Unravelling the complexity of a savoury fermented product using a holistic sensory-analytical approach

ARNE GLABASNIA and Joséphine Charve
Nestlé Research Center, 1000 Lausanne 26, Switzerland

Abstract

The fermented corn starch hydrolysate reveals more complexity in savoury flavour that goes beyond the umami-taste of MSG, despite the presence of glutamic acid amongst other amino acids. Glutamyl-dipeptides were identified as major class of known taste compounds present in the savoury paste but, at the found concentrations, their impact is not sufficient to match the product's taste. Fru-Glu was found as the major Amadori compound. However, its individual taste impact could not be proven. Additionally, sensory-guided fractionation has revealed a class of N-acyl derivatives of amino acids as possible taste-actives in that fermented product. Taste activity for some derivatives has already been described elsewhere but their final impact on the taste profile of the current product is currently under investigation.

Introduction

In culinary food products, umami compounds like monosodium glutamate (MSG) and 5′-nucleotides are often used to impart savoury taste. As consumers get increasingly more sensitive to the addition of such pure ingredients, which are classified as flavour enhancers, alternative natural sources have gained interest in the past years such as products obtained through the fermentation of different raw materials (e.g., wheat gluten, soybean). In the past years, several studies have been conducted to determine the presence of taste-active or modifying compounds in such products, mainly in soy sauce [1,2]. However, the link to the sensory characteristics of the products was studied less intensely and the role of the individual taste compounds on the overall flavour remains in many cases questionable [3]. The fermented savoury product herein investigated (Savoury Base 100) is produced by fermentation of hydrolysed corn starch using *C. glutamicum*, a Gram-positive bacterium. *C. glutamicum* has been widely used for industrial production of amino acids, such as L-glutamic acid, and fermented cereals. The aim of the study was to unravel the complex savoury flavour by combination of analytical and sensory approaches.

Experimental

The investigated product (Savoury Base (SB) 100) is produced by fermentation of hydrolysed corn starch using non genetically-modified proprietary strains of *Corynebacterium* sp. (*Corynebacterium glutamicum* ATCC 13032).

Sensory assessment was performed with a trained panel using nose-clips for descriptive and comparative profiling either in water or model broth.

Glutamyl-peptides and Amadori compounds were quantified by LC/MS in MRM mode on a BEH amide (Waters) using isotopically labelled standards. Nucleotides were quantified by LC-UV using a PBr column (Cosmosil) and external calibration. Determination of basic composition was performed by ion chromatography for sugars, amino acids, organic acids and minerals with external calibration.

B. Siegmund & E. Leitner (Eds): Flavour Sci., 2018, Verlag der Technischen Universität Graz
DOI: 10.3217/978-3-85125-593-5-18, CC BY-NC-ND 4.0

Ultrafiltration was performed using a stirring cell and membranes of 1 kDA cut-off. Further fractionation of the low molecular weight fraction was performed by 1D preparative HPLC and 2D-fractionation with a Sepbox (Sepiatec) using the polar set-up.

HR-mass spectroscopy of individual fractions was performed using a BEH amide column and MS detection was done on a Q Exactive Focus (Thermo) in full scan mode and auto MS/MS of the 3 most abundant ions at three different collision energies.

For structure elucidation, NMR of isolated peaks was performed on a 600 Mz NMR from Bruker using 1D/2D experiments.

Results and discussion

The savoury powder used in this study contains a specific intrinsic mix of various compounds, including amino acids, organic acids, and minerals. The considerable amounts of glutamic acid raised the question how the sensory profile of this novel ingredient is compared to a pure MSG solution. Sensory evaluation (Fig. 1) performed with a trained panel (n=6) wearing nose-clips comparing MSG and the savoury base at same glutamic acid level, revealed that the umami taste of the fermented savoury product is higher than a pure MSG solution. In addition, a simple recombinant including basic tastants (NaCl, glutamic acid, acetic acid, pH adjusted) did not match the initial product's taste either. Panellists described the taste of SB100 as being more complex and round.

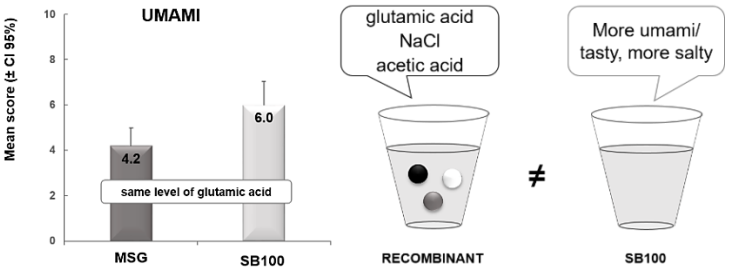

Figure 1: Sensory evaluation of MSG and savoury base providing same amount of glutamic acid (left) and comparison of simple recombinant with entire product SB 100 (right)

Based on the gap identified by the sensory panel, known taste-active molecules were quantified in the corresponding product and their individual contribution to overall taste was evaluated by calculating dose-over-threshold (DoT) values (Table 1). Glutamic acid was found to be the dominant amino acid with a DoT value of 6.4 followed by alanine and proline as second most abundant amino acids. Amongst the well-known γ-glutamyl-dipeptides, Glu-Glu and Glu-Gln were found as the most abundant members of that family, beside small amounts of other derivatives. The DoT values of these di-peptides were below their reported taste thresholds. However, it is known that these peptides have taste-modulating properties rather than showing taste-activity on their own. In addition, Amadori compounds were identified, with N-(1-deoxy-D-fructos-1-yl)-L-glutamic acid (Fru-Glu) as the main compound beside traces of other Amadori compounds. These Amadori compounds were also below individual threshold but might be of importance due to modifying properties. Other compounds that were identified were minerals and organic acids with NaCl and acetic acid, respectively, being the dominating ones, as well as sugars and ribonucleotides found in trace levels in SB100. Among those, most individual compounds were found well below their individual taste thresholds except for

NaCl, and acetic acid, which showed DoT-factors of 0.6 and 0.3, respectively. A recombinant sample, including compounds with DoT >0.1, still showed a gap in taste profile indicating the presence of other compounds contributing to the taste of the savoury powder.

Table 1: Concentrations and Dose-over-threshold factors of taste compounds found in SB 100

Taste	*Compound*	*Av. Conc. (% dm)*	*DoT at 0.2% solution*
umami	Glutamic acid	52.1	6.4
	Glutamyl-peptides	1.8	< 0.01
	Fru-Glu	0.7	0.03
	Ribonucleotides	0.1	< 0.01
	Other Amadori	0.2	n.c
salty	NaCl	4.9	0.6
	Other minerals	0.9	< 0.01
sour	Acetic acid	1.7	0.3
	Other acids	0.3	< 0.01
other	Alanine	1.8	0.05
	Proline	1.1	0.01
	Sugars	< 0.1	< 0.01
	Other free amino acids	0.9	< 0.01
	Nucleosides	0.2	< 0.01

As the recombinant of the known taste-active compounds showed no taste match with the initial powder, the product was submitted to a sensory-guide fractionation approach, using ultrafiltration (1 kDa) followed by preparative HPLC of the LMW fraction. Each fraction was then tested in water or model broth (MSG, NaCl, sucrose) for any taste activity (Fig. 2). Sensory evaluation of fractions revealed two fractions showing low taste activity when tasted alone in water, but having umami (F3 and F4) and salt modulating effects (F4), when tasted in a model broth.

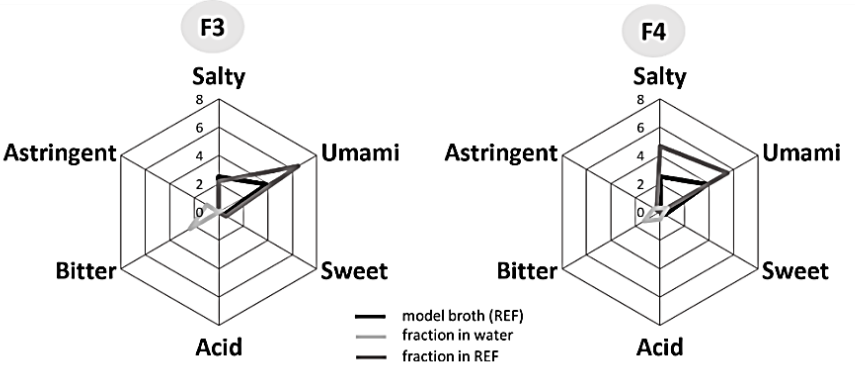

Figure 2: Sensory evaluation of most taste-active fractions F3 and F4

Taste-active fractions were then submitted to LC-HR-MS analysis and a library search was performed for first identification of peaks. This confirmed the presence of the already quantified glutamyl-peptides in fractions F3 and F4. Recombination of these peptides was nevertheless not sufficient to mimic the taste properties of fractions F3 and F4. Thus, the most prominent unknown peaks were isolated by preparative LC and structure elucidation was performed by means of NMR and HR-MS/MS. This led e.g. to the identification of N-acetyl-glutamine in fraction F4. By using molecular networks of the MS data several more of N-acyl derivatives of amino acids could be tentatively identified. Confirmation by reference molecules and sensory evaluation is currently ongoing and structure elucidation of further unknown compounds is also in progress. The results so far show that the fermented savoury base is a complex mixture of several taste-active and taste-modifying molecules, which probably contribute even in sub-threshold concentrations to the complex taste of the product by additive, synergistic and modulating effects.

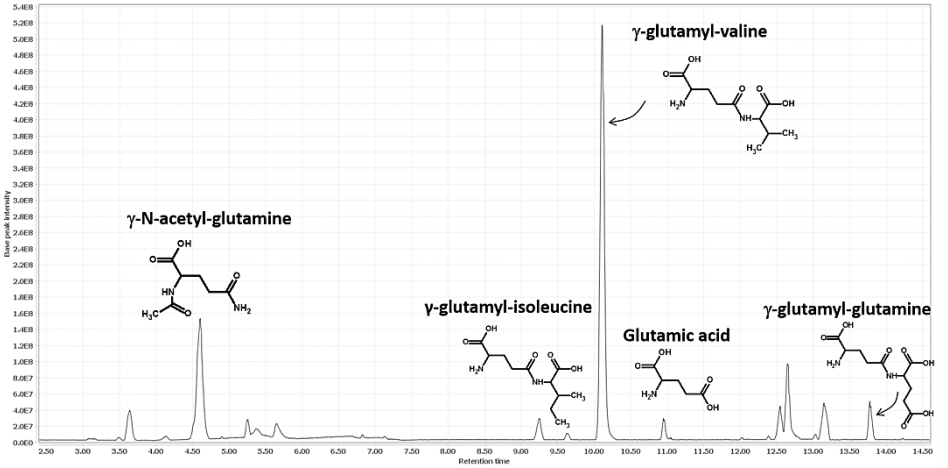

Figure 3: HR-MS chromatogram of fraction F4 containing taste-active/modifying molecules

References

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