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Determination of potential off-flavour in yeast extract

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ABSTRACT

Yeast extracts is a kind of food ingredient for enhancing flavor, but some yeast extract samples had yeasty odour (off-flavour). The aim of this study was to detect key aroma compounds leading to the yeasty odour. Volatile compounds of 3 yeast extracts (with yeasty odour), FA and FB, LC were extracted by solid phase micro-extraction (SPME) and solvent assisted flavour evaporation (SAFE) respectively, and analysed by gas chromatography-olfactometry-mass spectrometry (GC-O-MS) and aroma extract dilution analysis (AEDA) techniques. Thirty aroma-active compounds were detected, among them 4-methylphenol, 3-methylpyridine, 3-methylbutanoic acid, propanoic acid with high FD factors (FD = 125, 125, 625, 125, ≥25), were identified as the key off-flavours responsible for yeasty and their accurate quantification were done by external standard method in SIM mode. Calculation of the OAVs revealed 3-methylbutanoic acid, 3-methylpyridine, 4-methylphenol as the key odourants in the extract. For the verification of analyzed off-flavours, sensory evaluations of aroma model were conducted by eight panelists, and the result showed that aroma recombinate had the yeasty odour profile similar with the original yeast extracts.

The methods were successful for the detection of off-flavors in yeast extract and the four off-compounds analyzed were mainly responsible for the yeasty flavour.

1. Introduction

Yeast extracts were made from baker's yeast (Verduyn, Suksomcheep & Suphantharika, 1999), brewer's yeast, torula yeast or kluyveromyces by autolysis process produced under controlled conditions (Festring & Hofmann, 2010; Moresi, Orban, Quaglia, & Casini, 1995), the degradation of intracellular nucleic acid, protein and other macromolecular compounds produced the tasty substances such as amino acids and nucleotides (Chae, Joo, & In, 2001), the yeast extract products were usually in the forms of liquid, paste or powder. Yeast extracts were excellent and natural seasoning, widely used as ingredients for the production of savoury foods (Ikeda, 2002).

The 'yeast flavour', which was formed during products processing, affected the overall aroma quality of yeast extract products (which normally had basic and characteristic meaty or caramel notes), and was therefore sometimes the focus of consumers complaints. Although a study conducted by Lin et al. had reported aroma components that included off-flavour of yeast extracts (Lin et al., 2014), the specific compounds that produce yeasty odour are rarely the focus of studies. It is particularly important and urgent to identify the cause of the off-flavour that is always described as yeasty in yeast extract products. However, the intensity of the off-flavour and the overall aroma profile of yeast extract samples varied with different processing techniques.

Until now, only a range of studies on yeast extracts are available that identify with different aroma characteristics that enhance the food flavour, but the yeasty odour in samples has barely been investigated. Münch, Hofmann and Schieberle et al. (Münch, Hofmann & Schieberle, 1997) identified 2-furanmethanethiol, 4-hydroxy-2,5-dimethyl-3(2H)-furanone as the key odorants from the intensely roasted, sweet smelling solution of yeast extract. Mahadevan and Farmer (2006) compared three types of yeast extract pastes from two different suppliers and the key odorants such as 2-methyl-3-furanthiol, 2-methyl-3-methyldithiofuran, methional, 1-octen-3-one were identified as the cause for the meaty and roasted note of the samples. Werkhoff et al. reported

Abbreviations: YE, yeast extract; SPME, solid phase micro-extraction; SAFE, solvent assisted flavour evaporation; GC-O-MS, gas chromatograph-olfactometry-mass spectrometry; AEDA, aroma extract dilution analysis; FD, flavour dilution; SIM, selected ion monitoring; OAV, odour activity value.

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sulfur-containing volatiles generated by thermal treatment of yeast extract (Werkhoff, Bruening, Emberger, Guentert, Koepsel & Kuhn, 1990). Ames and MacLeod identified some aroma compounds with meaty odour properties from yeast extract (Ames & MacLeod, 1984), and Grosch (2001) investigated amino-carbonyl interactions and their possible contribution to the overall odor profile of yeast extract.

The aim of this study was to identify the key aroma compounds responsible for the 'yeasty' odour in yeast extract samples then verified the accuracy of detected compounds by the sensory evaluation of an aroma model. The aroma model was made by combining authentic standard compounds according to their natural concentrations present in the yeast extracts (Lawless & Heymann, 2010). The quantitative analysis of key off flavours compounds was completed using an external standard method in SIM (selected ion monitoring) mode (Steingass, Langen, Carle & Schmarr, 2015; Hoscheid, Reinas, Cortez, Costa, & Cardoso, 2012).

2. Materials and methods

2.1. Samples

Three kinds of yeast extracts were provided by Angel Yeast Co. Ltd. (Yichang, Hubei Province, China) and each extract had been the focus of complaints about off-flavour (yeasty) properties, in comparison with other yeast extract products. The yeast extract powers FA and FB were prepared by the enzymatic hydrolysis of yeast cells with protease/RNase, thermal-inactivation of enzymes, centrifugal separation, vacuum concentration and spray drying. The yeast extract LC was prepared by the same process except no vacuum concentration was used.

2.2. Chemicals

Diethyl ether (AR), n-pentane (AR), n-hexane (AR), anhydrous sodium sulfate (AR), sodium chloride (AR) were purchased from Yifengtiancheng Scientific Instruments Co. Ltd. (Beijing, China). n-Alkanes (C_7–C_{22}) (chromatographic reagent) and 2-methyl-3-heptanone (chromatographic reagent) were purchased from Sigma–Aldrich (St Louis, MO, USA). Nitrogen (99.995% purity) was supplied by Beijing Haipu Beifen Gas Industry Co. Ltd. (Beijing, China).

Distilled water was purified by the Millipore Simplicity system (Millipore Corp., Saint-Quentin, France) to obtain the ultrapure water. Standards of volatile compounds such as 3-methylbutanal, 3-methylthiophene, 2-methylthiazole, methylpyrazine, octanal, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, ethylpyrazine, 2,3-dimethylpyrazine, dimethyltrisulfide, 2-methyl-3-furanthiol, 3-(methylthio)propanal, nonanal, trimethylpyrazine, benzeneacetaldehyde, 2-methyl-5-(1-methylethyl)pyrazine, acetic acid, furfural, 2,6-diethylpyrazine, benzaldehyde, propanoic acid, 2,3-butanediol, 2-methylpropanoic acid, acetophenone, 3-methylbutanoic acid, 3-methylpyridine, phenylethyl alcohol, 4-methylphenol, and indole were purchased from Sigma–Aldrich Co. Ltd (Santa Clara, CA, USA).

2.3. Quantification of volatile compounds

The extraction/isolation of volatile substances was carried out by solid phase micro-extraction (SPME) and solvent assisted flavour evaporation (SAFE), respectively. 2-Methyl-3-heptanone was used as an internal standard because it eluted quickly (Song & Cadwallader, 2008), without interfering with the target compounds. For SPME and SAFE analysis, the content of internal standards as follows.

In this study, two quantitative methods were used, including an internal standard method for total aroma compounds and an external standard method in SIM mode for the four off-flavour compounds detected. For the internal standard method, the authentic standards listed in Table 1 were mixed well, then 2-methyl-3-heptanone (0.816 μg/μL in hexane) was added as the internal standard.

Response factor = (concentration of authentic standard/peak area of standard)/(concentration of internal standard/peak area of the internal standard)

Concentration of an odorant = concentration of internal standard × peak area of compound/(peak area of the internal standard/response factor).

For the external standard method in SIM mode, four standard solutions of 3-methylbutanoic acid, 3-methylpyridine, 4-methylphenol, and propanoic acid compounds were prepared at five levels: 50, 100, 200, 500 and 1000 μg/L in selected ion monitoring mode according to the mass spectra SIM modes of m/z 60, 41, 93, 66, 107, 108 and 74, 73, respectively.

2.4. Solid phase micro-extraction (SPME)

SPME analytical method was applied in previous research (Lin, Wong, & Kao, 2002). Yeast extract powder (4 g) was placed in a 40 mL headspace vial, then 1 μL internal standard 2-methyl-3-heptanone at the concentration of 0.816 μg/μL was added, and thermostated at 61°Cin a water bath for 20 min. The SPME needle coating divinylbenzene/carboxen/polydimethylsiloxane (75 μm) was inserted into the vial and adsorbed for 44 min, then desorbed at the gas chromatography inlet for 5 min before the GC-O-MS analysis.

2.5. Solvent assisted flavor evaporation (SAFE)

Five grams of the each of yeast extracts was weighed accurately, dissolved in 100 mL of ultrapure water, after which the internal standard compound 2-methyl-3-heptanone was added at a concentration of 0.816 g/mL. The YE sample was mixed with 120 mL combined solvent of diethyl ether and pentane (v:v = 2:1) and was extracted repeatedly three times, producing a total of 220 mL mixture. It was put on shaking plate, rotating at a speed of 180r/min at 4 °C for 8 h. The organic phase of 100 mL was obtained using a separatory funnel after static layering. The installation of the SAFE apparatus (Deutsche Forschungsanstalt für Lebensmittelchemie, Freising, Germany) and operation procedure were as follows: a 500 mL round bottom flask was put on a thermostatic water bath pot of 40 °C, another 250 mL round bottom flask used as a receiver was put into a liquid nitrogen environment; the cold trap was also filled with liquid nitrogen, the distilled water temperature was held at 50 °C, and the pressure under vacuum was 10^{-4}Pa by a combination of a high vacuum pump and a turbo pump (Edwards, UK). The 100 mL volume of organic phase extracted by ether/n-pentane was dropped into the distillation flask slowly and uniformly. The extracts were separated from organic solvent, then dried by anhydrous sodium sulfate two times, and concentrated to 2 mL by Vigreux column under 40 °C, and finally 0.5 mL concentrate was obtained by a gentle purge of nitrogen. All extracts being free of water and impurities were subsequently stored at −20 °C in a 2 mL glass vial equipped with Teflon-lined cap before undergoing GC-O-MS analysis.