



Instituto Politécnico de Castelo Branco
Escola Superior Agrária

Training Report

**“Application of two extraction methods: SPME and
SAFE for identification of volatile compounds
and key odorants in Tempeh”**

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“Application of two extraction methods: SPME and SAFE for identification of volatiles
compounds and key odorants in Tempeh”

*“The doctrines expressed in this document
are the sole responsibility of the author”*

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Abbreviations’ list

- 1D**- Tempeh with one day of fermentation
- 1DF**- Tempeh with one day of fermentation and fried
- 5D**- Tempeh with five days of fermentation
- 5DF**- Tempeh with five days of fermentation and fried
- AEDA**- aroma extract dilution analysis
- CAR/PDMS**- Carboxen™/ Polydimethylsiloxane
- CW/DVB**- Carbowax®/ Divinylbenzene
- DVB/CAR/PDMS**- Divinylbenzene/ Carboxen/PDMS
- FD**- Factor of Dilution
- GC**- Gas Chromatography
- GC-O**- Gas Chromatography- Olfactometry
- HVT**- High Vacuum Transfer
- LPME**-Liquid Phase Microextraction
- MS**- Mass Spectrometry
- PDMS**- Polydimethylsiloxane
- PDMS/DVB**-Polydimethylsiloxane/ Divinylbenzene
- R.o.**- *Rhizopus oligosporus*
- RI**- Retention Index
- SAFE**- Solvent Assistant Flavour Extration
- SDE**- Solvents Dislitation Extration
- SLF**- submerged-liquid fermentation
- SPME**- Solid Phase Microextraction
- SS**- Standard Sample
- SSF**- Solid State Fermentation
- ToF**-Time-of-flight
-

Abstract

Tempeh is a traditional Indonesian fermented product, made from soy seeds inoculated with mould, *Rhizopus oligosporus*.

Tempeh is highly nutritious, easily digestible and delicious food, and is especially interesting for consumers looking for products rich in healthy components for their diet. The aim of this study was to estimate the changes in the flavour compounds profile during fermentation and frying of tempeh. For tempeh production bean seeds (*Phaseolus vulgaris*) were boiled and inoculated by spores of *Rhizopus oligosporus*. Volatile compounds produced after the fermentation and frying processes, were isolated using SPME and SAFE techniques

SPME extraction allowed the monitorization of volatile compounds formed during tempeh fermentation such as pyrazines, ketones, aldehydes, alcohols and sulphuric compounds. It has been noticed that longer fermentation time increased formation of pyrazines, aldehydes, ketones and alcohols but had no influence on sulphuric compounds. On the other hand frying of 5 days fermented tempeh produced an increase of aldehydes and sulphuric compounds.

By application of SAFE extraction and GCO analysis, many compounds were identified in tempeh with 24 hours of fermentation, 5 days and in fried tempeh with highest FD for boiled potatoes and popcorn notes. Application of GC×GC/TOFMS analysis allowed for their identification as methional and methyl pyrazine.

Keywords: SPME; SAFE; Volatile Compounds; Fermentation; Tempeh

Resumo

O tempeh é um produto tradicional da Indonésia, feito a partir de sementes de feijão inoculadas com *Rhizopus oligosporus* e por fim fermentado.

O tempeh é altamente nutritivo, de fácil digestão e delicioso, e é de especial interesse para consumidores que procuram produtos ricos em compostos saudáveis para a sua dieta. O objectivo deste estudo foi estimar as mudanças no perfil dos compostos aromáticos durante a fermentação e a fritura do tempeh. Para a produção de tempeh, foram ferveridas e inoculadas sementes de feijão (*Phaseolus vulgaris*) com esporos de *Rhizopus oligosporus*. Os compostos voláteis formados durante os processos de fermentação e de fritura foram isolados usando técnicas de SPME e SAFE.

Extracção por SPME permitiu monitorizar o crescimento fúngico e o desenvolvimento dos compostos voláteis, tais como, pirazinas, cetonas, aldeídos, álcoois e compostos sulfúricos. Constatou-se que períodos mais longos de fermentação aumentavam a formação de pirazinas, aldeídos, cetonas e álcoois mas não têm notável influência nos compostos sulfúricos. Por outro lado, durante o processo de fritura do tempeh fermentado de 5 dias, houve um aumento da quantidade de aldeídos e compostos sulfúricos.

Por aplicação da extracção SAFE e análise GCO, muitos compostos foram identificados no tempeh após fermentação de 24 horas, de 5 dias e em tempeh frito com o maior FD para os aromas de batata cozida e pipoca. A análise por GC×GC/TOFMS permitiu a identificação do metional e da metilpirazina.

Palavras-Chave: SPME; SAFE; Compostos Voláteis; Fermentação; Tempeh
