Zhou et al. 2017)
Tricholoma lobayense	0.27	1.73	0.16	0.75	0.11	N/A	Boiling 15 min	(Zhou et al. 2017)
Cantharelus cibarius	1.29	3.78	0.38	0.19	0.22	N/A	Cooked 80 °C/ 10 min	(Manninen et al. 2018)
Cratellus tubaeformis	1.65	1.92	0.70	0.11	0.13	N/A	Cooked 80 °C/ 10 min	(Manninen et al. 2018)
Boletus edulis	2.85	2.62	1.39	0.60	0.35	N/A	Cooked 80 °C/ 10 min	(Manninen et al. 2018)
Lactarius camphoratus	3.35	4.69	0.08	-	n.d.	N/A	Cooked 80 °C/ 10 min	(Manninen et al. 2018)

[a]Amino acid abbreviations: Asp: Aspartic acid. Glu: Glutamic acid

N/A = not available

Glutamic acid has been reported as one of the major FAAs in both cultivated and wild edible mushrooms e.g. in fresh *A. bisporus*, (Tsai et al., 2007; Tseng & Mau, 1999), in *A. bisporus* mushroom soup (Li et al., 2011), in four Finnish forest mushrooms (Manninen et al., 2018) and in Shiitake mushrooms (*Lentinus edodes*) (Dermiki et al., 2013). In the literature, there were great variations in the presented glutamic acid concentrations (**Table 3**). For example, Tseng & Mau (1999) reported concentrations of 16.1 to 41.26 mg/g dry weight (dw) for freeze-dried *A. bisporus* mushrooms, while Ranogajec et al. (2010) reported glutamic acid concentration 1.39 mg/g, dw for *A. bisporus* dried in shade (**Table 3**). The large difference in concentrations is partly explained by the fact that Ranogajec et al. (2010) has used wild, edible *A. bisporus* and Tseng & Mau (1999) has used cultivated *A. bisporus*. Similarly, there was a great variation in the glutamic acid content of *B. edulis* species, since concentration in air dried mushroom 0.59 mg/g, dw (Tsai et al., 2008) was much lower than the concentration 39.1 mg/g, dw in freeze-dried mushroom (Beluhan & Ranogajec 2011). The differences in concentrations might be due to differences in regional growth conditions, processing conditions or storage time before analysis. Tsai et al. (2008) purchased dried mushrooms from a hypermarket in Taiwan while Beluhan and Ranogajec (2010) freeze dried mushrooms immediately after collecting from forest in Croatia. Relatively high concentrations of aspartic acid has been reported in fresh *A. bisporus* (Tseng & Mau, 1999) and in Finnish forest mushrooms (Manninen et al., 2018) (**Table 3**).

The content and relative proportion of 5′-nucleotides to each other seems to vary widely in different studies (**Table 3**). In particular, 5′-AMP, 5′-GMP and 5′-XMP are typically present (**Table 3**), but also detectable concentrations of 5′-IMP have been measured in some studies (Tseng & Mau, 1999; Yang et al., 2001). However, in the research of Phat et al. (2016), no 5′-GMP was detected in any of the mushroom samples. However, 5′-CMP and 5′-UMP have been reported in some mushroom studies, although they are not known to give taste stimuli (**Table 3**). As with free amino acids, there is a great variation in reported concentrations of of 5′-nucleotides between studies and species (**Table 3**). Tseng & Mau (1999) reported concentrations of 1.52-3.36 mg/g, dw for 5′-GMP in cultivated *A. bisporus*, while much a lower concentration, 0.21 mg/g, dw was reported by Ranogajec et al. (2010) for wild *A. bisporus* mushroom. Moreover, for *Botulis edulis*, concentrations have been analyzed between 0.04 mg/g, dw (Tsai et al., 2008) and 0.64 mg/g dw (Beluhan & Ranogajec, 2011) for air and freeze-dried mushrooms, respectively. In addition to different drying methods, the differences in concentrations might be explained by differences between cultivated and wild mushrooms as well as regional growth conditions, as mentioned in the previous chapter for FAAs.

2.6 Umami compounds in potatoes; factors affecting to the formation and changes in the chemical content of tubers

Raw potato tubers contain taste and flavor precursors, such as carbohydrates, amino acids, ribonucleotides and lipids, the levels of which are affected by a number of factors such as growth environment, plant genotype, stage of harvest and storage conditions (Jansky, 2010; Morris et al., 2007). Sun et al. (2018) and Muttucumaru et al. (2013) emphasized the significance of the selected potato cultivar on the chemical composition of potato tubers. The mild and pleasant taste that potatoes develop during cooking is formed by taste and flavor compounds and their precursors through enzymatic and chemical reactions (Jansky, 2010; Solms & Wyler, 1979).

Even though the crude protein content of potato tubers is low (2%) (Belitz et al., 2009), they have relatively high levels of free amino acids compared to many other vegetables (Eppendorfer & Bille, 1996). The share of free amino acids in potato tubers was 34% of the total nitrogen analyzed and much higher compared to kale, beans, spinach and cauliflower (Eppendorfer & Bille, 1996). Asparagine and glutamine, the amide derivatives of aspartic and glutamic acids, have a dominant share of the FAA pool of potato tubers, asparagine typically accounting for at least 30% of the FAA pool (Elmore et al., 2007; Eppendorfer & Bille, 1996). In the study of Morris et al. (2007), asparagine and glutamine constituted 20-50% (w/w) and 15-45% (w/w) of the sum of FAAs, whereas the the share of other FAAs remained low. Glutamic and aspartic acids are often among the five most abundant free amino acids in uncooked potato tubers (Elmore et al., 2007; Eppendorfer & Bille, 1996). In a study of Eppendorfer & Bille (1996), the content of aspartic acid and glutamic acids of total FAA pool in different potato cultivars varied between 1.8 and 3.9% and between 2.0 and 4.4%, respectively. Unlike the moderate concentrations of free umami AAs, only very low concentrations of free 5´-nucleotides have been detected in raw potato tubers (Jansky, 2010; Morris et al., 2007; Solms & Wyler 1979).

Table 4. Free amino acids in potato tubers (mg/g fresh weight).

Cultivar	Asn[a]	Asp	Gln	Glu	Treatment	Reference
Rikea	2.56	0.53	2.47	0.19	Uncooked	Aisala et al. (2016)
Rikea	2.53	0.65	2.49	0.54	Cooked	
Bintje	2.20	0.47	0.49	0.74	Boiled	Solms & Wyler (1979)
Ostara	1.87	0.36	0.78	0.36	Boiled	
Mayan Gold (*S.phureija*)	0.24	0.03	0.26	0.13	Uncooked	Morris et al. (2007)
Mayan Gold	0.24	0.03	0.22	0.15	Cooked	
Inca Sun (*S. phureija*)	0.35	0.07	0.36	0.12	Uncooked	
Inca Sun	0.36	0.06	0.42	0.11	Cooked	
Pentland Dell (*S. tuberosum*)	0.31	0.03	0.25	0.07	Uncooked	
Pentland Dell	0.31	0.03	0.20	0.07	Cooked	
Montrose (*S. tuberosum*)	0.14	0.03	0.25	0.04	Uncooked	
Montrose	0.15	0.03	0.24	0.04	Cooked	
Pentland Dell	2.07	0.03	1.76	0.15	At harvest	Brierley et al. (1997)
Pentland Dell	7.27	0.13	4.60	0.32	25 wks (5 °C)	
Pentland Dell	9.31	0.22	7.66	0.60	25 wks (10 °C)	
Pentland Dell	2.06-9.31	0.03-0.90	1.76-7.66	0.15-1.32	Uncooked	Brierley et al. (1996)

Record	1.38-8.23	<0.01-0.89	1.93-9.55	0.08-1.39	Uncooked	
Bintje	1.37	0.32	0.94	0.41	Uncooked, 6.0g N/pot	Eppedorfer & Bille 1996
	3.94	0.39	2.87	0.63	Uncooked, 12 g N/pot	
Italian (several)[b]	0.15-4.58	N/A	N/A	N/A	Uncooked	Vivanti et al. (1996)
American (several)[b]	0.50-7.62	N/A	N/A	N/A	Uncooked	
Several	2.01-4.25	N/A	0-2.06	N/A	Uncooked	Amrein et al. (2003)
		N/A		N/A		

[a]Amino acid abbreviations: Asn: Asparagine, Asp: Aspartic acid, Gln: Glutamine, Glu: Glutamic acid

[b]Recalculated from the original concentration of mmol/kg fresh weight

N/A = not available

2.6.1 Growth environment

During the growing season, the development of potato tubers is particularly influenced by light interception, temperature and day length (Kooman & Haverkort, 1995). According to the developmental responses, *Solanum tuberosum* spp. *tuberosum* is classified as a short day (SD) plant, the developmental responses being promoted by long nights (ca. 12h or longer) i.e. short day (SD) photoperiods (Jackson, 2009). A photoperiod interacting with light intensity and quality as well as temperature, may substantially affect the biosynthesis and partitioning of primary and secondary metabolites in potato plants (Rubio-Covarrubias et al., 2006; Stuttle et al., 1996). It is also likely to have an effect on the umami taste of potato tubers. Marwaha & Sandhu (2002) found lower FAA concentrations in potato tubers grown in autumn SD conditions than those grown in spring long day (LD) conditions.

Adequate fertilization is necessary for the growth of potato tubers and also affects the concentration of taste compounds. Sulfur deprivation has been found to increase the concentration of total free amino acids, including glutamic acid but excluding aspartic acid (Elmore et al., 2007), while increased nitrogen fertilization may cause a rise in free amino acid concentration (Eppendorfer & Bille, 1996) (**Table 4**).

2.6.2 Genotype and storage

In the study of Morris et al. (2007), the genotype of tubers had a strong influence on the concentrations of taste compounds at harvest and during storage. Immediately after harvest, the tubers of *Solanum phureja* cultivars contained significantly higher levels of glutamate and nucleotides 5′-AMP and 5′-GMP than tubers of *Solanum tuberosum* cultivars, whereas no significant difference in the level of aspartate was observed (**Table 4**). However, in a later study of Morris et al. (2010), it was found that during the three months of storage (both at 4 °C and 10 °C), the decrease in aspartate and glutamate concentrations was much stronger in *Solanum phureja* cultivars compared to *Solanum tuberosum* cultivars. However, the change in nucleotide concentrations of cooked tubers were stable or even increased a little. After three months of storage, no significant differences between these two cultivars could be detected anymore when calculated as EUC values, due to a much stronger decrease in glutamate and aspartate concentrations in *Solanum phureja* cultivars (Morris et al. 2010).

Based on the results of Brierley et al. (1997), the FAA content of potato tubers increases during storage, a higher storage temperature leading to a greater accumulation of compounds (**Table 4**). Of the FAAs, particularly the concentrations of asparagine and glutamine increased significantly ($P<0.05$) after 25 weeks of storage, the accumulation being greater at 10 than at 5 °C

(**Table 4**). Similarly, the contents of aspartate and glutamate increased ($P<0.05$) more at 10 °C, reaching a maximum after 33 weeks of storage.

2.6.3 Cooking

According to Aisala et al. (2016), compared to raw tubers, a notable increase in the concentrations of glutamic ($P<0.001$) and aspartic acids ($P<0.007$) were detected during cooking. Concentration of glutamic acid increased from 0.19 to 0.54 mg/g, fw and aspartic acid from 0.53 to 0.65 mg/g, fw between raw and cooked potato (**Table 4**). Cooking did not affect to the concentrations of the dominating FAAs, L-asparagine and L-glutamine.

In the study by Morris et al. (2007), higher levels of glutamate ($P\leq0.08$) were observed in *Solanum Phureja* cultivars compared to *Tuberosum* cultivars, both in raw and cooked tubers. The mature tubers were analyzed at harvest representing *S. phureja* cultivars Mayan Gold and Inca Sun and *S. tuberosum* cultivars Montrose and Pentland Dell. As an example, glutamate content in *Phureja* cultivar Mayan Gold was 910 nmol/g (fw) in the raw tuber and in the cooked tuber 999 nmol/g while in the *Tuberosum* cultivar Pentland Dell the content was 500 nmol/g when raw and 466 nmol/g when cooked (**Table 4**). There were no statistics representing raw and cooked tubers, only between *Phureja* and *Tuberosum* cultivar concentrations, but the concentrations of FAAs between raw and cooked tubers of the same cultivar were on the same level.

In the study of Morris et al. (2007), a sharp increase in the content of 5′-GMP and 5′-AMP was observed during the first 5 min of cooking the tubers before the concentrations stabilized (the exact numerical values were not presented). In the 1970s, Buri et al., (1971) had already suggested that the activity of RNA-degrading nuclease enzymes during the cooking process caused the accumulation of 5′-nucleotides in cooked potato tubers.

Unfortunately, the number of publications on the levels of umami compounds and their precursors in uncooked and cooked potato tubers is quite limited, whereas the suspected carsinogen acryamide, discovered in processed foods at elevated temperatures, has received much more attention by researchers. Since the primary precursors of acrylamide, reducing sugars glucose and fructose together with free AA L-asparagine, exist in relatively high concentrations in potato tubers (Halford et al., 2012; Rosen et al., 2018), several research reports have focused on the possibilities of reducing their content (Amrein et al., 2003; Elmore et al. 2007; Muttucumaru et al. 2017; Sun et al. 2018; Vivanti et al. 2006). However, these studies can also provide indications of the concentrations of taste compounds as well.

3 AIMS OF THE STUDY

The overall aim of the study was to investigate the effect of sous vide cooking conditions on the umami compound concentrations in meat and mushrooms (I-II) and the effect of growth conditions on potato quality (III). The research was done by comparing and evaluating the concentrations of taste compounds by chemical analysis. The research also focused on an investigation of the quantities of umami compounds i.e. free amino acids (FAAs) and nucleotides.

The specific aims were:

1. To investigate the effects of sous vide cooking time and cooking temperature on the concentration of umami taste-related compounds in pork meat and in the released juice (Publication I)
2. To examine the effect of sous vide cooking temperature on the concentration of umami taste-related compounds in mushrooms and the released juice (Publication II).
3. To study the quantity of taste compounds in released cooking juice (Publications I and II)
4. To investigate the concentrations of taste compounds in Nordic mushroom species (Publication II)
5. To evaluate the differences in potato taste quality due to shorter and longer day length conditions by studying the chemical composition of potato tubers (Publication III)

The hypothesis in the pork study was that at different cooking temperatures and cooking times, the concentration of the umami compounds in the meat varies and the compounds are released to the cooking juices. According to the published literature, the cooking time was expected to have less importance. Based on the results of the pork study, the mushroom study concentrated on the effects of cooking temperature. Also in the mushroom research, the hypothesis was that cooking affects the concentration of umami compounds both in the mushrooms and in the released juices. Based on the published literature, it was hypothesized that there would be differences in the concentrations of umami compounds in the mushroom species. Based on the previously published research literature, the genotype of the potato tubers and the growth conditions, e.g. day length was expected to affect the concentrations of potato metabolites. The hypothesis of the potato research was that the length of the day affects the concentration of free amino acids in the potato tubers. It was assumed that there were also differences in the concentrations of free amino acids between potato cultivars.

4 MATERIALS AND METHODS

4.1 Studied raw materials and analyzed compounds

Table 5. Studied raw materials and analyzed compounds

Material	Analyzed compounds	Variables
Pork meat and cooking juice: Pork loin (*longissimus dorsi)*	umami FAAs, nucleotides, nucleosides	Cooking time Cooking temperature
Mushrooms: One cultivated and four forest mushroom species	FAAs, nucleotides, nucleosides	Cooking temperature
Potato tubers: Seven cultivars	FAAs	Two different growing conditions (daily light integrals)

4.2 Pretreatment and chemical analysis

4.2.1 Pretreatment of pork

Pork loin (*longissimus dorsi*) samples were purchased from a local supermarket on the same day they were cooked. All three samples were of Finnish origin, supplied by Atria Finland Ltd. (Seinäjoki, Finland). Before heat treatment, the pork loins were cut into 2 cm thick slices constituting a complete cross-section of the muscle. The fresh weight of the slices was 117 ± 11 g and all visible fat and connective tissues were removed. Vacuum-packed slices were heated in a water bath in an immersion circulator at temperatures of 60, 70, and 80 °C and at three different cooking times (Table 1, Publication I). The internal temperature of the meat was measured by a Core Temperature Sensor (Pt100, for Fusion Chef Diamond by Julabo) in the circulator. There were four replications in every experimental unit i.e. three temperatures and three cooking times. Every replication was analyzed once, both meat and juice. After cooking, the samples were stored in a refrigerator at 4 °C for 24 h before analysis.

The amount of separated cooking juice was measured and juice was stored in plastic tubes at –40 °C before analysis. The cooked meat was cut into three pieces, the middle section was homogenized with a kitchen blender, and 1.8 to 2.0 g of the homogenized meat was extracted with deionized water. An identical hot water extraction method was used for both FAAs and 5′-nucleotides and

corresponding nucleosides. The extraction procedure is described in more detail in Chapter 4.2.4.

4.2.2 Pretreatment of mushrooms

The mushroom species examined in the studies were a cultivated species *Agaricus bisporus* (white button mushroom, fresh) and four forest mushroom species: *Lactarius trivialis* (Nordic milkcap, frozen), *Cantharellus cibarius* (chantarelles, fresh), *Cantharellus tubaeformis* (funnel chanterelle, frozen) and *Suillus variegatus* (velvet bolete, frozen). The common commercial edible fungus *A. bisporus* was chosen as a reference material for the study as well as the test material for evaluating the effect of sous vide cooking temperatures. Cultivated *A. bisporus* were purchased from a local market in April 2016, supplied by Mykora Ltd, Finland. All forest mushrooms were collected in September 2016 and bought from the same retailer in Eastern Finland. The forest mushroom species were chosen according to their commercial availability.

Fresh mushrooms were delivered and transported at 4 °C and pre-treated within 24 h of their delivery. The fresh mushrooms were cut into 0.5 cm slices, a proportion were freeze dried as fresh and a proportion were vacuum packed in plastic pouches for cooking. The frozen mushrooms were also cut in pieces, pooled and freeze dried as such or were vacuum packed and thawed overnight at 4 °C and kept at room temperature before *sous vide* cooking.

There were three replications in every experimental unit i.e. non-cooked mushroom and four cooking temperatures for *A. bisporus*. For forest mushrooms the experimental units were non-cooked mushroom and cooking at temperature 90 °C. Every replication was analyzed once, both mushroom and juice. Three separate subsamples of *A. bisporus* slices were vacuum packed for each cooking temperature or freeze dried as such. The subsamples were treated with sous vide method at 60 °C, 70 °C, 80 °C and 90 °C temperatures for 10 min each. Similarly, for all the four forest mushroom species three separate subsamples were vacuum packed or freeze dried as fresh or frozen. In the sous vide treatment of forest mushrooms, the temperature of the water bath was 90 °C for 10 min. After cooking treatment, the samples were immediately immersed in icy water (2–4 °C) for 5 min, after which mushrooms and cooking juice were separated and both were frozen at −40 °C. The mushrooms were freeze dried for 26–29 h in a vacuum at −40 °C. Before nucleotide and free amino acid analysis the freeze dried mushroom powder was extracted with deionized water. An identical hot water extraction method was used for both FAAs and 5′-nucleotides and corresponding nucleosides. The extraction method is described in more detail in Chapter 4.2.4.

4.2.3 Pretreatment of potato tubers

The tubers of the seven cultivars 'Jazzy', 'Rock', 'Jussi', 'Gala', 'Soraya', 'Jessica' and 'Annabelle' were grown in controlled grown chambers with two different photoperiods. All seven potato cultivars are commercially available in Finland. Six of the cultivars were developed by Central European potato breeders, while the cultivar Jussi was developed in Finland. The details of the experimental setup of the cultivars are described in Table 1 of Study III. Photoperiodic treatments were adjusted to short day (SD) conditions (8 h light, 16 h dark) and long day (LD) conditions (15 h light, 9 h dark). The tubers were grown until a senescence growth stage, which was determined according to Hack et al. (1993). At harvest, the tubers of each cultivar from the same treatment were combined into one biological sample and stored in the dark in paper bags at + 4 ± 1 °C. The maximum storage time before lyophilization was one month and was always the same for all tubers of the same cultivar.

There were three replications in every experimental unit i.e. two photoperiodic treatments. Every replication was analyzed once. Fresh tubers of a similar size from both treatments (SD and LD) were sliced and divided into three subsamples and frozen at –40 °C before freeze drying for 30 h in a vacuum at –40 °C. To obtain dry matter content, the slices were weighed before and after freeze-drying. The dried slices were stored in a freezer (–20 °C) before extraction. An identical hot water extraction method was used for both FAAs and sugars. The extracts were stored at –20 °C if not analyzed immediately. The extraction method is described in more detail in Chapter 4.2.4.

4.2.4 Extraction procedure

In all three studies (Publications I-III), an identical hot water extraction method was used for all analyzed compounds based on the extraction method of Ranogajec et al. (2010). Homogenized fresh or freeze-dried samples were extracted three times with 10 to 20 mL of deionized water in centrifuge tubes and the obtained supernatants were combined and the volume was adjusted to 50 mL. The extraction procedure included heating the suspension in a water bath (100 °C) for 1 min, which was followed by an ultrasonic bath for a period of 15 min and centrifugation (RCF 2525 x *g*) for 15 min (at 15 to 20 °C). The supernatants were stored at –40 °C, if not analyzed immediately.

4.2.5 HPLC analysis of pork

The two most important amino acids contributing to the intensity of the umami taste, aspartic and glutamic acids, were analyzed from pork loins. The main focus was on glutamic acid concentrations, as aspartic acid concentrations were mainly

below the quantification limit. The methods for the analysis of FAAs as well as for nucleotides and nucleosides were modified from the procedure of (Ranogajec et al., 2010).

A Waters HPLC instrument, consisting of two 515 HPLC pumps, a degasser, a 717plus autosampler and a column oven (Waters Co., Milford, Mass., U.S.A.) was used for the analysis of FAAs, nucleotides and nucleosides. For nucleosides and 5′-nucleotides, a Synergi Hydro column (150 × 3.0 mm, 4 µm, Phenomenex) and Waters 2487 ultraviolet-visible (UV/VIS) spectroscopy at a wavelength of 254 nm was used. For FAAs, a Waters 2475 fluorescence detector and a Kinetex XB-Phenomenex C18 column (100 × 3.00 mm, 2.6 µm, 100 Å, Phenomenex) at the excitation and emission wavelengths of 340 and 450nm was used. Empower 3 software was utilized for data acquisition and analysis. A more detailed information of the analysis is described in Publication I.

4.2.6 UHPLC analysis of mushrooms

A total of 26 amino acids, five nucleotides and five nucleosides were quantified using a Shimadzu Nexera X2 UHPLC (Kyoto, Japan) instrument. The method for the analysis of nucleotide and nucleoside content was modified from the studies of Ranogajec et al. (2010) and Rotola-Pukkila et al. (2015). The amino acid analysis was based on the technical notes of Shimadzu (Shimadzu Corporation).

For the analysis of nucleotides and nucleosides, a Synergi Hydro-RP column (150×3.0 mm, 4 µm; Phenomenex) and an SPD-M20A photodiode array detector (DAD) at a wavelength of 254 nm was used. For FAA analysis, a Kinetex C18 column (100×4.6 mm, 2.6 µm; Phenomenex) and a RF-20AXS fluorescence detector was used. Compounds were detected at the excitation and emission wavelengths of 340/450 nm used for primary amino acids and 266/305 nm for secondary amino acids. Lab-Solutions software (Shimadzu) was used for data acquisition and analysis. The derivatization chemicals used were *o*-phthaldehyde (OPA) and 3-mercaptopropionic acid (3-MPA) in 0.1M borate buffer and FMOC in acetonitrile, and acidic phosphate buffer (pH 2.1). A more detailed information of the analysis is described in Publication II.

4.2.7 Chromatographic analysis of potato tubers

The analysis of potato tubers was done in two batches, referred to as Experiments 1 and 2. The FAA compositions of cultivars in both batches, Experiment 1 and 2 were analyzed by liquid chromatographic methods.

In Experiment 1, FAAs were identified using an identical UHPLC method described in Publication 2 for mushrooms. In Experiment 2, the separation of free amino acids was performed using a Zorbax Eclipse AAA column (Rapid

Resolution 4.6 × 150 mm, 3.5 µm, Agilent) and compounds were analyzed and identified according to the Eclipse AAA protocol described in Technical Note 5980-1193 (Agilent Technologies). A Shimadzu HPLC equipped with an RF-20AXS fluorescence detector was used to confirm the FAAs from 3-MPA/ OPA derivatized supernatants. Fluorescence detection was set at wavelengths of 340 nm for excitation and 450 nm for emission. A more detailed information of the analysis is described in Publication III.

4.3 Statistical analysis

All the statistical analyses were done using the IBM SPSS Statistics (versions 21.0, 24.0 and 26.0, IBM Corporation, Armonk, NY, USA) and Unscrambler X (version 10.3, Camo Software, Oslo, Norway). All the statistical analyses used during the studies and the purpose for their use are described in **Table 6.** A non-parametric Kruskal-Wallis test was used instead of an analysis of variance (ANOVA) because the groups examined were small. Therefore, the normality assumption and equality of variances did not match in all cases. Pairwise comparison was done using the Mann-Whitney U test.

Table 6. Statistical analyses performed in the studies I-III. Differences were considered statistically significant if *p*-value was below 0.05.

Study	Method	Purpose
I	Non-parametric Kruskal-Wallis test	To test the differences between cooking temperatures and cooking times of pork samples
I	Non-parametric Mann-Whitney U test	The pairwise comparison of cooking times and temperatures of pork samples
II	Non-parametric Kruskal-Wallis test	To test the differences between the fresh and cooked mushrooms and released cooking juices at different temperatures
II	Non-parametric Mann-Whitney U test	The pairwise comparison of cooking temperatures of mushrooms and juice
III	paired T-test	Comparison of the means of the two treatments for every analyzed compound in potato tubers
III	Levene's test	The test the equality of variances for ANOVA
III	Principal Component Analysis (PCA)	Dependence of the experimental results. To determine correlations between the photoperiodic treatments and the analyzed chemical properties

5 RESULTS AND DISCUSSION

5.1 Effect of cooking temperature and time on umami compounds in pork

The concentration of umami compounds in cooked pork meat and the released juice was dependent on the cooking temperature and the length of cooking time, though the cooking temperature played a more important role. The most predominant FAAs, nucleotide and nucleoside in cooked pork meat and cooking juice were free glutamic acid, 5′-IMP and its corresponding degradation product inosine. The highest concentrations of glutamic acid and inosine were measured in meat and juice cooked at 80 °C, while no change in 5′-IMP content was observed between the three cooking temperatures 60, 70 and 80 °C.

5.1.1 Effect of cooking on free amino acid (FAA) concentration in pork meat and cooking juice

There were statistically significant differences in glutamic acid concentrations between the meat samples cooked at different temperatures. A significantly lower concentration (0.38 μmol/g) was detected in the meat samples cooked at 70 °C compared with the samples cooked at 60 or 80 °C (0.58 and 0.64 μmol/g, respectively) (**Figure 3**). Numerical values are represented in Table 3 of Study I. In the cooking juice, the concentration of glutamic acid was significantly higher when heated to 80 °C (1.42 μmol/g) as compared to the juice samples cooked at lower temperatures 60 °C (0.64 μmol/g) and 70 °C (0.67 μmol/g, at time point 0). Time 0 is the time when meat has reached the target temperature, time 60 and 120 indicate minutes cooked after time 0. Since there were no statistically significant differences between the cooking times at the three different temperatures either in the meat or juice, the statistical analysis between the cooking temperatures was done only from the cooking time point 0. Aspartic acid concentrations were near or below the LOQ in most samples in both meat and cooking juice, but concentrations followed the pattern of glutamic acid concentrations between different cooking temperatures, although the differences were not significant (Study I, Table 3).

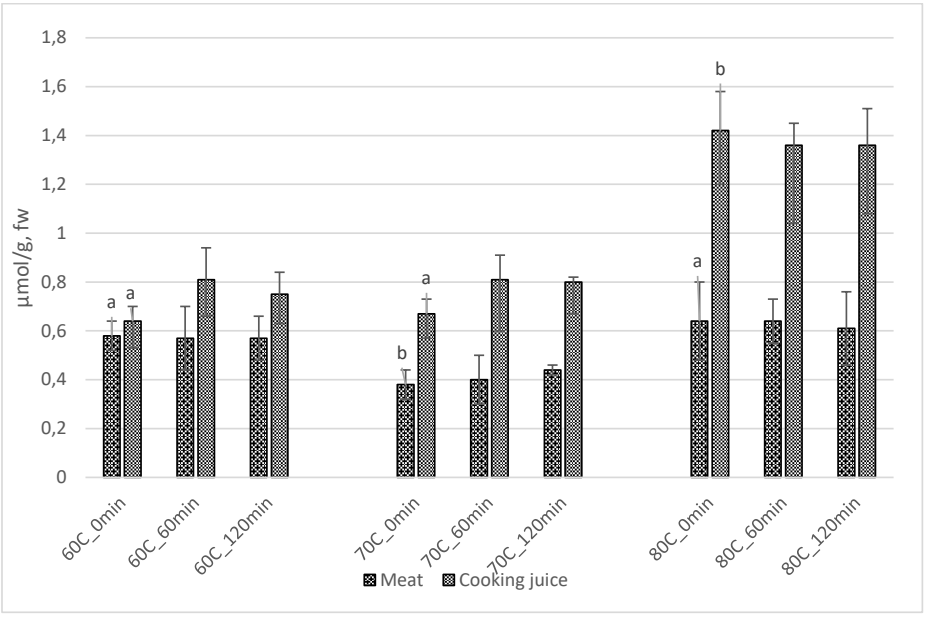

Figure 3. Mean values (n=4) of glutamic acid concentrations in the cooked meat and meat juices (µmol/g) at three different temperatures. Significant differences ($p \leq 0.05$) have been marked with letters [a] and [b]. Different letters indicate statistically significant differences. Statistically significant effects of cooking temperatures are viewed only from the cooking time point 0.

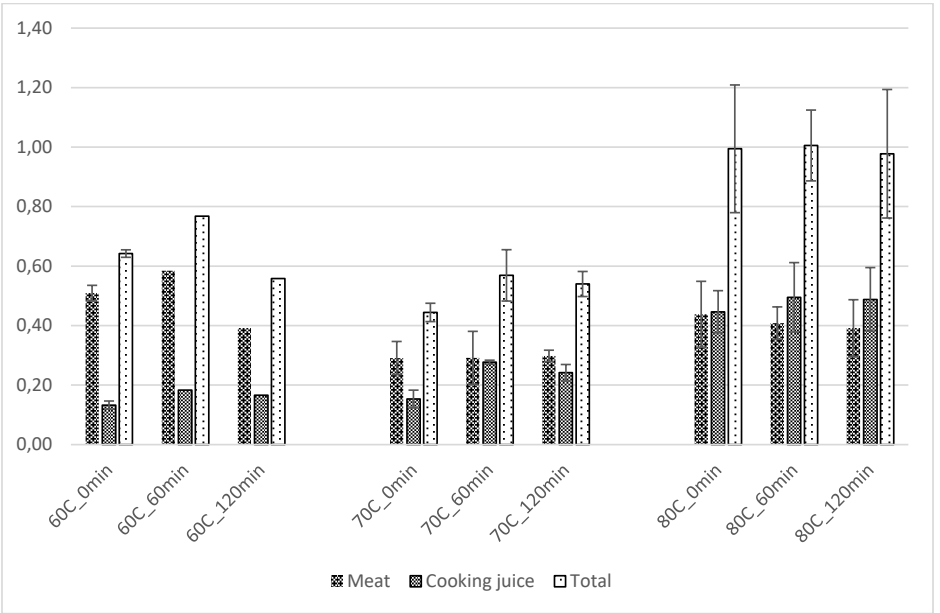

Figure 4. Content of glutamic acid in one gram of total sample calculated by using the relative proportions of meat and juice (%, w/w) in one gram of total sample multiplied by their concentrations in µmol/g.

The relative precentage (%) of cooking juice from the total sample at 60 °C was 18-24% (w/w), at 70 °C 20-33% and at 80 °C 30-37%, respectively. Our research results clearly showed the release of umami compounds from meat into cooking juice during cooking at these three studied cooking temperatures. The effect of cooking temperature on the concentration of glutamic acid in the whole sample including both meat and juice was calculated based on the percentual proportions of meat and juice (%, w/w) multiplied by their absolute concentrations of μmol/g (from **Figure 3)**. Concentration in the total sample c_{tot} (μmol/g, total sample) is represented by the equation (2),

$$c_{tot} = \left(\frac{c_m \times \%_m}{100} + \frac{c_j \times \%_j}{100}\right) \quad (2)$$

where c_m and c_j are the absolute concentrations of meat and juice (μmol/g) and $\%_m$ and $\%_j$ are the percentual concentrations of meat and juice. The results of these calculations are shown in **Figure 4**, which shows the distribution of glutamic acid concentration between meat and juice, indicating the release of compounds from meat to cooking juice.

From **Figure 3** and **Figure 4** it can be seen that at temperatures 60 °C and 70 °C, where the absolute content of glutamic acid in meat (μmol/g) (**Figure 3**) is lower than in juice, but as the relative proportion of meat (%, w/w) is greater in total sample, most of the glutamic acid is in meat. At temperature 80 °C, both the relative proportion (%) of juice of the total sample and the absolute concentration of glutamic acid (μmol/g) in the juice increases. This leads to the role of juice as a source of glutamic acid in the total sample to increase. From **Figure 4** it can be seen that the total concentration of glutamic acid in the whole sample increases at 80 °C compared to lower cooking temperatures. The concentration in meat cooked at 80 °C is at the same level as the meat cooked at 60 °C. Observed increase in total concentration is due to high concentration in the cooking juice. A higher cooking temperature in our study resulted in increased release of glutamic acid from meat to cooking juice but also increased the content of glutamic acid in the total sample.

The unexpected variation in glutamic acid concentration of pork meat in our study might be partially explained by chemical and enzymatic reactions and the release of compounds from meat into the cooking juice, but there may also be natural variation in raw materials. It is likely that FAAs participate in the complex, thermally induced reactions with other compounds in meat during cooking as suggested by Mottram (1998). A significant increase in glutamic acid concentrations in the cooking juice released from meat cooked at 80 °C may indicate increased protein denaturation in the meat, resulting in higher amount

of juice and water-soluble components released from the structures compared to meat cooked at lower temperatures.

The levels of quantified compounds in our study were mostly of the same order of magnitude as in other reported research results. The variations between studies were most likely due to differences in breeds, selected cuts of the meat and the extraction and analysis methods used. The levels of glutamic acid in pork meat in our study (0.38-0.64 μmol/g) were at the same level as reported in the earlier studies. A glutamic acid content of 0.42 μmol/g has been measured for grilled goat meat by Madruga et al. (2010), for moist cooked pork (0.22 – 0.54 μmol/g) by Sasaki et al. (2007) and for vacuum-packed pork cooked at 70 °C (0.36 μmol/g) by Chikuni et al. (2002).

According to other research articles, the content of glutamic acid in meat has mainly been reported to be relatively stable or as decreasing during cooking experiments. In the study of Sasaki et al. (2007), the total content of glutamic acid did not change during the moist heat cooking of pork meat, although the content in the meat decreased but increased in broth indicating the removal of compounds from meat. The broth in the study of Sasaki et al. (2007) was a water extraction of meat, not cooking juice like in our research, and therefore the results are not directly comparable. In the pilot study of Clausen et al. (2018), no statistically significant change in glutamate (glutamic acid) concentration was detected in beef tenderloin during 8 h of sous vide cooking at temperatures of 54 °C or 64 °C compared to raw meat; this result was found when the contents were measured in the total sample including both meat and juice. In contrast, Madruga et al. (2010) reported a statistically significant decrease ($p<0.001$) in the concentration of FAAs between raw and grilled goat meat, with 57% of glutamic acid and 69% of total FAAs remaining after grilling at 80 °C (**Table 2**). Furthermore, in the study of Chikuni et al. (2002), glutamic acid contents decreased significantly between raw and pork meat cooked at 70 °C (from 0.44 to 0.36 μmol/g respectively). Conversely, concentration of aspartic acid was reported to be low and stable during the cooking of goat meat (101% remaining after cooking) (Madruga et al. 2010).

5.1.2 Effect of cooking on nucleotide and nucleoside concentration in pork meat and cooking juice

Of the identified and separated five 5′-nucleotides (5′-AMP, 5′-CMP, 5′-GMP, 5′-IMP and 5′-UMP) and five nucleosides (adenosine, cytidine, guanosine, inosine and uridine), 5′-AMP, 5′-IMP and inosine were quantified. The dominant 5′-nucleotide in cooked pork meat samples was 5′-IMP, the concentration of which did not vary significantly with time or temperature. The concentrations of 5′-IMP in meat remained between 2.77 and 3.12 μmol/g and in the cooking juice between 3.99 and 4.77 μmol/g (Study I, Table 4 and **Figure**

5). The levels of 5′-IMP in cooked meat in our study were close to those in cooked pork meat reported by Sasaki et al. (2007) and Chikuni et al. (2002) and grilled or cooked goat and sheep meat, reported by Madruga et al. (2010) and by Arya and Parihar (1979). Concentrations of inosine, the degradation product of 5′-IMP, first decreased in our study from 60 to 70 °C and then increased from 70 to 80 °C, both in the meat and the juice (Study I, Table 4). The higher concentration of inosine at 80 °C compared to lower cooking temperatures might indicate an earlier deactivation of the inosine degrading enzyme at 80 °C, rather than at lower temperatures. Although the concentration of 5′-AMP was close to the detection limit of the analysis method, increasing the cooking temperature significantly increased the concentration of 5′-AMP, especially in meat, from 60 °C (0.07 μmol/g) to 80 °C (0.96 μmol/g). Moreover, 5′-AMP was the only quantified compound, whose concentrations increased significantly with increased cooking time. The measured 5′-AMP concentrations in our study were in line with previous studies on cooked goat and sheep meats (Arya and Parihar (1979).

The effect of temperature on the concentration of 5′-IMP in the whole sample including both, meat and juice, as well as the transfer of compounds from meat to cooking juice, was evaluated in a similar manner than for glutamic acid in the earlier chapter. The results of these calculations are shown in **Figure 6**, showing the distribution of 5′-IMP concentration between meat and juice. At temperatures of 60 °C, 70 °C and 80 °C, where the absolute content of 5′-IMP in meat (μmol/g) is lower than in juice (**Figure 5**), but as the relative proportion of meat (%, w/w) is greater in total sample, most of the 5′-IMP is in meat (**Figure 6)**.

From **Figure 6** it can be seen that the total concentration of 5′-IMP in the whole sample does not notably change between cooking temperatures, although the relative proportion of 5′-IMP in the cooking juice seems to increase slightly and the relative proportion in the meat seems to decrease. Since the absolute 5′-IMP concentrations of meat and juice do not change due to the change in cooking temperature (**Figure 5**), and the difference in the absolute 5′-IMP concentration between meat and juice is not very large, the role of juice as a source of 5′-IMP at higher cooking temperatures does not increase as much as with glutamic acid. A higher cooking temperature in our study resulted in increased release of 5′-IMP from meat to cooking juice but did not increase the content of 5′-IMP in the total sample.

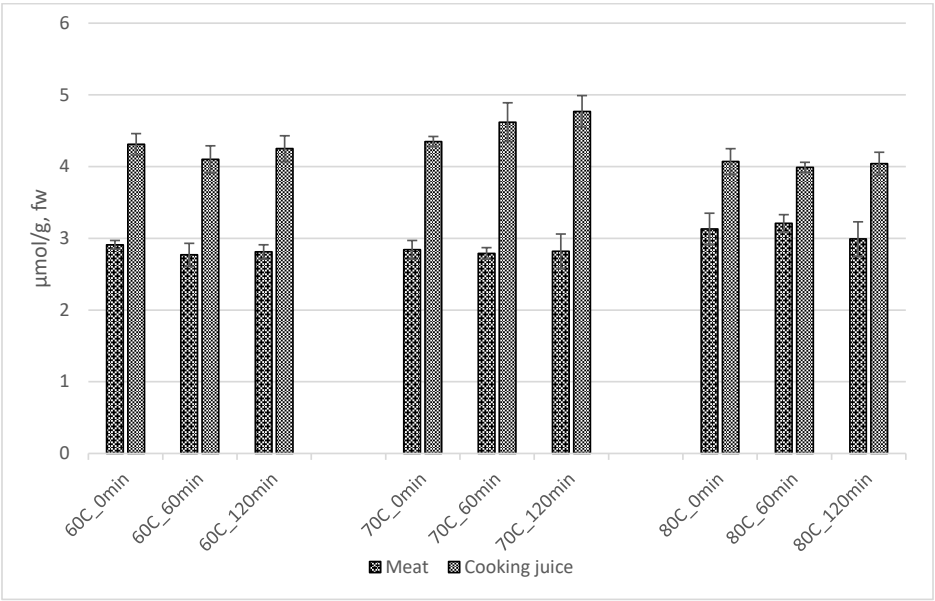

Figure 5. Mean values (n=4) of 5′-IMP concentrations in the cooked meat and meat juices (µmol/g) at three different temperatures and cooking times. There were no statistically significant ($p \leq 0.05$) differences between cooking times or cooking temperatures.

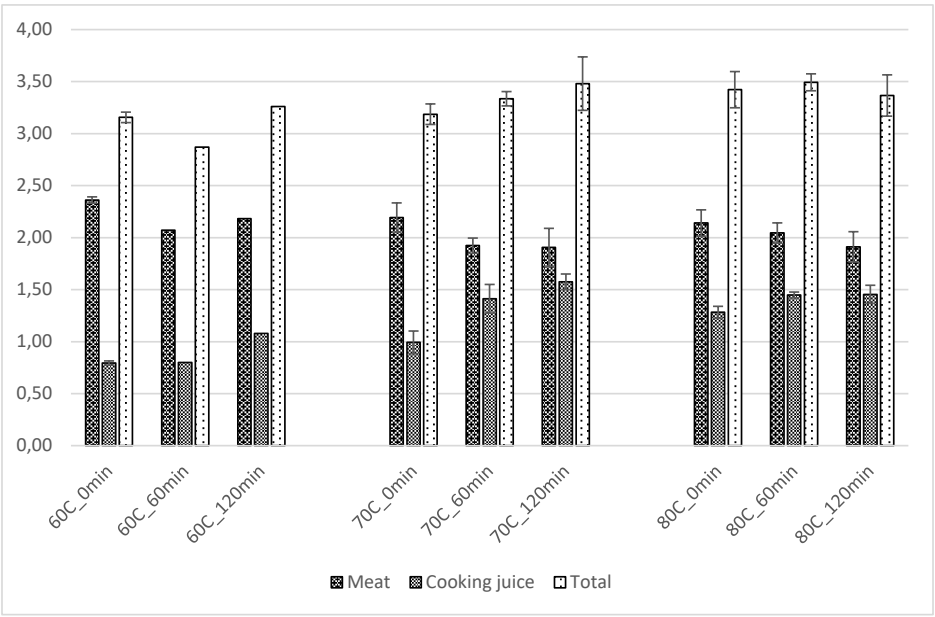

Figure 6. Content of 5′-IMP in one gram of total meat sample calculated by using the relative proportions of meat and juice (%, w/w) in one gram of the total sample multiplied by their concentrations in µmol/g.

Although in our study, neither the cooking time nor the temperature affected the concentration of 5′-IMP in meat or juice, the earlier studies of Arya & Parihar, 1979 and Madruga et al. 2010, where the cooking juice was not analyzed, the concentration of 5′-IMP in meat was reported to have decreased as a result of heating. Moreover, concentration of the degradation products of 5′-IMP, inosine and hypoxanthine, has been stated to vary due to heat treatment (Arya & Parihar, 1979). In the study of Arya & Parihar (1979), after a cooking time of 30 mins the 5′-IMP content in cooked goat meat (0.82 - 2.03 μmol/g) and cooked sheep meat (0.91-2.32 μmol/g) were lower than in raw goat meat (2.58 μmol/g) and in raw sheep meat (3.10 μmol/g) (no statistics represented). In addition, a simultaneous increase in the concentrations of inosine and hypoxanthine were observed in goat and sheep meats during cooking (Arya & Parihar, 1979). Madruga et al. (2010) reported a statistically significant decrease in the 5′-IMP and inosine concentrations of grilled (80 °C) goat meat, 66% of 5′-IMP and 75% of inosine remaining when grilled (**Table 2**). However, in the research of Sasaki et al. (2007), where cooking broth was analyzed separately, increased levels of total 5′-IMP were reported in the early stages of moist heat cooking at 95 °C, while after 10 min the concentrations remained unchanged, although the concentration in meat decreased and in cooking broth increased. In the study of Cambero et al. (2000), where only broth was analyzed, an enhanced movement of umami nucleotide 5′-IMP from meat to the broth was observed, when the cooking temperature was increased. The researchers also found that the cooking temperature had a greater effect on concentrations than the cooking time Cambero et al., 2000).

The unchanged concentration of 5′-IMP in our study might indicate a similar deactivation of the 5′-IMP degrading enzyme at all three cooking temperatures 60, 70 and 80 °C. It can be assumed that the decomposing enzyme was inactivated during the time the temperature in meat reached the temperature of the water bath, suggesting the lowest temperature 60 °C was also sufficient for deactivation. According to Ishiwatari et al. (2013) a temperature of 64.1 °C was needed to completely inactivate the enzyme decomposing 5′-IMP. The researchers also reported the decomposition rate of 5′-IMP to be strongest at a cooking temperature of 40 °C, while 5′-IMP concentration was constant when beef was cooked at temperatures 65 and 70 °C, indicating enzyme deactivation. Similarly, in the study of Arya & Parihar (1979), a lower concentration of 5′-IMP in meat cooked at 60 °C and 80 °C compared to higher temperatures 100 and 120 °C, and simultaneous increase of inosine and hypoxanthine, indicated a slower inactivation of the IMP-degrading enzyme at lower temperatures. Furthermore, the decrease in 5′-IMP at 120 °C might, according to Arya & Parihar (1979), be due to temperature-induced degradation. The rate of the heat transfer also affects to the concentrations of umami compounds in food. If the

temperature rises sharply throughout the whole raw material, the enzyme activity will discontinue as well. In the study of Ishiwatari et al. (2013), the amount of 5′-IMP at the surface region of meat was much higher than in the interior of meat, indicating the inactivation of the degradation enzyme of 5′-IMP on the surface region at the very beginning of the cooking process.

The proportion of nucleotide 5′-AMP in our study was low compared to the content of 5′-IMP, even though the 5′-AMP concentration in meat increased with increased cooking temperature. Likewise, Arya & Parihar (1979) and Madruga et al. (2010) have reported an increase in 5′-AMP concentration in meat due to the effect of increasing heat. In the study of Madruga et al. (2010), a significant increase ($p<0.001$) from raw (0.01 umol/g) to grilled (2.51 umol/g) goat meat was observed. Similarly, in the study of Arya & Parihar (1979), an increase in 5′-AMP concentrations was found during the first 30 min of cooking in goat and sheep meats at all four cooking temperatures (60, 80, 100, 120 °C) compared to raw meat concentrations (**Table 2**). An increased release of 5′-AMP depending on increased heat was also observed in the study of Cambero et al. (2000), where 5′-AMP was detected only in broth cooked at a temperature of 65 °C or higher. Macy et al. (1970) also reported that the concentration of 5′-AMP in lamb meat doubled during the first 30 min of heating at 60 °C, following a decrease after 45 and 60 min. The apparent reason for the increase of 5′-AMP at the beginning of cooking was probably due to the presence of 5′-AMP precursors ATP and ADP in the tissue and their hydrolysis during the first minutes of heating (Arya & Parihar, 1979; Macy et al., 1970). When ATP and ADP were depleted, the concentration of AMP also began to decrease in subsequent heating (Arya & Parihar, 1979; Macy et al., 1970).

5.2 Effect of cooking temperature on umami compounds in mushrooms

Despite the fact that the concentrations of quantified umami compounds in *A. bisporus* cooking series were highest at cooking temperatures from 70 to 80 °C, the highest test temperature of 90 °C was chosen to be used for the processing of forest mushroom species. This was done to minimize the negative effects of the expected enzymatic activity in umami compound concentrations, and especially to deactivate the umami nucleotide degrading enzymes by using high enough cooking temperature, when cooking time was relatively short. In addition, mushrooms cooked at 90 °C had a similar content of glutamic acid compared to mushrooms cooked at 70 and 80 °C. Fresh *A. bisporus* is known to be very sensitive to enzymatic browning, leading to surface discoloration (Czapski & Szudyga, 2000). In our research, when air-tight SV pouches were opened after the cooking period of 10 minutes, the color changes were notable in the slices

cooked at 60 °C and 70 °C. This phenomenon indicated at least partial enzymatic activity still remaining in the mushrooms, suggesting the use of higher cooking temperatures with such a short cooking time.

5.2.1 Effect of cooking on free amino acids

The total FAA content of *A. bisporus* gradually decreased in mushrooms during the cooking series as the cooking temperature increased (Study II, Table 2). The highest concentration of total FAAs was measured in fresh mushrooms (1060 g/ 100g, fw) and the lowest from both, SV 90 °C cooked mushrooms (609 mg/100g, fw) and the corresponding juice (554 mg/100g, fw); with the total FAA concentration of mushroom and corresponding cooking juice being at the same level. A similar total FAA content was found for cooked mushrooms at 70, 80 and 90 °C and their corresponding juices (Study II, Table 2). A 43% decrease from fresh to a 90 °C SV cooked mushroom indicates equal distribution of amino acids between mushrooms and cooking juice at all three cooking temperatures.

The decrease in umami FAA content in *A. bisporus* mushrooms during cooking was significant but smaller than that of total FAAs. The highest concentration of glutamic acid was in the fresh (123 mg/100g) and the 60 °C cooked mushroom (127mg/100g) and corresponding juice (123mg/100g) and lowest in mushrooms cooked at 80 and 90 °C (102-104 mg/100g, fw) (Study II, Table 2), (**Figure 7**). For aspartic acid, the highest concentration was measured in fresh mushrooms and those cooked at 80 °C cooked. Glutamic acid concentration decreased by 15.4% from fresh (123 mg/100g fw) to SV 90 °C cooked mushrooms (104 mg/100g fw), and the L-aspartic acid concentration by 9.2 % from fresh mushrooms (75.1 mg/100 g, fw) to SV 90 °C cooked mushroom (68.2 mg/100g, fw)) (**Figure 7**).

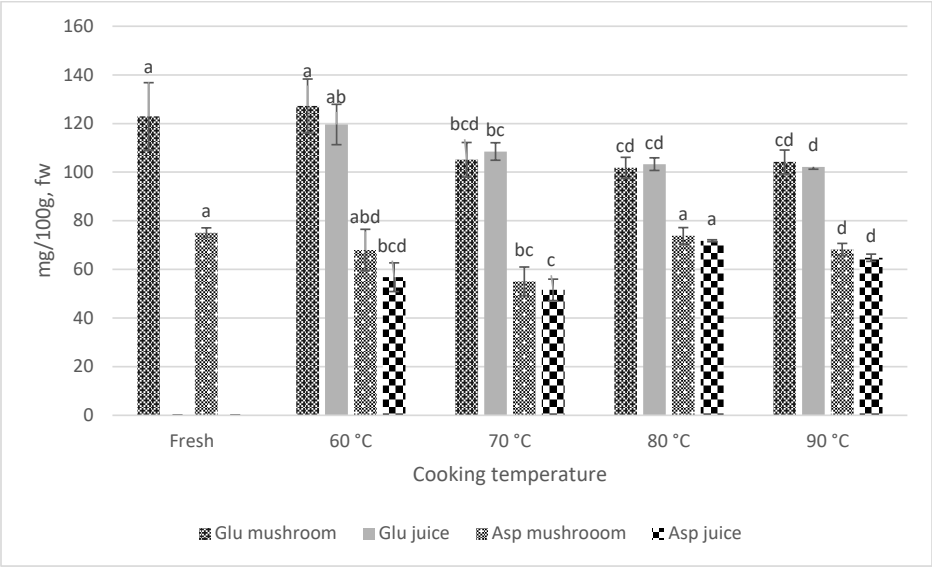

Figure 7. Glutamic and aspartic acid concentrations (mg/100g, fw) in *A. bisporus* cooking series of fresh and at 60, 70, 80 and 90 °C temperatures with a 10 min cooking time. Mean values (n=3). Significant differences ($p \leq 0.05$) are marked with the letters a-d. Different letters indicate statistically significant differences.

There were no statistically significant differences in the concentration of glutamic or aspartic acids in *A. bisporus* between the mushroom and its corresponding juice at any of the cooking temperatures used (**Figure 7**). Since the concentration of glutamic acid decreased in both the mushroom and juice as the temperature increased, the glutamic acid concentration of the total sample also decreased.

In our study, the umami FAA content of fresh *A. bisporus* was in accordance with the study of Li et al. (2019), Poojary et al. (2017) and Tseng & Mau (1999), while concentrations reported by Ranogajec et al. (2010) were significantly lower (**Table 2**). The sum of aspartic and glutamic acid, 25.6 mg/g (dw) in *A. bisporus* after three days of storage reported by Tseng & Mau (1999), 25.2 mg/g (dw) for non-treated *A. bisporus* in the study of Li et al. (2019), and 16.4 mg/g (dw) for white champignons (*A. bisporus*) in the study of Poojary et al. (2017), were on the same compared to concentration in our study (26.1 mg/g, dw). The sum of umami FAAs, 1.75 mg/g (dw), reported by Ranogajec et al. (2010) was measured from wild *A. bisporus*, not the cultivated one, explaining the significantly lower concentration.

Furthermore, the sum of the 15 FAAs in our study was 108 mg/g (dw) three days after harvesting, and this was at the same concentration level compared to the concentration of Tseng & Mau (1999), 101 mg/g (dw) of *A. bisporus,* three days after storage. If divided into taste groups (**Table 1**), the contents of sweet (34.9), bitter (41.6) and tasteless (5.79 mg/g, dw) FAAs in our research were in

accordance with the corresponding concentrations of sweet (35.3), bitter (33.2) and tasteless (7.3 mg/g (dw) FAAs in the study of Tseng & Mau (1999).

Based on the results of *A. bisporus*, the forest mushrooms were all cooked at 90 °C for 10 minutes. A considerable variation in total FAA and umami FAA concentrations of forest mushrooms between the four different species and between the fresh and cooked mushrooms of the same species was observed. Among the forest mushrooms, the highest concentration of total FAAs was in fresh *S. variegatus* (642 mg/100g) and the lowest in fresh *C. tubaeformis* (117 mg/100g) (Study II, Table 5). Compared to the total FAA content of fresh, cultivated *A. bisporus* (1060 mg/100g), the total FAA content of fresh forest mushrooms was much lower.

There were no statistically significant differences in the concentrations of glutamic and aspartic acids between SV 90 °C cooked mushroom and corresponding cooking juice in any of the five mushroom species (Study II, Table 5), indicating equal distribution of compounds between mushroom and juice. The highest concentration of glutamic acid in forest mushrooms was observed in *S. variegatus*, both in the fresh (67.5 mg/100g, fw) and those cooked at 90 °C SV (64.9 mg/100g, fw) (**Figure 8**). Similarly, the concentrations of aspartic acid in *S. variegatus* remained at the same level between fresh (17.4 mg/100, fw) and cooked mushroom (18.0 mg/100g, fw) (Study II, Table 5). However, compared to the cultivated *A. bisporus* cooked at 90 °C, the content of glutamic acid was 38% and the aspartic acid 74% lower in the cooked (90 °C SV) forest mushrooms *S. variegatus*.

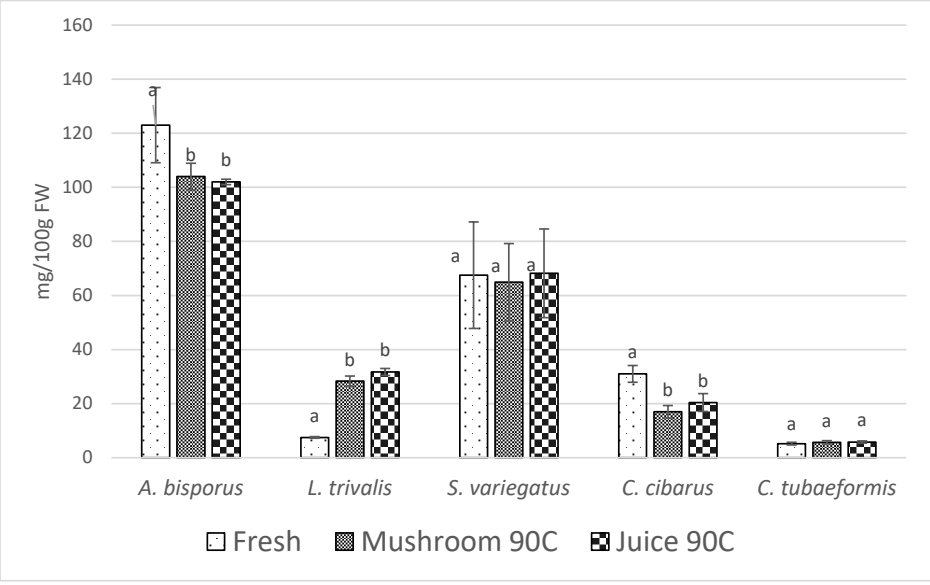

Figure 8. Glutamic acid (mg/100g, fw) in fresh and cooked (90 °C, 10 min) mushrooms and cooking juice in five mushroom species. Mean values (n=3). Significant differences ($p \leq 0.05$) are marked with the letters a-b. Different letters indicate statistically significant differences.

Cooking affected the FAA concentrations of different mushroom species in different ways. The most noticeable increase in glutamic acid concentration caused by cooking was seen in *L. trivialis*, where the concentration increased 2.8 fold from fresh (7.5 mg/100g, fw) to cooked SV90 °C mushroom (28.3 mg/100g fw**) (Figure 8).** Similarly, aspartic acid concentration increased 4.8 fold from fresh (6.5 mg/100g, fw) to SV90 °C cooked *L. trivialis* mushroom (37.8 mg/g, fw) (Study II, Table 5). The most noticeable decrease in the glutamic acid concentration was seen in the case of *C. cibarius*, where the concentration in cooked mushroom (17.0 mg/100g, fw) was 45% lower than that of the fresh ones (31.0 mg/100g, fw). Correspondingly, the aspartic acid content decreased by 38% between fresh (9.7 mg/100g, fw) and cooked (6.0 mg/100g, fw) *C. cibarius*. In *C. tubaeformis*, no change was seen in the concentration of glutamic acid (5.2-5.7 mg/100g, fw) and a decrease in the content of aspartic acid (7.4 – 12.2 mg/ 100g, fw) between fresh and cooked mushroom, but the concentrations were relatively low (**Figure 8**).

The reported FAA contents of *C. cibarius* vary considerably between different studies (Beluhan et al., 2011; Manninen et al., 2018; Ranogajec et al., 2010). In our research, glutamic and aspartic acid concentrations in fresh *C. cibarius* were 3.6 and 1.1 mg/g (dw), respectively, which were at the same level as the concentrations of 3.8 and 1.3 mg/g (dw) reported by Manninen et al. (2018). However, Beluhan et al. (2011) reported notably higher concentration of glutamic acid 30.0 mg/g (dw) and lower concentration of aspartic acid 0.06 mg/g (dw) for *C. Cibarius*, while Ranogajec et al. (2010) reported concentrations of 1.5 and 0.29 mg/g (dw), respectively. In the study of Manninen et al. (2018), the reported glutamic and aspartic acid contents for *C. tubaeformis*, 1.65 mg/g (dw) and 1.92 mg/g (dw), were slightly higher than the contents 1.1 and 0.75 mg/g (dw) in our study. For the other two forest mushrooms included in our study, *L. trivialis* and *S. variegatus*, no comparative results were found in the literature.

The effect of cooking on FAA content vary between mushroom studies with different cooking times and temperatures. In the research of Zhou et al. (2017), a short 15 minutes boiling of the fruiting body of *Tricholoma lobayense* did not affect the total content of FAAs or the content of glutamic acid (**Table 3**). The concentration of aspartic acid, on the other hand, increased slightly as a result of cooking, compared to a control sample that was not heated. However, a significant 32% decrease in the level of total FAAs, as well as 20% decrease in glutamic acid levels was observed in *A. bisporus* mushroom soup as a result of cooking for 92 min at 60 °C(Li et al. 2011). Glutamic acid concentration decreased from 21.1 to 17.0 mg/g, dw and aspartic acid from 3.95 to 1.42 mg/g, dw, respectively, compared to control material not heated (Li et al. 2011).

The relative proportion of umami FAAs compared to other free amino acids varied in the studied mushroom species in our study. Glutamic and aspartic acids

were among the five most abundant FAAs both in fresh and SV 90 °C cooked *A. bisporus* mushrooms (Study II, Table 2). In forest mushrooms, umami FAA concentrations were not so abundant.

5.2.2 Effect of cooking on nucleotides

In the cultivated material *A. bisporus,* concentrations of 5′-nucleotides and corresponding nucleosides varied throughout the cooking series at different temperatures (60, 70, 80 and 90 °C) and compared to the fresh mushroom (Study II, Table 1). The concentration of total 5′-nucleotides and umami nucleotides 5′-GMP and 5′-AMP increased *in A. bisporus* as a result of cooking. In our research, heat treatment was needed for the appearance of umami nucleotide 5′-GMP, since it was detected only in mushroom samples cooked at 70 °C and above, but the concentration was negligible in the fresh mushroom. The most predominant nucleotide in raw and cooked *A. bisporus* mushrooms, 5′-CMP, is not known to have umami taste enhancing properties, while/on the other hand umami nucleotide (umami taste enhancing) 5′-IMP was not detected in any of the samples.

The concentration of total 5′-nucleotides and 5′-GMP in *A. bisporus* were highest in the mushroom samples processed at 70 °C (Study II, Table 1), while mushrooms cooked at 80 °C had 18% and at 90 °C 22% lower content of 5′-GMP compared to mushrooms cooked at 70 °C. The highest concentration of 5′-AMP was observed in the *A. bisporus* mushrooms cooked at temperatures of 70 and 80 °C (Study II, Table 1). In general, the 5′-AMP, 5′-CMP and 5′-GMP concentrations in cooked *A. bisporus* mushrooms were higher than in the corresponding cooking juice and in fresh mushroom. This indicates the necessity of cooking to release nucleotides and might also indicate the effect of hot water extraction releasing more nucleotides from *A. bisporus* mushrooms after cooking experiment compared to released juice.

The 5′-GMP content of SV90 °C cooked *A. bisporus* mushrooms (0.57 mg/g, dw) in our study was on the same level as that of boiled *A. bisporus* (0.35 mg/g, dw) reported by Li et al. (2011). However, the 5′-GMP content was notably lower than in the study of Tseng & Mau (1999), who reported 5′-GMP concentrations of 1.5-2.5 mg/g (dw) in non cooked *A. bisporus* mushrooms. The results of Tseng & Mau (1999) are significantly different from our results, since the 5′-GMP concentration in our study was negligible in fresh *A. bisporus*. Similarly, 5′-AMP contents in fresh (0.64 mg/g, dw) and SV 90 °C cooked mushrooms (1.30 mg/g, dw) of *A. bisporus* in our study were lower than in fresh mushrooms (1.46-2.76 mg/g, dw) analyzed by Tseng & Mau (1999), but higher than in boiled *A. bisporus* (0.351 mg/g, dw) analyzed by Li et al. (2011). The contents of 5′-AMP and 5′-GMP in SV 90 °C cooked *C. cibarius* in our research were similar to the reported values for non-cooked *C. cibarius* by Beluhan et al.

(2011) and Ranogajec et al. (2010) and for cooked *C. cibarius* by Manninen et al. (2018), (**Table 3**)

The 5′-nucleotide and nucleoside concentrations of the four forest mushroom species studied varied considerably. Umami taste enhancing nucleotides in forest mushrooms were represented mainly by 5′-GMP, which was detected in all four SV 90 °C cooked mushrooms, but not in any of the fresh mushrooms, as noted with *A. bisporus* in the previous chapter (Study II, Table 4). 5′-IMP was not detected in any of the forest mushroom species. The highest concentration of total 5′-nucleotides and 5′-GMP were detected in forest mushroom *L. trivialis*, cooked at 90 °C (71.1 and 17.2 mg/100g, respectively) and corresponding juice (83.4 and 19.8 mg/100g, respectively) (Study II, Table 4) (**Figure 9**). The concentrations of total nucleotides and 5′-GMP of *L. trivialis* were even higher than those of cooked *A. bisporus* mushrooms (63.7 and 5.3 mg/100g) and juice (32.8 and 3.8 mg/100g), respectively. Similarly, the highest concentration of 5′-AMP (17.8 mg/100g, fw) was found in SV 90 °C cooked *L. trivialis*, the concentration even higher than those of cooked *A. bisporus* (12.0 mg/100g, fw) (Study II, Table 4). The other three SV 90 °C cooked forest mushrooms and juices had remarkably lower concentrations of total nucleotides (1.6-16.5 mg/100g) and total nucleosides (0.0-4.0 mg/100g).

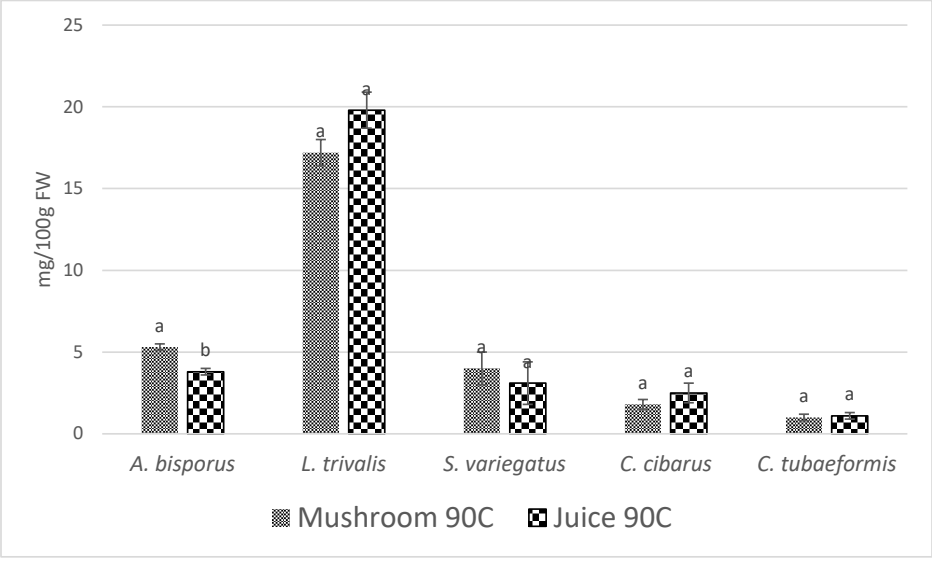

Figure 9. 5′-GMP (mg/100g, fw) in cooked (90 °C) mushrooms and the cooking juices of five mushroom species. Mean values (n=3). Significant differences ($p \leq 0.05$) are marked with the letters a-b.

5.2.3 Effect of cooking on nucleosides

In all five mushroom species, the degradation products of nucleotides, nucleosides, were present both in fresh and cooked mushrooms, but the total nucleoside content was higher in fresh mushrooms compared to cooked (90 °C) mushrooms and the corresponding juices (Study II, Table 4). The concentrations of total nucleosides were between 60.6 and 77.8 mg/100g, fw in four fresh mushroom species, only the concentration of *fresh C. tubaeformis* being significantly lower (18.5 mg/100g). Most of the total nucleosides in the fresh samples were constituted of adenosine and uridine. Although 5′-CMP was the most predominant nucleotide, concentrations of its degradation product cytidine were negligible both in fresh and cooked samples. The presence of nucleosides in fresh mushrooms indicates the degradation processes of nucleotides, and the lack of nucleosides in the cooked samples might indicate possible degradation and further reactions of nucleosides under the influence of or enhanced by temperature.

5.2.4 Classification of mushrooms according to their taste properties

Mushrooms can be classified according to their taste compounds in several different ways. According to the classification presented by Yang et al. (2001) the concentration of umami FAAs or MSG-like FAAs can be divided into three ranges; high (>20 mg/g, dw), medium (5-20 mg/g) and low (<5mg/g). Of the studied mushrooms, only fresh *A. bisporus* had a high range concentration of umami amino acids (26.1 mg/g). Cooked (SV 90 °C) *A. bisporus* and cooked *L. trivialis* together with fresh and cooked *S. variegatus* had medium concentrations of umami amino acids. In all other mushroom species, concentrations were classified as low. There is also a similar classification for umami enhancing 5′-nucleotides, the term used for compounds 5′-GMP and 5′-IMP (Yang et al. (2001). A high range concentration (> 5 mg/g, dw) of umami-enhancing 5′-nucleotides was not observed in any of the mushrooms analyzed and only in SV 90 °C cooked *L. trivialis* (1.86 mg/g, dw) was a middle range concentration (1-5 mg/g dw) measured. All the other mushrooms had a umami-enhancing 5′-nucleotide concentration in a low range. However, it should be noted that in *L. trivialis*, the proportion of aspartic acid of MSG-like FAAs was more than half in SV cooked mushroom.

Several mushroom-related publications (Beluhan & Ranogajec, 2011; Mau et al., 2001; Tsai et al., 2007; Tseng & Mau 1999; Yang et al. 2001) (**Table 1**) have used a classification in which FAAs are divided into groups based on their taste characteristics. The classification is explained in more detail in Chapter 2.2.1 and **Table 1**. When calculated according to this classification, it appeared that in the three mushroom species with the highest concentration of total FAAs, i.e. *A.*

bisporus, L. trivialis and S. variegatus, the content of bitter FAAs decreased the most during cooking compared to other taste characteristics. Moreover, the content of umami FAAs decreased the least or even increased (Study II, Table 6), (**Figure 10**). The content of bitter FAAs of *A. bisporus* decreased 70% from fresh to cooked mushroom (Study II, Table 6), **Figure 10**, while sweet FAAs decreased 52% and tasteless FAAs 50%. However, the content of umami FAAs only decreased by 13%. In the mushroom *S. variegatus*, the decrease of bitter (23%) and sweet FAAs (13%) from fresh to cooked mushroom was more notable than a 1.5% decrease in umami FAA concentration. A 3.7-fold increase in the content of umami FAAs in *L. trivialis* from fresh to cooked mushroom was remarkable with a simultaneous decrease of the concentration of bitter (31%) and sweet (29%) FAAs. In *C. cibarius,* a decrease from fresh to cooked mushroom and in *C. tubaeformis,* an increase in the concentration of all four FAA groups of taste was observed.

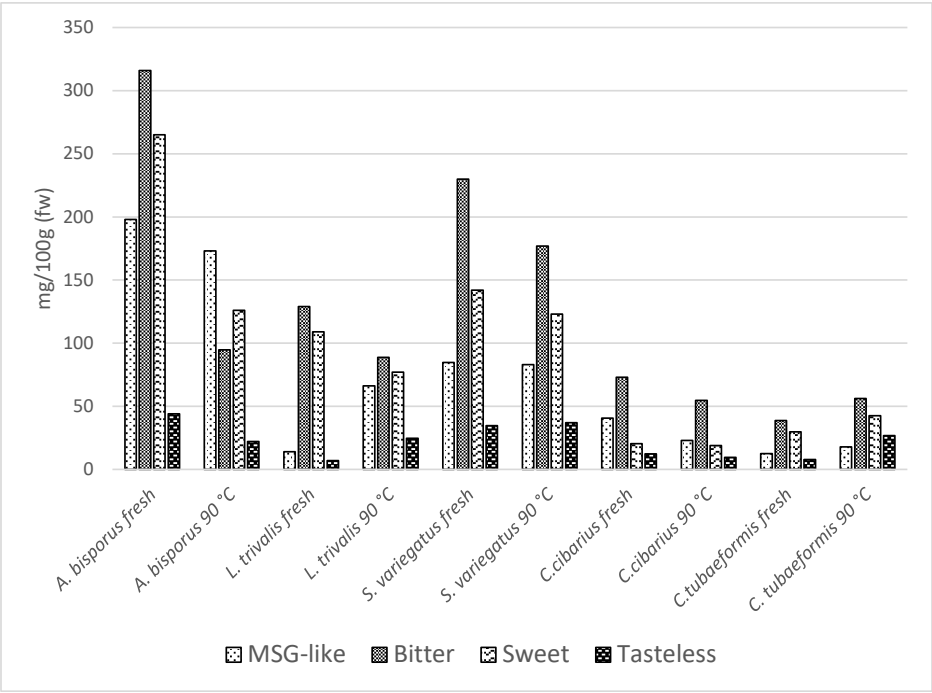

Figure 10. Taste characteristics of fresh and cooked mushrooms of five different species.

5.2.5 Equivalent umami concentration (EUC)

The synergistic interaction between umami amino acids and 5′-nucleotides was evaluated by calculating equivalent umami concentration (EUC) using the equation derived from the sensory evaluation (Yamaguchi et al., 1971). EUC

(grams of MSG per 100 g) is the concentration of MSG equivalent to the umami intensity given by a mixture of MSG and the 5′-nucleotide and is represented by the addition equation (3):

$$Y = \sum a_i b_i + 1218\left(\sum a_i b_i\right)\left(\sum a_j b_j\right) \quad (3)$$

In the equation, Y is the EUC of the mixture, a_i is the concentration of each umami amino acid (Glu or Asp), a_j is the concentration of each umami 5′-nucleotide (5´-IMP, 5′-GMP or 5′-AMP), b_i is the relative umami concentration (RUC) for each umami amino acid to MSG (Glu = 1, Asp = 0.077), b_j is the RUC for each umami 5′-nucleotide to 5′-IMP (5′- GMP = 2.3, 5′-AMP = 0.18), and 1218 is a synergistic constant. All concentrations must be in grams per 100 g (Morris et al. 2007).

Table 7. Equivalent umami concentrations (EUC) from fresh and SV90 °C cooked mushrooms of the five mushroom species.

	Fresh mushroom	SV90 °C cooked mushroom
	EUC (g/100g dw)	EUC (g/100g dw)
A. bisporus	25.5	223
L. trivialis	0.46	191
S. variegatus	20.9	67.2
C. cibarius	0.2	2.6
C. tubaeformis	0.4	10.2

In our study, the EUC values for fresh mushrooms were in the range of 0.2-25.5 g MSG/100g dry weight and for sous vide 90 °C cooked mushrooms in the range of 2.6 – 223 g MSG/100g (**Table 7**). Equivalent umami concentration (EUC) indicated cooked *A. bisporus* and *L. trivialis* had a higher umami concentration than cooked *S. variegatus* and much higher than cooked *C. cibarus* and *C. tubaeformis*. (**Table 7**). In fresh mushrooms, EUC values were low.

The range of reported EUC values vary widely. For example, EUC values for *A. bisporus* at different harvest stages varied from 204-284 g MSG /100g (Tsai et al., 2007), while EUC value 11.75 g MSG/100g was calculated for wild *A. bisporus* by Ranogajec et al. (2010). Similarly, Beluhan & Ranagajec, (2011) reported EUC value 248.58 g MSG/100g for *C. cibarus* while a much lower value, 9.36 g MSG/100g was calculated by Ranogajec et al. (2010).

5.3 Challenges in optimizing umami taste during cooking

The partly reverse behavior of umami compounds, FAAs and 5′-nucleotides, during cooking brings challenges to optimizing the umami taste in meals. In addition to cooking studies of mushrooms, the changes in the concentration of umami compounds due to an increase in temperature have been investigated in the extraction process studies optimizing the content of umami compounds (Dermiki et al., 2013; Poojary et al., 2017). These extraction studies support the idea of the cooking studies that the concentration of FAAs mainly decreases as a result of cooking while extraction of certain nucleotides require heat. In the extraction studies of Dermiki et al. (2013) and Poojary et al. (2017), the best recovery of FAAs for cultivated mushrooms *A. bisporus* and *L. edodes* were obtained when the extraction temperature was kept relatively low, i.e. close to room temperature and with long extraction times; 180 min and 360 min were used (**Table 3**). In contrast, for nucleotides 5′-AMP and 5′-GMP, a more suitable extraction temperature was 70 °C compared to room temperature, since negligible concentrations of 5′-nucleotides were detected when lower extraction temperatures were used (Dermiki et al., 2013; Poojary et al., 2017). The differences in nucleotide concentrations between the tested cooking times were negligible (Poojary et al., 2017).

Additionally, in our study, all five mushroom species required cooking in order to increase the concentration of umami nucleotides. On the other hand, the FAA profile of fresh mushrooms and the effect of cooking on the levels of FAAs varied more between the studied mushroom species. Even though the cultivated mushroom *A. bisporus* had clearly the highest concentration of umami FAAs, *L. trivialis* showed the potential of forest mushrooms as a source of umami by having the highest content of 5′-AMP and 5′-GMP in cooked mushroom. There were also differences between the studied forest mushrooms, since *S. variegatus* and *L. trivialis* proved to have a higher content of umami compounds compared to species *C. tubaeformis* and *C. cibarus*. The different umami profiles of mushroom species should be taken into account when using them in cooking. Based on our results, *L. trivialis* with its high nucleotide content could be used in meals together with another raw material containing glutamate to achieve a synergistic interaction and a rich umami taste sensation. Instead, *S. variegatus* and especially *A. bisporus* offer plenty of glutamate as such, e.g. to vegetarian dishes.

In our pork meat study, the opposite trend of umami FAAs and 5′-nucleotides due to cooking was not as clear as with mushrooms. Relatively uniform concentrations of both glutamate and 5′-IMP were present at all cooking temperatures. To optimize the synergistic effect of glutamate and nucleotide 5′-

IMP in meat, the 5′-IMP degrading enzyme should be rapidly deactivated at a sufficiently high temperature.

5.4 Free amino acids in potato tubers

The taste of cooked potato is perceived mainly as mild and pleasant, which is why it is one of the most popular vegetables in the world. The presence of umami compounds as a source of pleasant taste for cooked potato has been emphasized by Solms (1971), Solms & Wyler (1979) and Morris et al. (2007). According to Morris et al. (2007) glutamate together with 5′-nucleotide 5′-GMP are the most important sources of the umami taste in potatoes. However, the 5′-GMP content in raw potato tubers is negligible, as most of the 5′-GMP has been found to be formed during cooking (Solms, 1971; Morris et al., 2007). Therefore, 5′-nucleotides of raw potato tubers have not been analyzed in our study.

Due to the limitations of temperature and frost conditions, potato cultivation in Finland is possible only in summer, under long day (LD) conditions. However, most of the potato cultivars grown in Finland have been bred in Central Europe, where the potato production conditions, including the short day (SD) length, differ from those in Finland. The emphasis in potato breeding programs have traditionally been mostly on tuber yield, processing quality as well as disease resistance (Morris & Taylor, 2019). Less attention has been paid to taste, other sensory quality and nutritional value of tubers. However, growing consumer demand has directed the focus of breeding towards this direction as well (Drapal et al., 2023). Therefore, it is important to examine the possible effect of day length on the concentrations of potato flavor compounds.

In our study, the tubers grown in short day (SD) conditions displayed a higher concentration of free amino acids than tubers in long day (LD) conditions. The total concentration of FAAs in the studied cultivars ranged from 657 to 1118 mg/100g (FW) in SD and from 484 to 763 mg/100g in LD treatment (**Table 8)**. Thus, within the same cultivar, the sum of FAAs were 15-46% lower in LD tubers than in SD tubers. The concentration of total FAAs in tubers of the seven cultivars in our study, 484-1118 mg/100g fw, was somewhat lower than that previous research results 1110-1878 mg/100g fw of Brierley et al. (1997).

Table 8. Free amino acid concentrations (mean ± standard deviation of three technical replicates) of potato tubers as mg $100g^{-1}$ fresh weight as a result of short day (SD) and long day (LD) treatments. Table modified from Table 3 in Publication III.

Cultivar	Jazzy		Rock		Jussi	
Treatment	SD	LD	SD	LD	SD	LD
Aspartic acid	37.9 ± 1.2	39.1 ± 0.4	47.1 ± 1.9	43.4 ± 3.4	32.7 ± 0.7	36.4 ± 0.1
Glutamic acid	30.3 ± 0.9	13.7 ± 0.3	44.8 ± 1.9	28.7 ± 2.0	45.3 ± 0.8	31.5 ± 0.4
Asparagine	425 ± 11	264 ± 2	202 ± 7	175 ± 16	378 ± 8	241 ± 1
Glutamine	243 ± 7	149 ± 2	153 ± 6	100 ± 9	308 ± 7	191 ± 0.4
Sum of 19 FAAs	1062	718	657	484	1118	727

Cultivar	Gala		Soraya		Jessica		Annabelle	
Treatment	SD	LD	SD	LD	SD	LD	SD	LD
Aspartic acid	56.0 ± 0.5	65.5 ± 4.6	52.8 ± 2.7	77.5 ± 1.7	41.4 ± 2.3	50.7 ± 1.2	41.4 ± 1.0	49.4 ± 0.8
Glutamic acid	31.2 ± 0.5	26.0 ± 3.6	28.1 ± 2.0	22.2 ± 1.0	34.0 ± 2.0	27.9 ± 0.9	26.5 ± 0.2	22.4 ± 0.1
Asparagine	304 ± 3	259 ± 18	263 ± 15	183 ± 4	237 ± 13	223 ± 6	353 ± 8	174 ± 3
Glutamine	284 ± 3	136 ± 9	215 ± 12	97.9 ± 2.3	168 ± 9	115 ± 3	157 ± 3	58.3 ± 0.5
Sum of 19 FAAs	922	763	856	618	735	622	907	496

FAA = free amino acid

The most abundant FAA in all seven cultivars was asparagine and the second-most abundant FAA glutamine, these constituted 28-40% (w/w) and 11-30% (w/w) of the total free amino acid pool, respectively. The other predominant amino acids both in SD ja LD tubers were arginine, aspartic acid, glutamic acid, GABA, lysine and valine, with some cultivar-dependent variations. In the long day (LD) treatment, the free asparagine concentrations in the tubers were 6-51% and the glutamine concentrations were 31-63% lower than in the SD treatment. The predominance of these FAAs were supported by the findings of Elmore et al. (2007) and Eppendorfer & Bille (1996), who showed asparagine as typically accounting for at least 30% of the total content of FAAs in fresh potato tubers. Due to the high content of asparagine and glutamine, the percentual proportions of other FAAs regarding the total amount of FAAs were relatively low. Thus, the umami FAAs, glutamic and aspartic acids comprised only 5-15% (w/w) of the total FAA pool.

The statistical analysis was performed by comparing the means of the two treatments (SD and LD) for every FAA by using different cultivars as replicates. Our research results showed a clear difference between the two photoperiodic treatments with different DLIs, resulting in differences in the concentration of FAAs in potato tubers. In 14 of the 19 FAAs, the concentrations were significantly lower in LD tubers than in SD tubers except for aspartic acid, which had significantly higher content in LD tubers (**Table 9**). As with most of the FAAs, also glutamic acid concentration was 15-55% lower in LD tubers compared to SD tubers. The average glutamic acid concentration was 24.6 mg/100g in LD tubers and 34.3 mg/100g in SD tubers (**Table 9**). The concentration of aspartic acid was exceptional compared to other proteinogenic amino acids, as in six of the seven cultivars its concentration was 3-47% higher in LD tubers than in SD tubers. The average aspartic acid concentration was 51.7 mg/100g in LD tubers and 44.2 mg/100g in LD tubers (**Table 9**).

The concentrations of aspartic and glutamic acids in our study were at the same level as that reported by Aisala et al. (2016). Aisala et al. (2016) analyzed glutamic acid concentration as being 19 mg/100g in uncooked potato, while Brierley et al. (1996) reported concentrations of 15-132 and 8-139 mg/100g for the cultivars Pentland Dell and Record, respectively.

Table 9. Free amino acids of potato tubers grown in SD and LD photoperiods. Average of seven potato cultivars (with three technical replicates each), in mg $100g^{-1}$ (FW). Table modified from Table 4 in Publication III.

Compound	SD mean ± sd	LD mean ± sd	Treatment *p* value
FAA			
Alanine	6.9 ± 1.9	5.4 ± 1.0	0.004
Arginine	61.1± 16.5	38.0 ± 11.9	<0.001
Asparagine	309± 77	217 ± 38	<0.001
Aspartic acid	**44.2± 8.0**	**51.7 ± 14.2**	**0.043**
GABA	32.8± 10.4	37.3 ± 8.7	-
Glutamic acid	**34.3± 7.4**	**24.6 ± 5.7**	**<0.001**
Glutamine	218± 60	121 ± 41	<0.001
Glycine	1.7± 0.6	1.1 ± 0.6	0.002
Histidine	13.7± 5.1	10.5 ± 2.8	0.018
Isoleucine	20.1 ± 5.8	14.1 ± 3.9	<0.001
Leucine	8.3 ± 3.3	6.3 ± 2.7	0.037
Lysine	26.8 ± 9.2	18.1 ± 8.3	0.003
Methionine	13.8 ± 5.0	8.5 ± 2.8	<0.001
Phenylalanine	15.0 ± 12.5	10.2 ± 7.4	-
Serine	15.4 ± 4.0	11.5 ± 2.9	0.001
Threonine	11.1 ± 2.5	8.6 ± 1.4	<0.001
Tryptophan	7.9 ± 7.7	7.6 ± 7.0	-
Tyrosine	13.5 ± 4.8	11.5 ± 4.3	-
Valine	40.1 ± 8.9	29.4 ± 7.4	<0.001

SD = short day, LD = long day, sd = standard deviation.

The possible difference between SD and LD groups within the cultivars was investigated by performing a principal component analysis (PCA) using concentrations of the 19 analyzed free amino acids (**Table 9**). Sugars, which are described in more detail in Publication III, were also included in this analysis and represented in **Figures 11 and 12**. Altogether 69% of the variability of the analyzed properties were explained by the components PC1 and PC2 (**Figure 11**). SD treatment of each cultivar was always to the right of the LD treatment in the direction of PC1, indicating the distinction between the groups. In the correlation loading plot (**Figure 12**), glucose, sucrose and aspartic acid were located to the left of the PC1 axis. In contrast, asparagine, glutamine, leucine, isoleucine, methionine, valine, histidine and serine were located to the right along the PC1 axis, correlating highly positively with each other but having a highly negative correlation with glucose, sucrose and aspartic acid.

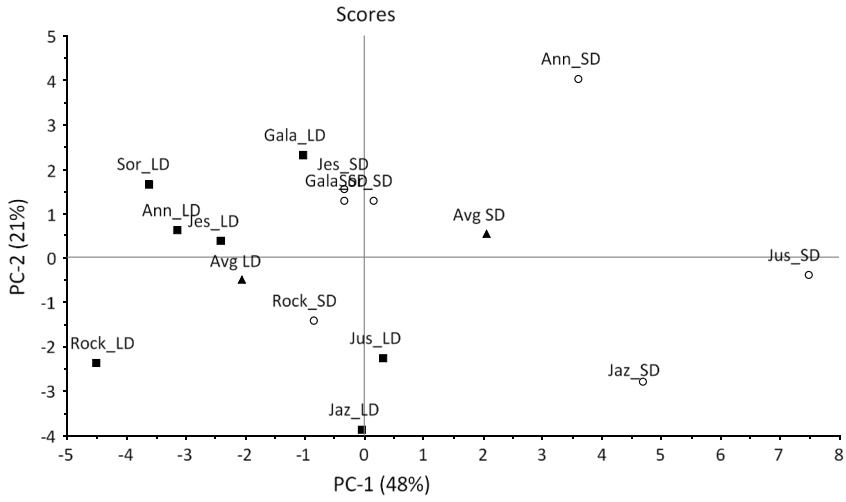

Figure 11. PCA Scores plot. The SD ("8h") photoperiod samples are marked with open circles and LD ("15h") photoperiod samples are marked with squares. The averages of all SD ("Avg SD") and LD ("Avg LD") samples are marked with triangles in the figure. (Figure 1 in Publication III, reprinted)

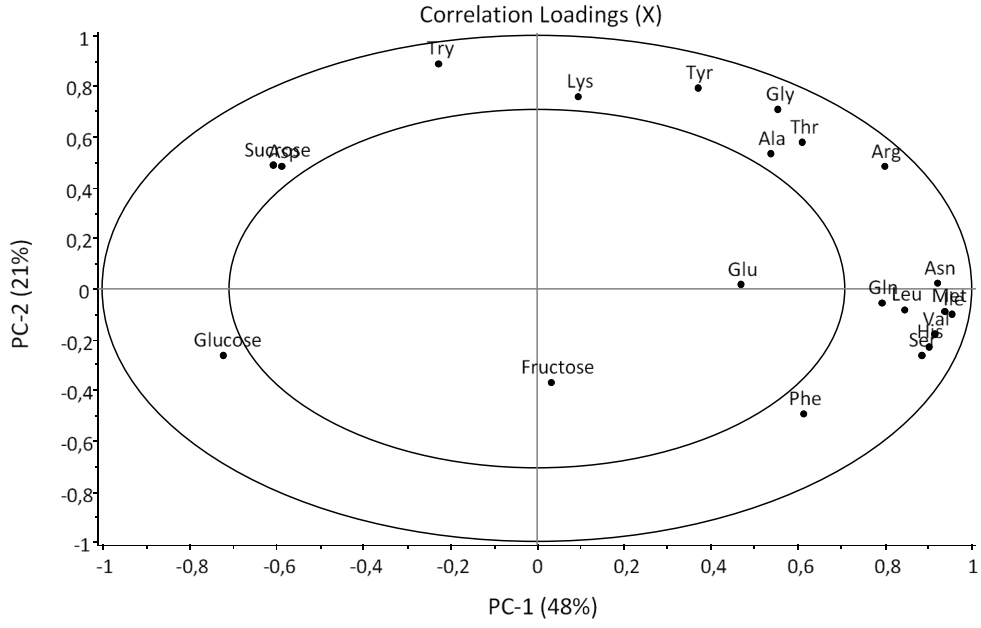

Figure 12. PCA Correlation Loadings plot. Three sugars and 19 free amino acids are presented as variables. (Figure 2 in Publication III, reprinted).

In the correlation loadings plot (**Figure 12**), sucrose, glucose and aspartic acid are located to the left along the PC1 axis, correlating (highly) positively with each other. In contrast, the FAAs, especially asparagine, glutamine, leucine, isoleucine, methionine, valine, histidine and serine are located to the right along the PC1 axis, correlating highly positively with each other and having a highly negative correlation with compounds on the left of the axis. The compounds located in the opposite directions of the PC1 axis explain the differences between the results of the SD and LD treatments. In **Figure 12**, glutamic acid is located inside the inner ellipse and therefore does not explain the variation as strongly as the amino acids mentioned above. Based on the results of the PCA analysis, it can be estimated that there are differences in the FAA concentrations between the potato tubers of the same cultivar grown under SD and LD conditions.

Several researchers have found variation in the content of taste- and health related components between plant crops cultivated at high latitudes compared to those grown further South (Hårdh & Hårdh 1977; Rosenfeld et al., 1997; Zheng et al., 2009). Studies have also been conducted under controlled growth conditions with contrasting day lengths and temperatures (Mølmann et al., 2018; Steindal et al., 2013). In addition to DLI and daily mean temperature, the differences in the quality of light source used, might also affect the concentration of primary and secondary metabolite profiles in the tubers of potato cultivars (Paradiso et al., 2019). Even though the concentrations of individual compounds alone do not explain the taste or flavor, the differences in FAA content can affect potato quality in multiple ways. Higher FAA content in tubers might indicate a favorable umami taste but also contribute to an unpleasant bitter taste. The presence of reducing sugars and FAAs, especially asparagine, are known to participate in the complex set of the Maillard reactions, giving color and aroma to the product but also enabling the formation of carcinogen acrylamide in favorable conditions as well.

6 SUMMARY AND CONCLUSION

Multiple factors affect the levels of umami compounds in food raw materials and in cooked products or meals. In our research, the factors considered were limited to processing conditions including the effect of cooking time and cooking temperature and the growth conditions i.e. photoperiodic treatment or day-length conditions. The potential differences in taste compound concentrations between different mushroom species was also evaluated. According to our research results and the literature review, the cooking temperature has been shown to have a greater affect on the content of umami compounds than the cooking time. The changes in the concentration of taste compounds during cooking are influenced by e.g. enzyme activity, further chemical reactions and structural changes to the food raw material.

During cooking, several chemical and enzymatic reactions take place at the same time. Therefore, optimizing the cooking conditions to maximize the desired properties, such as the synergistic interaction of free glutamate and 5′-nucleotides, is challenging. The effect of cooking on umami FAA and nucleotide concentrations varied depending on the raw material. An important finding was the role of the liquid released from the raw material during cooking. A separate analysis of the cooked material and the released cooking juices in sous vide cooked food showed changing concentrations and relative proportions of umami compounds when the cooking temperature and time changed.

In the pork loin study, an increase in the cooking temperature increased the amount of umami FAA and glutamic acid in the cooking juice. In further studies, the concentrations of FAAs in raw meat should also be analyzed. In the mushroom study, the variation in the concentration of umami FAAs in fresh mushrooms between different species was clearly seen. In addition, the effect of cooking on the umami FAA concentrations in different mushroom species varied by either decreasing or increasing the concentration.

Cooking had partly different effects on nucleotide concentrations in the two different types of raw materials. In meat and in the released cooking juice, the concentration of the dominating nucleotide 5′-IMP did not change due to the effect of cooking time or temperature. However, in mushrooms, the concentration of 5′-GMP, increased as the temperature increased. According to our study, the 5′-AMP concentration increased both in meat and mushrooms as a result of cooking.

The growth conditions i.e. day length, were found to have an effect on FAA content of potato tubers. In general, FAAs including glutamic acid were significantly higher in short day (SD) conditions compared to long day (LD) conditions. As a result, in SD conditions, the sensation of an umami taste may increase due to the increased level of glutamic acid but sweet and bitter FAAs

may increase sweetness and bitterness as well. According to the literature review, the 5′-GMP and 5′-AMP contents in potato tubers also increased as a result of cooking, indicating enzymatic activity and structural changes in tubers during cooking.

In recent years, interest in umami taste peptide research has increased, and several potential taste peptides have been isolated and identified from various food raw materials due to improved separation, purification and characterization techniques. In addition, new methods such as molecular docking are constantly being developed to evaluate the effectiveness of umami taste active compounds, umami taste enhancers and potential receptors. Moreover, an electronic tongue has become more common in sensory evaluation. Further research is needed to evaluate the umami taste sensation given by known and recently found umami compounds or umami taste enhancers and their possible synergy. There is still a considerable amount of study to be conducted, especially in the complex reactions of these compounds during thermal treatment. Therefore, research is needed on both the raw and the cooked material, including the released cooking juice. Fortunately, new technologies are now contributing to the investigations on these interesting and important but complicated research questions/topics.

The research in this thesis was limited to a chemical analysis of free amino acids and nucleotides. These analyses provided the basis for examining the factors influencing the umami taste properties of food. The relationship between the changes in the contents of umami-related FAAs and nucleotides needs to be clarified with further studies. Sensory evaluation and other related techniques provide a better evaluation the effect of these compounds and their synergy with the umami taste intensity. With these tools, food preparation processes can be improved in the future. The relatively low cooking temperatures used in our study are interesting and challenging in terms of both enzyme activity, water holding capacity and protein coagulation. To achieve the synergistic effect of umami compounds, even a lower concentration of glutamic acid and umami 5′-nucleotides might be sufficient, as long as they are released to the taste receptors at the same time. In the optimization of synergistic effect on umami compounds, the use of different raw materials naturally rich in umami can help to achieve the desired result. Thus, it is important to know the dominating umami compounds or umami taste enhancers in the raw materials and the potential reactions of compounds when heated.

Altogether, the results of this doctoral thesis demonstrated the dominant umami compounds and their behavior under different conditions in three different food raw materials. The concentrations and release of umami compounds during cooking were examined in the temperature range where many enzymatic, chemical and structural changes occur. The thesis provides a basis for continuing the research of Nordic food raw materials, some of which have

been relatively scarcely studied in terms of taste compounds. The potential of Nordic forest mushrooms and as a source of umami was established and potato tubers as a source of FAAs was demonstrated. To extent the further utilization of mushrooms and to promote the consumption of potato products, more investigation is required on other species and cultivars and the umami taste-related compounds they contain, especially under different processing conditions.

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APPENDIX: ORIGINAL PUBLICATIONS

DOCTORAL THESES IN FOOD SCIENCES AT THE UNIVERSITY OF TURKU

1. **REINO R. LINKO (1967)** Fatty acids and other components of Baltic herring flesh lipids. (Organic chemistry).
2. **HEIKKI KALLIO (1975)** Identification of volatile aroma compounds in arctic bramble, *Rubus arcticus* L. and their development during ripening of the berry, with special reference to *Rubus stellatus* SM.
3. **JUKKA KAITARANTA (1981)** Fish roe lipids and lipid hydrolysis in processed roe of certain *Salmonidae* fish as studied by novel chromatographic techniques.
4. **TIMO HIRVI (1983)** Aromas of some strawberry and blueberry species and varieties studied by gas liquid chromatographic and selected ion monitoring techniques.
5. **RAINER HUOPALAHTI (1985)** Composition and content of aroma compounds in the dill herb, *Anethum graveolens* L., affected by different factors.
6. **MARKKU HONKAVAARA (1989)** Effect of porcine stress on the development of PSE meat, its characteristics and influence on the economics of meat products manufacture.
7. **PÄIVI LAAKSO (1992)** Triacylglycerols – approaching the molecular composition of natural mixtures.
8. **MERJA LEINO (1993)** Application of the headspace gas chromatography complemented with sensory evaluation to analysis of various foods.
9. **KAISLI KERROLA (1994)** Essential oils from herbs and spices: isolation by carbon dioxide extraction and characterization by gas chromatography and sensory evaluation.
10. **ANJA LAPVETELÄINEN (1994)** Barley and oat protein products from wet processes: food use potential.
11. **RAIJA TAHVONEN (1995)** Contents of lead and cadmium in foods in Finland.
12. **MAIJA SAXELIN (1995)** Development of dietary probiotics: estimation of optimal *Lactobacillus* GG concentrations.
13. **PIRJO-LIISA PENTTILÄ (1995)** Estimation of food additive and pesticide intakes by means of a stepwise method.
14. **SIRKKA PLAAMI (1996)** Contents of dietary fiber and inositol phosphates in some foods consumed in Finland.
15. **SUSANNA EEROLA (1997)** Biologically active amines: analytics, occurrence and formation in dry sausages.
16. **PEKKA MANNINEN (1997)** Utilization of supercritical carbon dioxide in the analysis of triacylglycerols and isolation of berry oils.
17. **TUULA VESA (1997)** Symptoms of lactose intolerance: influence of milk composition, gastric emptying, and irritable bowel syndrome.
18. **EILA JÄRVENPÄÄ (1998)** Strategies for supercritical fluid extraction of analytes in trace amounts from food matrices.
19. **ELINA TUOMOLA (1999)** *In vitro* adhesion of probiotic lactic acid bacteria.
20. **ANU JOHANSSON (1999)** Availability of seed oils from Finnish berries with special reference to compositional, geographical and nutritional aspects.
21. **ANNE PIHLANTO-LEPPÄLÄ (1999)** Isolation and characteristics of milk-derived bioactive peptides.
22. **MIKA TUOMOLA (2000)** New methods for the measurement of androstenone and skatole – compounds associated with boar taint problem. (Biotechnology).
23. **LEEA PELTO (2000)** Milk hypersensitivity in adults: studies on diagnosis, prevalence and nutritional management.
24. **ANNE NYKÄNEN (2001)** Use of nisin and lactic acid/lactate to improve the microbial and sensory quality of rainbow trout products.
25. **BAORU YANG (2001)** Lipophilic components of sea buckthorn (*Hippophaë rhamnoides*) seeds and berries and physiological effects of sea buckthorn oils.
26. **MINNA KAHALA (2001)** Lactobacillar S-layers: Use of *Lactobacillus brevis* S-layer signals for heterologous protein production.
27. **OLLI SJÖVALL (2002)** Chromatographic and mass spectrometric analysis of non-volatile oxidation products of triacylglycerols with emphasis on core aldehydes.
28. **JUHA-PEKKA KURVINEN (2002)** Automatic data processing as an aid to mass spectrometry of dietary triacylglycerols and tissue glycerophospholipids.
29. **MARI HAKALA (2002)** Factors affecting the internal quality of strawberry (*Fragaria x ananassa* Duch.) fruit.
30. **PIRKKA KIRJAVAINEN (2003)** The intestinal microbiota – a target for treatment in infant atopic eczema?
31. **TARJA ARO (2003)** Chemical composition of Baltic herring: effects of processing and storage on fatty acids, mineral elements and volatile compounds.
32. **SAMI NIKOSKELAINEN (2003)** Innate immunity of rainbow trout: effects of opsonins, temperature and probiotics on phagocytic and complement activity as well as on disease resistance.
33. **KAISA YLI-JOKIPII (2004)** Effect of triacylglycerol fatty acid positional distribution on postprandial lipid metabolism.
34. **MARIKA JESTOI (2005)** Emerging *Fusarium*-mycotoxins in Finland.
35. **KATJA TIITINEN (2006)** Factors contributing to sea buckthorn (*Hippophaë rhamnoides* L.) flavour.
36. **SATU VESTERLUND (2006)** Methods to determine the safety and influence of probiotics on the adherence and viability of pathogens.
37. **FANDI FAWAZ ALI IBRAHIM (2006)** Lactic acid bacteria: an approach for heavy metal detoxification.
38. **JUKKA-PEKKA SUOMELA (2006)** Effects of dietary fat oxidation products and flavonols on lipoprotein oxidation.
39. **SAMPO LAHTINEN (2007)** New insights into the viability of probiotic bacteria.

40. **SASKA TUOMASJUKKA (2007)** Strategies for reducing postprandial triacylglycerolemia.
41. **HARRI MÄKIVUOKKO (2007)** Simulating the human colon microbiota: studies on polydextrose, lactose and cocoa mass.
42. **RENATA ADAMI (2007)** Micronization of pharmaceuticals and food ingredients using supercritical fluid techniques.
43. **TEEMU HALTTUNEN (2008)** Removal of cadmium, lead and arsenic from water by lactic acid bacteria.
44. **SUSANNA ROKKA (2008)** Bovine colostral antibodies and selected lactobacilli as means to control gastrointestinal infections.
45. **ANU LÄHTEENMÄKI-UUTELA (2009)** Foodstuffs and medicines as legal categories in the EU and China. Functional foods as a borderline case. (Law).
46. **TARJA SUOMALAINEN (2009)** Characterizing *Propionibacterium freudenreichii* ssp. *shermanii* JS and *Lactobacillus rhamnosus* LC705 as a new probiotic combination: basic properties of JS and pilot *in vivo* assessment of the combination.
47. **HEIDI LESKINEN (2010)** Positional distribution of fatty acids in plant triacylglycerols: contributing factors and chromatographic/mass spectrometric analysis.
48. **TERHI POHJANHEIMO (2010)** Sensory and non-sensory factors behind the liking and choice of healthy food products.
49. **RIIKKA JÄRVINEN (2010)** Cuticular and suberin polymers of edible plants – analysis by gas chromatographic-mass spectrometric and solid state spectroscopic methods.
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59. **SOILI ALANNE (2012)** An infant with food allergy and eczema in the family – the mental and economic burden of caring.
60. **MARKO TARVAINEN (2013)** Analysis of lipid oxidation during digestion by liquid chromatography-mass spectrometric and nuclear magnetic resonance spectroscopic techniques.
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65. **JAAKKO HIIDENHOVI (2015)** Isolation and characterization of ovomucin – a bioactive agent of egg white.
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69. **JUHANI AAKKO (2016)** New insights into human gut microbiota development in early infancy: influence of diet, environment and mother's microbiota.
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71. **LEENAMAIJA MÄKILÄ (2017)** Effect of processing technologies on phenolic compounds in berry products.
72. **JUHA-MATTI PIHLAVA (2017)** Selected bioactive compounds in cereals and cereal products – their role and analysis by chromatographic methods.
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74. **XUEYING MA (2018)** Non-volatile bioactive and sensory compounds in berries and leaves of sea buckthorn (*Hippophaë rhamnoides*)
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78. **MAIJA PAAKKI (2020)** The importance of natural colors in food for the visual attractiveness of everyday lunch.

79. **SHUXUN LIU (2020**) Fermentation with non-*Saccharomyces* yeasts as a novel biotechnology for berry wine production.
80. **MARIKA KALPIO (2020)** Strategies for analyzing the regio- and stereospecific structures of individual triacylglycerols in natural fats and oils.
81. **JOHANNA JOKIOJA (2020)** Postprandial effects and metabolism of acylated anthocyanins originating from purple potatoes.
82. **NIINA KELANNE (2021)** Novel bioprocessing for increasing consumption of Nordic berries.
83. **NIKO MARKKINEN (2021)** Bioprocessing of berry materials with malolactic fermentation.
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91. **AMRUTA KULKARNI (2023)** Effect of omega-3 deficiency and positional distribution of docosahexaenoic acid in triacylglycerols on tissue lipids in rats.
92. **LIZ A. GUTIÉRREZ QUEQUEZANA (2023)** Effect of cultivar, growth environment and developmental stage on phenolic compounds and ascorbic acid in potato tubers grown in Finland.
93. **MINNA ROTOLA-PUKKILA (2024)** The umami compounds in Nordic food raw materials and the effect of cooking.

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