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# Monitoring the ripening process of Cheddar cheese based on hydrophilic component profiling using gas chromatography-mass spectrometry

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## ABSTRACT

We proposed an application methodology that combines metabolic profiling with multiple appropriate multivariate analyses and verified it on the industrial scale of the ripening process of Cheddar cheese to make practical use of hydrophilic low-molecular-weight compound profiling using gas chromatography-mass spectrometry to design optimal conditions and quality monitoring of the cheese ripening process. Principal components analysis provided an overview of the effect of sodium chloride content and kind of lactic acid bacteria starter on the metabolic profile in the ripening process of Cheddar cheese and orthogonal partial least squares-discriminant analysis unveiled the difference in characteristic metabolites. When the sodium chloride contents were different (1.6 and 0.2%) but the same lactic acid bacteria starter was used, the 2 cheeses were classified by orthogonal partial least squares-discriminant analysis from their metabolic profiles, but were not given perfect discrimination. Not much difference existed in the metabolic profile between the 2 cheeses. Compounds including lactose, galactose, lactic acid, 4-aminobutyric acid, and phosphate were identified as contents that differed between the 2 cheeses. On the other hand, in the case of the same salt content of 1.6%, but different kinds of lactic acid bacteria starter, an excellent distinctive discrimination model was obtained, which showed that the difference of lactic acid bacteria starter caused an obvious difference in metabolic profiles. Compounds including lactic acid, lactose, urea, 4-aminobutyric acid, galactose, phosphate, proline, isoleucine, glycine, alanine, lysine, leucine, valine, and pyroglutamic acid were identified as contents that differed between the 2 cheeses. Then, a good sensory prediction model for "rich flavor," which was defined as "thick and rich, including umami taste and soy sauce-like flavor," was constructed based on the metabolic profile during ripening using partial least squares regression analysis. The amino acids proline, leucine, valine, isoleucine, pyroglutamic acid, alanine, glutamic acid, glycine, lysine, tyrosine, serine, phenylalanine, methionine, aspartic acid, and ornithine were extracted as ripening process markers. The present study is not limited to Cheddar cheese and can be applied to various maturation-type natural cheeses. This study provides the technical platform for designing optimal conditions and quality monitoring of the cheese ripening process.
**Key words:** metabolomics, metabolic profiling, cheese ripening, gas chromatography-mass spectrometry

## INTRODUCTION

Natural cheese is made from cow milk by first adding rennet and lactic acid bacteria starter. The final cheese is obtained after a multi-step manufacturing process. Biochemical changes occur in the cheese, especially during ripening, resulting in the development of a specific flavor, aroma, and texture (McSweeney, 2004). Several factors, including the composition of the ingredients (added salts, enzymes, and lactic acid bacteria) and the degradation and catabolism that occur during ripening, result in considerable diversity in the compounds that lead to the phenotype expressed as cheese quality. Sensory characteristics of natural cheese are expressed in terms of synergistic nonlinear interactions between various components, making these characteristics difficult to elucidate and control scientifically by targeting a single or just a few components. In the cheese industry, it is difficult to scientifically grasp the complex components affecting the quality of cheese and the dynamic change during maturation in relation to the quality of cheese; thus, artisan skills based on long-term experience are still required to manage the quality and manufacturing.

A variety of metabolites are formed during the vital activity of microorganisms, plants, and animals. The whole metabolites are called metabolomes and the area of comprehensive analytical study of metabolomes is metabolomics. Because metabolomics can analyze

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many kinds of massive compounds uniformly and exhaustively, it helps to reveal the high resolution of phenotype and metabolic fluctuation. Metabolomics is an effective post-genomic research tool that has been applied to many disciplines, including the study of human diseases, nutrition, drug discovery, and plant physiology (Wishart, 2008; Drexler et al., 2011; Herrero et al., 2012; Ma et al., 2012; Nadella et al., 2012). Because metabolic profiling based on metabolomics is an extremely effective tool not only for the analysis of biological metabolisms, but also for the analysis of food science processes, the application of metabolic profiling for monitoring the quality, processing, and safety of both raw materials and final products in food science has recently attracted attention (Cevallos-Cevallos et al., 2009; Aiello et al., 2011; Cevallos-Cevallos and Reyes-De-Corcuera, 2012; Kuang et al., 2012; Mannina et al., 2012; Putri et al., 2013). Metabolomics holds major promise as an effective tool for solving the problem of qualitatively evaluating the complexity of cheese.

Recent studies have focused on the hydrophilic taste-active compounds in cheese, such as the water-soluble components in Cheddar cheese (Andersen et al., 2010), Gouda cheese (Toelstede and Hofmann, 2008), Swiss cheese (Biede and Hammond, 1979a,b), Comte cheese (Salles et al., 1995), and goat milk cheese (Engel et al., 2000). Therefore, the application of metabolic profiling of the hydrophilic components in cheese represents a major advance toward scientifically expressing the sensory characteristics of cheese. It is meaningful if we can understand the sensory qualities of cheese in terms of the components of the metabolome and use this understanding to design optimal manufacturing conditions for quality.

Based on this idea, we conducted metabolic profiling using GC-MS, targeting hydrophilic low-molecular-weight components in Cheddar and Gouda cheeses with various degrees of ripening, successfully resulting in the construction of highly precise sensory prediction models for 2 sensory attributes expressing important parts of maturation, "rich flavor" and "sour flavor" (Ochi et al., 2012b). Additionally, for using the metabolomics-based cheese quality evaluation tool at manufacturing sites, we conducted metabolic fingerprinting using a gas chromatography-flame ionization detector and successfully reconstructed the sensory prediction model with metabolic profiling from GC-MS as previously reported (Ochi et al., 2012a). Our previous reports revealed that metabolomics-based component profiling, focusing on hydrophilic low-molecular-weight components, was able to predict the important aspects of the sensory characteristics related to ripening, identify the contributing compounds, and be expanded practically to manufacturing sites.

The results obtained from diverse cheese samples with various degrees of ripening could be applicable to cases in which a given kind of natural cheese changes its metabolome during the ripening process, resulting in changing sensory characteristics. However, no published research exists validating this. The focus of the present study is not so much to precisely describe the specific case of Cheddar cheese manufacturing in terms of the transition of component compounds as to demonstrate the methodology applicable to universal cases, which means the novel framework for monitoring cheese ripening, by linking metabolome analysis to multivariate analysis and validate its effectiveness.

In this study, we conducted metabolic profiling targeting hydrophilic low-molecular-weight compounds with GC-MS for cheese samples with different sodium chloride contents or kinds of lactic acid bacteria starter, changing over ripening time under different ripening temperatures. The objectives of this study were (1) to reveal the differences in metabolic profile caused by manufacturing conditions such as ingredients, (2) to construct a model corresponding to sensory characteristics using metabolic profiling, and (3) to investigate the compounds that may work as markers of cheese maturation.

## MATERIALS AND METHODS

### *Cheddar Cheese Samples*

Three kinds of Cheddar cheese were manufactured by Morinaga Milk Industry Co. Ltd. (Higashihara Zama, Kanagawa, Japan) in the usual way (Scott, 1998) and coded as A, B, and C. They were manufactured at the same production site using the same sort of raw milk. The differences in these samples were as follows: the same lactic acid bacteria starter was used for A and B, but A was a normal salt type and cheese B was a salt-free type. Cheese C was a normal salt type, whereas the lactic acid bacteria starter used was a different kind from that of cheese A and cheese B. Thirteen samples, consisting of 1 sample before ripening and 12 samples under different ripening temperatures and ripening periods, were collected for A, B, and C. The ripening conditions were set as follows: the ripening temperatures were 5, 10, and 15°C and the ripening periods were 1, 3, 6, and 9 mo. Ripening was conducted as a 20-kg block of cheese. Collected samples of a predetermined period under each temperature condition were reserved at a superchilling temperature (−3°C) to stop maturation until sensory evaluation.

The composition of cheeses A, B, and C (before ripening) was as follows: 34.8% moisture content, 26.5% protein content, 34.2% fat content, and 1.6% sodium