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THE KINETICS OF MILD ACID HYDROLYSIS OF GLUTEN
AND THE FUNCTIONAL PROPERTIES OF THE MODIFIED PROTEINS
AT VARIOUS LEVELS OF HYDROLYSIS

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ABSTRACT

Gluten is the mixture of proteins remaining in wheat flour after starch and water soluble components have been extracted by washing. Its unique dough forming properties are due to the structure of the proteins. A feature of the protein is the high glutamine content, about 30% of the total amino acids. A number of studies have shown that gluten's properties of insolubility and water-binding can be substantially modified by mild acid hydrolysis. The principal effect of the mild acid treatment is to hydrolyse the amide side chain of glutamine such that the amide group is replaced by a carboxyl group. In addition, it is known that hydrolysis of peptide bonds can have a large influence on the functional properties of proteins.

The aims of this study were to determine the kinetics of the acid catalysed deamidation and peptide bond hydrolysis reactions, and to comment on the resultant changes in functional properties.

A statistically designed experiment was used to determine the effect of temperature, hydrogen ion concentration and gluten concentration. An initial rate analysis of the results showed that reactions could be described by equations of the form:

$$\text{Rate of amide bond hydrolysis} = k_1[\text{Amide}][\text{H}^+]$$

$$\text{and Rate of peptide bond hydrolysis} = k_2[\text{Peptide}][\text{H}^+]$$

$$\text{where } k = k_0 e^{-\frac{E}{R} \cdot \frac{1}{T}}$$

A stoichiometric analysis of the experimental data confirmed that hydrogen ions were consumed in both reactions.

A numerical solution was developed to predict the extent of reaction with time. A computer program incorporating the solution was used to simulate the reaction and test the

solution. The simulation results appeared to overestimate the progress of the reaction with time.

A series of ten gluten powders, hydrolysed to different extents was prepared at small pilot scale. The composition of the samples was determined and compared with the extent of hydrolysis predicted by the reaction simulation. Reasonable agreement was achieved.

A selection of the functional properties of the prepared samples was examined.

The quantity of alkali required to dissolve each preparation to the extent of its solubility at pH 7.6 increased markedly with the extent of hydrolysis due to the additional carboxyl groups requiring neutralization.

The flavour of each preparation was examined. A cereal flavour was found to decrease with the extent of hydrolysis. A lingering bitter flavour was found to increase with the extent of hydrolysis.

The solubility of all preparations at pH 7.6 in 0.1 M phosphate buffer increased with the extent of treatment so that the most hydrolysed samples were almost completely soluble. No (significant) difference was found between freeze dried and spray dried samples. Samples prepared without dialysis showed no solubility difference from those prepared with dialysis at a similar extent of hydrolysis.

The hydrophobicity of the preparations was measured using two different fluorescent probes and was found to increase with the extent of hydrolysis. The emulsion-forming properties of the preparations were found to depend on the oil used in the test, as would be expected if hydrophobicity was equivalent to the hydrophile lipophile balance, which is commonly used to classify emulsifying agents. The preparations did not, however, show the additivity properties of emulsifiers. It was also shown

that only the soluble portion of the preparations was responsible for emulsion formation.

The possibility of achieving deamidation of gluten using the enzymes peptidoglutaminase I and II was examined. No activity against gluten or partially hydrolysed gluten was found.

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* In fiche

SYMBOLS AND ABBREVIATIONS

a	activity (e.g. a_{H^+} is hydrogen ion activity)
A_{340}	Absorbance measured at a wavelength of 340 nm
ANS	8-anilino-1-naphthalenesulfonate
°C	degrees centigrade
cc	cubic centimetre
CI	confidence interval
CPA	cis parinaric acid
E	activation energy
E	the observed electrode potential in the Nernst equation
E°	the stable fixed potential including reference potential
F	the Faraday
GDH	L-glutamate dehydrogenase
g or gm	gram
HLB	hydrophile lipophile balance
k	reaction rate constant
°K	degrees Kelvin
L-glu	L-glutamic acid

\ln	logarithm to base e
LOF	lack of fit
\log_e	logarithm to base e
\log_{10}	logarithm to base 10
M	molar
ml	millilitre
mm	millimetre
mM	millimolar
N_A	number of moles of component A
N_{A0}	initial number of moles of component A
NAD	nicotinamide adenine denucleotide
NADH	nicotinamide adenine denucleotide reduced
nm	nanometre
PCA	pyrrolidone carboxylic acid
SDS	sodium dodecyl sulphate
t	statistic calculated in Students t test
T	temperature, °K
TN	total nitrogen content
TNBS	trinitrobenzene sulfonic acid

R	ideal gas law constant
W	watt
w/v	weight per unit volume
X_A	mole fraction of component A
v	the difference in voltage measured by a pH electrode over the range of pH
σ_{n-1}	standard deviation estimated using a population sample of size n
μ	ionic strength
μl	microlitre