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HEADSPACE ANALYSIS OF NATURAL YOGHURT USING HEADSPACE SOLID PHASE MICROEXTRACTION

A THESIS

PRESENTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

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IN FOOD TECHNOLOGY

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GEEDHA SIVALINGAM-REID

2001

ERRATA

Page xxi, lines 23 & 24	"PA polyacrylate PA polyacrylate" should read "PA polyacrylate"
Page 10, line 2	"quantified" should read "were quantified"
Page 25, line 5	"or" should read "as"
Page 51, line 20	"was" should read "were"
Page 69, line 31	The sentence beginning "Variations to the sampling....." is missing some text and should read "Variations to the sampling procedures are detailed in the Materials and Methods sections in each chapter."
Page 70, line 1	The first sentence is missing some text and should begin with "Before headspace sampling, each sample ("
Page 82, Fig. 4.1 (A-F)	When regression lines are forced through the origin in Fig. 4.1, R^2 values do not change a great deal, with the majority changing by less than 1%. All trends in R^2 values are retained, thus discussions based on these values are unaffected.
Page 104, line 24	"were determined" should be deleted
Page 105, line 1	"were determined" should be deleted
Page 109, line 27	"very to 1" should read "very close to 1"
Page 143, line 25	"were" should be deleted
Page 147, line 3	"unfavorable" should read "unacceptable"
Page 177, line 10	"that" should read "those"

This thesis is dedicated to my darling daughter,

Sophia Jeevaranee Campbell Reid.

ABSTRACT

The Solid Phase Microextraction (SPME) method was originally developed to extract volatile and semivolatile compounds from wastewater samples but has since been applied to flavour compounds in foods and beverages. Research using the HS-SPME in related areas such as cheese and skim milk powder has been carried out but, to date, no work has been done on yoghurt flavours. The main objective of this study was to devise a methodology for the Headspace Solid Phase Microextraction (HS-SPME) technique to investigate and quantify six flavour analytes in natural, set yoghurts made from recombined milk.

The relevant literature was reviewed and from it, a research proposal for this work on yoghurts was drawn. The first step in analysing and quantifying the yoghurt volatiles was to set up a working methodology for the HS-SPME method. The 100 μm polydimethylsiloxane (PDMS) fibre was chosen along with 20 minutes being the optimum fibre adsorption time. General equipment, materials and methods used throughout this thesis are also detailed. The external standard (ES) method was used to calibrate the GC and quantify the analyte concentrations in this study. The internal standard (IS) method was not used as a quantitative tool in this study.

Once the HS-SPME methodology had been set up for the analysis of yoghurts, the classical Static Headspace (SH) method was compared with the HS-SPME method for extraction efficiency. The results suggested that the two methods were complementary in that the SH method extracted the more volatile compounds (acetaldehyde, acetone and 2-butanone) whereas, the HS-SPME method extracted the semi- to non-volatile compounds (ethanol, diacetyl and acetoin) more readily. However, the HS-SPME was found to be the more sensitive and effective method of the two techniques tested.

The next step in the thesis was to investigate the presence of the six analytes in milk and cultured yoghurt. The effects of the sample matrix, fat levels and incubation on the volatile concentrations were also examined. The results suggested that the six analytes were inherently present in milks but at low concentrations. No conclusive effects were found for the sample matrix, fat levels and incubation. However, it was evident that

fermentation of the milks using bacterial starter cultures resulted in a large increase in some of the volatiles being investigated.

Following this, the effects of fat levels, storage time and storage temperature on the six volatiles in yoghurts were examined. The results indicated that significant fat level effects were only seen for diacetyl and acetoin, while temperature effects were only observed for ethanol. In both trials, only general trends for the analytes concentrations were drawn because the data varied from day to day. The results suggested that most of the compounds decreased with time except for diacetyl, which seemed to increase.

The final part of this study looked at applying the devised HS-SPME methodology to a series of commercial yoghurts as a preliminary trial, with a view to investigating a potential application for the HS-SPME method. Fourteen commercial yoghurts were analysed and the six analytes quantified. The data obtained was analysed using Principle Component Analysis (PCA), which divided the yoghurts into groups based on their analyte concentrations. From these groupings, eight yoghurts were selected and fresh samples were analysed using HS-SPME and PCA. This was carried out parallel with an untrained consumer panel, which had to distinguish differences between the yoghurts in a series of triangle tests by smelling the headspace on opening the yoghurt containers. The conclusions drawn were that, unlike the HS-SPME method with PCA, the average consumer could not differentiate the yoghurts based on smell alone. PCA also showed that the HS-SPME results obtained were fairly reproducible.

In conclusion, the HS-SPME method was shown to be a useful analytical technique, which can be used to analyse and quantify flavour compounds in natural, set yoghurts. This area of investigation has a lot of scope, with the results from this study providing a basis or starting point for further investigations in this area. Future studies may lead to potential applications for the HS-SPME method, one of which may be quality control where correlation of sensory data with HS-SPME analytical data is required.

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and lastly, my darling daughter, Sophia.
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ABBREVIATIONS

A	peak area ratio
AnalaR	analytical reagent
ANOVA	analysis of variance
B	Biofarm
BA	Biofarm *Aciophilus*
BP	boiling point
BTEX	benzene, toluene, ethyl benzene and xylene isomers
CO_2	carbon dioxide
conc.	concentration
CW/DVB	carbowax/divinylbenzene
DA	descriptive analysis
DDI	distilled, deionised water
DH	dynamic headspace (Purge and Trap)
DH-GC	dynamic headspace - gas chromatography
DNA	deoxyribonucleic acid
ES	external Standard
FA	factor analysis
FID	flame ionisation detector
FN	Fresh 'n' Natural
g	gramme
GC	gas chromatography
GC-MS	gas chromatography-mass spectrometry
GC-O	gas chromatography-olfactory
HF	high fat
HPLC	high performance liquid chromatography
hr	hour
HS	headspace
HS-GC	headspace-gas chromatography
HS-SPME	headspace solid phase microextraction
IFNHH	Institute of Food Nutrition and Human Health
IR	infra-red

IS	internal Standard
k	partition coefficient
kg	kilogram
L	litre
L/hr	litre per hour
LDA	linear discriminant analysis
LF	low fat
min	minute
MITC	methyl isothiocyanate
mL	millilitre
MS	mass spectrometry or mass spectroscopy
N	Naturalea
N_2O	nitrogen oxide
NA	Naturalea *Acidophilus*
Na_2SO_4	sodium sulphate
NaCl	sodium chloride
NMR	nuclear magnetic resonance
NS	not significant
NZDRI	New Zealand Dairy Research Institute
O_2	oxygen
°C	degree Celsius
OFN	oxygen-free nitrogen
PA	polyacrylate
~~PA~~	~~polyacrylate~~
PCA	principal component analysis
PDMS	polydimethylsiloxane
PDMS/DVB	polydimethylsiloxane/divinylbenzene
ppb	parts per billion
ppm	parts per million
psi	pounds per square inch
QDA	qualitative descriptive analysis
R	regression coefficient
rep.	replicate

R_T	retention time
SAM	standard addition method
SC	Slimmers' Choice
SDA	stepwise discriminant analysis
secs	seconds
SFE	Supercritical fluid extraction
SH	static headspace (direct injection)
SH-GC	static headspace - Gas chromatography
SMP	skim milk powder
SPE	Solid phase extraction
SPME	Solid Phase Microextraction
Std.	standard
TSNF	total solids non-fat
UHT	ultra high temperature
units ^{2}	peak area units
VA	Verona *Acidophilus*
VB	Verona Bulgarian
WMP	whole milk powder
WW	Weight Watchers
YAB	Yoplus *Acidophilus Bifido bacterium*
YABC	Yotrim *Acidophilus Bifido bacterium Caseii*
YHF	Yoplait high fat
YLF	Yoplait low fat
µL	microlitre

CHAPTER ONE

INTRODUCTION

Volatile organic compounds (VOC) in food have significant impacts on the flavour quality. In order to identify which compounds significantly influence the flavour of a product, flavour analysis has to be undertaken. This involves separating and isolating the volatile flavour compounds, quantifying them and lastly, identifying them.

In flavour analysis, gas chromatography (GC) coupled with mass spectrometry (MS) have been the most important flavour-separating and identifying tools, to date. Various isolation and extraction methods have been developed to extract the flavour compounds from the sample matrix. The traditional methods include the classical (direct injection) static headspace (SH) method, the dynamic (Purge and Trap) technique (DH), supercritical fluid extraction (SFE), solid phase extraction (SPE) and the membrane extraction (ME) methods. The area of flavour analysis research has grown recently with the development of new and improved analytical tools.

One such analytical tool is the Solid Phase Microextraction (SPME) method, which was originally developed to extract volatile and semi-volatile compounds from wastewater samples (Arthur and Pawliszyn, 1990; and Arthur *et al.*, 1992b). It has since been applied to flavour compounds in foods and beverages (Yang and Peppard, 1994; Elmore *et al.*, 1997; and Chin *et al.*, 1996).

The SPME technique is a simple analytical tool. It is relatively cheap to run and easily automated with convenient on-site sampling capabilities. It is a solventless extraction method with advantages such as high precision and efficiency with increased sensitivity. The method can be used to extract analytes from the aqueous sample directly (SPME) or from the headspace above the sample matrix (headspace SPME [HS-SPME]). The SPME fibre is small and cylindrical with an analyte-specific coating on it. The fibre is easily incorporated into a syringe-like device, which makes it more durable and less fragile.

The main objective of this study was to devise a methodology for the HS-SPME technique to investigate six flavour volatiles in natural, set yoghurts. Yoghurt is produced when bacterial starter cultures are added to milk and incubated. The subsequent fermentation results in acidification of the milk and development of favourable flavours and organoleptic properties in the product.

Very little flavour analysis work has been done in the dairy area using HS-SPME (Chin *et al.* 1996; and Stevenson and Chen, 1997). To date, no work has been carried out on yoghurts and their flavour volatiles. The work in this study is a pilot study in the area and the results obtained provide a good starting point for further investigations in HS-SPME.