Studies on Interactions of Milk Proteins with Flavour Compounds

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ABSTRACT

Milk proteins are known to bind volatile flavour compounds to varying extents, depending on the nature of the protein and flavour compound. Processing conditions, such as temperature and pH, are also known to have an influence on the interactions between milk proteins and flavour compounds. These interactions cause a great challenge for flavour scientists because they influence the perceived aroma profile of food products significantly, in particular in low-fat food products.

The objectives of this research were to develop a headspace solid-phase microextraction (SPME) method followed by gas chromatography with flame ionisation detection (GC-FID) for the investigation of protein-flavour interactions, and to determine binding parameters of the hydrophobic flavour compound, 2-nonanone, to individual milk proteins – namely, β-lactoglobulin (β-lg), α-lactalbumin (α-la), bovine serum albumin (BSA), α_{s1}-casein, and β-casein –, whey protein isolate (WPI), and sodium caseinate. Secondly, it was the aim to compare the binding of the structurally similar flavour compounds – 2-nonanone, 1-nonanal, and *trans*-2-nonenal – to WPI in aqueous solution, and to investigate the effect of heat and high pressure treatment, and pH on the extent of protein-flavour binding. The final objective was to investigate the *in vivo* release of the reversibly bound flavour compound, 2-nonanone, from WPI and sodium caseinate using proton-transfer-reaction mass spectrometry (PTR-MS), and to understand the effect of viscosity on flavour release *in vivo*.

The binding of the model flavour compound 2-nonanone to individual milk proteins, WPI, and sodium caseinate in aqueous solutions was investigated, using headspace SPME followed by GC-FID. The 2-nonanone binding capacities decreased in the order: BSA > β-lg > α-la > α_{s1}-casein > β-casein, and the binding to WPI was stronger than the binding to sodium caseinate. All proteins appeared to have one binding site for 2-nonanone, except for BSA which possessed two classes of binding sites.

The influence of heat treatment, high pressure processing and pH of the protein solutions on the binding of 2-nonanone, 1-nonanal, and *trans*-2-nonenal to WPI was determined. The binding of these compounds to WPI decreased in the order: *trans*-2-nonenal > 1-nonanal > 2-nonanone. The binding of 2-nonanone appears to involve hydrophobic interactions only, whereas the aldehydes, in particular *trans*-2-nonenal, also react through covalent binding. Upon both heat and high pressure denaturation, the binding of 2-nonanone to WPI decreased, the binding of 1-nonanal remained unchanged, while the binding of *trans*-2-nonenal

increased. The binding affinity of the flavour compounds and WPI increased with increasing pH, which is likely to result from pH dependent conformational changes of whey proteins.

The *in vivo* flavour (2-nonanone) release from solutions of WPI and sodium caseinate was investigated using proton-transfer-reaction mass spectrometry. During consumption, 2-nonanone was partly released from WPI, whereas there was no significant release from sodium caseinate. Even after swallowing of the samples, a substantial amount of flavour was detected in the breath, suggesting that the milk proteins interact with the mucosa in the mouth and throat, resulting in a further release of flavour from mucosa-bound proteins. An increase in viscosity of the protein solutions by the addition of carboxymethylcellulose enhanced the release of 2-nonanone from WPI, and resulted in 2-nonanone release from sodium caseinate. This may be due to a thicker coating of the mucosa with the sample solution after swallowing due to the higher viscosity, resulting in additional release of protein-bound flavour.

These findings contribute to the knowledge of the interactions that occur between flavour compounds and proteins, which is required to improve food flavouring and to make protein based foods, e.g., low-fat dairy products, sensorily more acceptable to the consumer. The results also emphasize a careful choice of food processing conditions, such as temperature, high pressure or pH to obtain a desirable flavour profile.

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∞	At equilibrium
α-la	α-Lactalbumin
β-lg	β-Lactoglobulin
ε	Extinction coefficient ($l \cdot mol^{-1} \cdot cm^{-1}$)
λ	Wavelength (nm)
θ	Temperature (°C)
A	Absorptivity
APCI	Atmospheric pressure chemical ionisation
APS	Ammonium persulphate
AUC	Area under the curve
BSA	Bovine serum albumin
BTEX	Benzene, toluene, ethylbenzene, and xylenes
c	Concentration (M)
C	Cross-linker as percentage of total monomer concentration (%)
CAR	Carboxen
CD	Circular dichroism
CMC	Carboxymethylcellulose
conc.	concentrated
cps	Counts per second
CV	Coefficient of variation (%)
Da	Dalton ($g \cdot mol^{-1}$)
DCCLC	Dynamic coupled column liquid chromatography
DEAE	Diethylaminoethyl
DSC	Differential scanning calorimetry
DVB	Divinylbenzene

E	Electric field
[F]	Concentration of flavour (M)
FID	Flame ionisation detector
GC	Gas chromatograph(y)
GC-O	GC-olfactometry
h	Hour(s) or Hill coefficient or headspace
HHP	High hydrostatic pressure
HPLC	High performance liquid chromatography
HPMC	Hydroxypropyl methyl cellulose
IEC	Ion-exchange chromatography
I_{max}	Maximum intensity of flavour perception
IR	Infrared
K	Binding constant (M^{-1})
K_d	Dissociation constant
K_{fh}	Partition coefficient between SPME fibre coating and headspace
K_{fw}	Partition coefficient between SPME fibre coating and water
K_{hw}	Partition coefficient between headspace and water
[L]	Concentration of free ligand (M)
$[L]_{tot}$	Total ligand concentration (M)
Log P	Logarithm of the partition coefficient between water and n-octanol
LTPRI	Linear temperature programmed retention index
M	$Mol \cdot l^{-1}$
min	Minute(s)
MPC	Milk protein concentrate
MS	Mass spectrometry / spectrometer
MW	Molecular weight

m/z	Mass to charge ratio
n	Number of binding sites per mole of protein
nK	Global binding constant (M^{-1})
NMR	Nuclear magnetic resonance
NOE	Nuclear Overhauser effect
[P]	Protein concentration
PAGE	Polyacrylamide gel electrophoresis
PAH	Polycyclic aromatic hydrocarbons
PDMS	Polydimethylsiloxane
PFG	Pulsed field gradient
PG	Propylene glycol (1,2-propanediol)
ppbV	Parts per billion by volume
ppm	Parts per million
PTFE	Polytetrafluoroethylene
PTR	Proton transfer reaction
RAS	Retronasal aroma simulator
RI	Refractive index
rpm	Revs per minute
sccm	standard centimeter cube per minute; 1 cm^3 of gas per minute at 0 ^{o}C and at atmospheric pressure.
SD	Standard deviation
SE	Standard error
SEM	Secondary electron multiplier
SDS	Sodiumdodecylsulphate
s	Second(s)
SEC	Size exclusion chromatography

SPME	Solid-phase microextraction
t	Time
T	Total monomer concentration (%)
TCA	Trichloroacetic acid
TEMED	N, N, N', N'-Tetramethylethylenediamine
t_{eq}	Equilibration time
t_{ex}	Extraction time
TI	Time-intensity
t_{max}	Time at which maximum flavour intensity is perceived
Tris	Tris(hydroxymethyl)methylamine
UV	Ultraviolet
V	Volume (l) or Volts
v	Number of moles of flavour bound per mol of protein
var	Variance
WPC	Whey protein concentrate
WPI	Whey protein isolate
Y	Fractional saturation of binding sites on the protein