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Objective Differentiation of Cheese Type and Maturity



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**A THESIS PRESENTED IN PARTIAL FULFILMENT OF
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BY

CHRISTINA JUNE PURYER COKER

(RIDDET CENTRE AND INSTITUTE OF FOOD, NUTRITION
AND HUMAN HEALTH, COLLEGE OF SCIENCES,
MASSEY UNIVERSITY, PALMERSTON NORTH)

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*This thesis is dedicated to my parents Eric and Sylvia,
my husband Neil
and children Nicholas, Michael, Justin and Timothy*

ABSTRACT

The main objectives of this study were to develop instrumental methods for determining cheese maturity and for differentiating variety irrespective of maturity. Traditionally this has been done by sensory (texture and taste) evaluation, but it is desirable to systematise the process, for both non-varietal cheese that is destined to become an ingredient in a complex food and for table cheese.

An initial study developed a size exclusion (high performance liquid) chromatography (SE-HPLC) method for the characterisation of proteolysis in cheese. The method provided predictable elution of peptide and amino acid standards on the basis of hydrodynamic volume (which equates well with molecular weight), although the presence of a net positive charge caused smaller peptides and amino acids to elute slightly earlier than anticipated. The repeatability of the elution time and peak intensity of a range of molecular weight standards was excellent, and their elution time was used to develop a standard curve for determining molecular weight.

The potential of the SE-HPLC method for characterising proteolysis in cheese was compared with commonly used methods by examining seven cheeses of different types. Non-casein nitrogen and non-protein nitrogen were useful for demonstrating differences in the overall amounts of protease and peptidase activity; alkaline urea-polyacrylamide gel electrophoresis (urea-PAGE) of the urea-soluble fraction (USF) of cheese was useful for comparing the amounts of chymosin and plasmin action; urea-PAGE of the water-soluble fraction (WSF) of cheese was useful for comparing the amounts of several large peptides (>~5000 Da) and reverse phase (RP)-HPLC of the WSF was useful for comparing patterns of peptides in different cheese types. SE-HPLC was useful for demonstrating differences in the pattern of molecular weight distribution of the peptides and amino acids present in the WSF of different cheese types, but was less useful for demonstrating the molecular weight distribution of the caseins (which eluted as a single peak), peptides and amino acids present in the USF. Good repeatability of the extraction of the WSF was demonstrated.

A larger study compared the SE-HPLC (WSF) method with alternative methods (urea-PAGE of the USF and WSF, and RP-HPLC of the WSF) for following the maturation of five cheese types (New Zealand-style Cheddar, Elsberg, Gouda, Mozzarella and Swiss) from pressed curd to beyond normal maturity. Principal component analysis (PCA) was used to reduce the data from a large number of peaks to a few principal components that accounted for most of the variation in the data set. Depending on the method of sample analysis, the correlation form of PCA accounted for between 72% and 83% of the variation in the data within the first three principal components, and generally provided better differentiation of cheese type than the covariance form of PCA which accounted for between 85 and 97% of the variation in the data and was better for determining

maturity. Plots of the first three principal components showed that urea-PAGE of the USF and SE- and RP-HPLC of the WSF could be used to differentiate the cheese types throughout ripening; urea-PAGE of the USF, which measured primary proteolysis, provided useful maturity trends in the early stages of ripening of most cheese types and throughout the ripening of slowly maturing cheese while RP- and SE-HPLC of the WSF, which mainly measured secondary proteolysis, provided useful maturity trends throughout the ripening of each cheese type.

The final study used RP- and SE-HPLC of the WSF to examine the diversity of a range of first grade mild to mature commercial cheese of one type (Cheddar), and to examine the potential of the instrumental methods for establishing a cheese maturity index that could accurately predict a sensory maturity score (SMS; 1.00 – 9.00 scale) provided by a trained sensory panel. Each cheese sample (77 samples of Cheddar cheese manufactured at 8 New Zealand factories over 2 seasons and ripened at 5, 10 or 13°C for 3, 6, 12 or 15 months) was analysed using both methods, as well as traditional methods for determining maturity (non-protein nitrogen (NPN), water-soluble nitrogen (WSN)). The relationship between each set of instrumental results and the SMSs was modelled using multivariate statistics and equations were developed for predicting cheese sensory maturity. Multiple linear regression (MLR) analysis showed that the simplest measures of maturity provided a reasonable correlation with the SMS ($R = 0.88$, R^2 (adj) = 0.77 for NPN and total nitrogen (TN); and $R = 0.90$ and R^2 (adj) = 0.80 for WSN and TN). The covariance form of PCA accounted for the most variation in the data (PC1 to PC3 = 88% for RP-HPLC; and PC1 to PC3 = 93% for SE-HPLC) and provided trends that were more closely associated with sensory maturity. MLR analysis showed the close correlation between the first three principal components and the SMSs (RP-HPLC: $R = 0.94$, R^2 (adj) = 0.87 for PC1, PC2, PC3 and TN; and SE-HPLC: $R = 0.92$, R^2 (adj) = 0.84 for PC1, PC2, PC3 and TN). The PCA factor loading scores were used to select influential peaks and MLR was used to demonstrate their close correlation with the SMS (RP-HPLC: $R = 0.99$, R^2 (adj) = 0.98 for 32 peaks and TN (Model K); and SE-HPLC: $R = 0.96$, R^2 (adj) = 0.90 for 15 'peaks' and TN (Model M)). The regression equations for Models K and M were validated. RP-HPLC or SE-HPLC could be used to provide effective indices of maturity.

It was possible to conclude that: varietal differences could be captured using either the SE-HPLC method or RP-HPLC; differentiation of the source of cheese within a variety was not possible using SE- or RP-HPLC methods; a standardised SE-HPLC procedure or a RP-HPLC procedure could be developed for accurately predicting the sensory maturity Cheddar cheese; and that the SE-HPLC procedure provided the most practical instrumental means of estimating the sensory maturity of Cheddar cheese.

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