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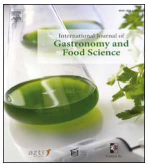

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Scientific paper

Flavour of fermented fish, insect, game, and pea sauces: Garum revisited

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Abstract

Fermentation of dairy products, legumes, fish, shellfish, molluscs, and meat are considered to lead to some of the most flavourful products in cuisines across the world. The deliciousness of these fermented products is to a large extent due to compounds like free amino acids and free nucleotides, formed during fermentation, which impart umami taste, often in a synergistic fashion. We have prepared fermented fish sauces based on mackerel, using procedures as in the ancient Roman cuisine, and used similar techniques to produce experimental fermented sauces from insects (moths and grasshoppers), game (pheasant), and pulses (peas). In some cases, the fermentation has been facilitated by fungal inoculation based on a Japanese *koji* mother. We have performed chemical analysis of these experimental fermentation products, together with a comparative analysis of a series of commercial fish sauces, with particular focus on free amino acids and free nucleotides in order to assess the umami potential of the various products. Whereas all of the 21 different investigated sauces are found to have high amounts of glutamate and aspartate, no significant amounts of free nucleotides are found in any of the samples. The investigated sauces where characterized by quantitative sensory evaluation. Although high in glutamate, umami synergy is not expected to play any significant role for the flavour of these fish, insect, game, and pea sauces. The sensory analysis shows a fairly good prediction of sensory properties from the chemical characterization of the sauces. However, the relationship between glutamate/aspartate concentration and intensity of umami taste is not simple. It demonstrates that in the complex solutions that constitute these sauces, there may be other perceptions that interfere with the main umami-tasting compounds.

Keywords: Fermentation; Fish sauce; Insects; Grasshopper; Wax moth; Pheasant; Peas; Umami; Glutamate; Aspartate: Amino acids; Nucleotides

Introduction

Some of the most flavourful and highly appreciated foodstuffs in many cultures around the world owe their deliciousness to taste compounds developed during fermentation and ageing processes, either by use of yeast, bacteria, moulds, and enzymes or combinations hereof (Mouritsen and Styrbæk, 2014). Fermented products are often considered an integral part of a food culture (Katz, 2012). Well-known examples include fermented milk (cheeses, sour-milk products), fermented beans (soy sauce, miso, *nattō*, *douchi*, *furu*), fermented grapes, fruit, berries, and grains (wine, beer, cider, brandy, bread), fermented vegetables (*sauerkraut*, *kimchi*, *tsukemono*), fermented leaves (tea), fermented fish (*katsuobushi*, fish- and shellfish sauces, Swedish surströmming), and fermented meats (sausages, hams). In most cases, the liking of fermented foodstuff requires adaptation and in some cases it is an acquired taste.

In the classical culinary triangle proposed by Lévy-Strauss (1983), the raw foodstuff can be changed either by cooking (temperature) into edible foodstuff or by microbiological degradation into rotten and inedible stuff. 'The 'cooked' and 'the rotten' then represent culture and nature, respectively. However, the borderline between 'the cooked' (prepared) and

Abbreviations: AA, amino acid; AAS, atomic absorption spectroscopy; AMP, adenosine-5′-monophosphate (adenylate); ANOVA, analysis of variance; A–PLSR, ANOVA–partial least squares regression; GMP, guanosine-5′-monophosphate (guanylate); HPLC-MS, high-pressure liquid chromatography mass spectrometry; IMP, inosine-5′-monophosphate (inosinate); MSA, monosodium aspartate (aspartate); MSG, monosodium glutamate (glutamate); PLSR, partial least squares regression; UMP, uridine-5′-monophosphate

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'the rotten' is not sharp, and different cultures as well as cultures at different times have varying perceptions regarding what is considered edible and what is not. Moreover this 'culinary borderland' is a flexibly boundary that is constantly subject to negotiation and reinterpretation (Højlund et al., 2014). Manipulating and mastering fermentation processes is a way to navigate this borderland. As pointed out by Vilgis (2013), by considering fermentation and long-time cooking as a desirably way to degrade proteins and carbohydrates in prepared food, in much the same way as spontaneous degradation in undesirable putrefaction, the borderland between 'the cooked' and 'the rotten' tends to vanish.

Fermentation releases a range of flavourful taste compounds and aroma substances. Often a repulsive smell can overpower the desire to taste the fermented foodstuff. In many cases the fermentation processes produce a bounty of nutritional elements that are more easily digestible and provide for enhanced bioavailability in the gastro-intestinal system. At the same time, properly conducted fermentation leads to preservation and prolonged lifetime of the prepared foodstuff, e.g., via dehydration or production of alcohol and lactic acid that suppress undesired fungal and bacterial growth as well as putrefaction. In some cases, enzymatically facilitated low-temperature Maillard reactions during the fermentation may release additional flavourful browning products (Nursten, 2005), as is well known, e.g., from soy sauce and black garlic, although the process at low temperatures take very long time.

The success of a fermentation process applied to foodstuffs is from the point of view of microbiology a delicate balance involving the risks of cross-contamination, wild fermentation outcompeting culturing, and growth of unwanted and potentially hazardous, poisonous fungal and bacterial cultures (Katz, 2012). Cultured fermentation typically requires good measures of cleanliness, temperature control, possible sterilization, and the use of starter cultures. High levels of salt (sodium chloride) and low pH are the most important parameters suppressing the growth of undesired microorganisms and leading way to the degradation of proteins, carbohydrates and nucleic acids.

There is a significant difference in the approaches used to ferment plant matter and meat from animals (Katz, 2012). Plants and foodstuff derived from plants contain large amounts of carbohydrates and sugars that are rapidly turned over by lactic bacteria and yeasts, producing alcohol and lactic acid, which suppress the growth of potentially hazardous bacteria and fungi. In contrast, meat and fish contain very little carbohydrates. Milk as an animal derived product is an exception that is readily susceptible to lactic fermentation. Fermentation of meat can be a risky business due to pathogenic microorganisms that may produce dangerous toxins, in particular when these organisms get access to the interior of the meat that in the living state is sterile. Fermentation of meat therefore usually involves pre-treatment by salt and application of drying or smoking techniques. In the case of fish, halotolerant visceral enzymes along with various aerobic and anaerobic salt-loving bacteria are able to ferment the pre-treated flesh and prevent putrefying bacteria to get into action. In this case, it is basically a principle of establishing conditions that selects the desirable bacterial and fungal cultures.

Whereas there is a substantial literature about fermentation of plants and plant materials (Katz, 2012), meat (Ockerman and Basu, 2008), as well as fish (Lopetcharat et al., 2001), there is very little published in the scientific literature about insects and in particular fermented insects as foodstuff (van Huis et al., 2014). In the present paper we describe an experimental attempt to produce a gastronomically acceptable fermented sauce from grasshoppers and wax moth larvae and deliver some of the first chemical analysis of insects sauces, combined with the first detailed sensory analysis of them. In the context of the Western cuisine, the particular combination of fermentation and insects represent an ultimate example of the challenging and less edible corner of Lévy-Strauss' culinary triangle.

In this paper we shall focus on the flavour of the fermented sauces, in particular from taste components derived from proteins and nucleic acids. Being large molecules, proteins and nucleic acids have no taste of their own since they cannot be detected by the taste receptors. Only when broken down into smaller constituents, in particular free amino acids and free nucleotides, respectively, can they stimulate appropriate taste receptors, such as the umami receptor (Mouritsen and Khandelia, 2012). In certain cases, small peptides can also elicit taste, e.g., *kokumi* taste by the tripeptide glutathione (Maruyama et al., 2012; Kuroda et al., 2012). During fermentation, the proteins and nucleic acids in the raw materials are subject to degradation, and large amounts of free amino acids and nucleotides can potentially be formed. However, the temporal evolution of the contents of these compounds is often highly complex and may be non-monotonic because the produced free nucleotides and amino acids may be consumed at a later stage in the fermentation process, which is the case in beer production. Similarly, high levels of free inosinate in fresh fish may be diminished by post-mortem autolytic decay and vanish almost completely during fermentation of fish sauces leading way to less-well tasting compounds, such as inosine or hypoxanthine (Gill, 1990).

Free glutamate and free nucleotides, in particular inosinate, guanylate, and adenylate, enter a unique synergic relation due to an allosteric action on the T1R1/T1R3 umami receptor (Zhang et al., 2008; Mouritsen and Khandelia, 2012). Being highly non-linear, this synergy implies that small amounts of one component, e.g., inosinate, can enhance the sensory perception of glutamate manifold (Yamaguchi and Ninomiya, 2000). This pairing of food items is hence based on a scientific principle in contrast to the claimed food pairing principle based on chemical identity, a principle that proven to be based on culture rather than a scientific principle (Ahn et al., 2011). The proposal of umami as a fifth basic taste (Ikeda, 2002) was based on the finding of large amounts of free glutamate in the brown algae *konbu* used for production of the Japanese soup stock *dashi* (Ninomiya, 1998; Yoshida, 1998). The synergetic action and the umami-pairing principle in traditional *dashi* are mediated by inosinate from a fish product, *katsuobushi*, and in

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