Rapid and Simultaneous Measurement of Organic Acids and Amino Acids in Cheddar Cheese Using Spectroscopy

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ABSTRACT

The flavor of Cheddar cheese influences its acceptance, price and application. Organic acids and amino acids are important flavor compounds and hence their quantification is essential. Chromatographic methods are complicated, time-consuming, and expensive. The potential of using Fourier transform infrared (FTIR) spectroscopy to rapidly and simultaneously measure organic acids and amino acids was investigated.

Twelve different Cheddar cheese samples were sampled on days 7, 15, 30, 45 and 73 during ripening and the water soluble compounds were extracted using organic solvents. The extracts were analyzed by reverse phase liquid chromatography for 3 organic acids, gas chromatography for 15 amino acids, and FTIR to collect the spectra (4000-700 cm^{-1}). The organic acid and amino acid concentrations were correlated with the FTIR spectra and analyzed by multivariate regression analysis to build prediction models.

The developed models showed excellent fit with coefficient of correlation >0.95 and could simultaneously determine the levels of organic acids and amino acids in cheese samples in less than 20 min. The estimated standard errors for predicting unknown samples were less than 4% of the actual value. Absorptions from organic acids and amino acids (1800-900 cm^{-1}) were found to be very important factors influencing the PLS models. Lactic acid, glutamic acid, leucine, asparagine, phenylalanine and valine are some of the compounds that exhibited significant changes during ripening.

This method shows great promise as a rapid analytical tool for simultaneous determination of organic acids and amino acids in cheese. It can save time, labor and operational costs for the industry and cheese research.

INTRODUCTION

About **9.13 billion pounds** of cheese is produced in the US every year, of which 34% is cheddar cheese. Flavor of cheddar cheese significantly influences its consumer acceptance, price and food processing application. **Organic acids and amino acids are important flavor compounds.** Therefore determination of their levels is important to understanding their influence on cheese quality. Currently available chromatography based methods are complicated, time-consuming, expensive, and require method-specific accessories. **Hence, rapid and cost-effective analytical methods that can simultaneously determine organic acids and amino acids are being sought by the industry as well as research institutions.** However, heterogeneous composition of cheese make it difficult to develop rapid and reliable instrumental methods for flavor quality analysis.

Fourier transform Infrared (FTIR) spectroscopy, which utilizes the light absorbing properties of chemical compounds, can be used as a rapid, inexpensive, and sensitive method to analyze cheese flavor. Unlike many chromatographic techniques, FTIR spectroscopy provides unique chemical fingerprints (spectra) of cheese samples. The spectra represents the overall composition of several functional groups. This exclusive feature of **FTIR when combined with a multivariate statistically technique can enable simultaneous determination of multiple components.** Biochemical information can also be extracted, which could help in understanding the flavor-related changes occurring during ripening.

OBJECTIVE

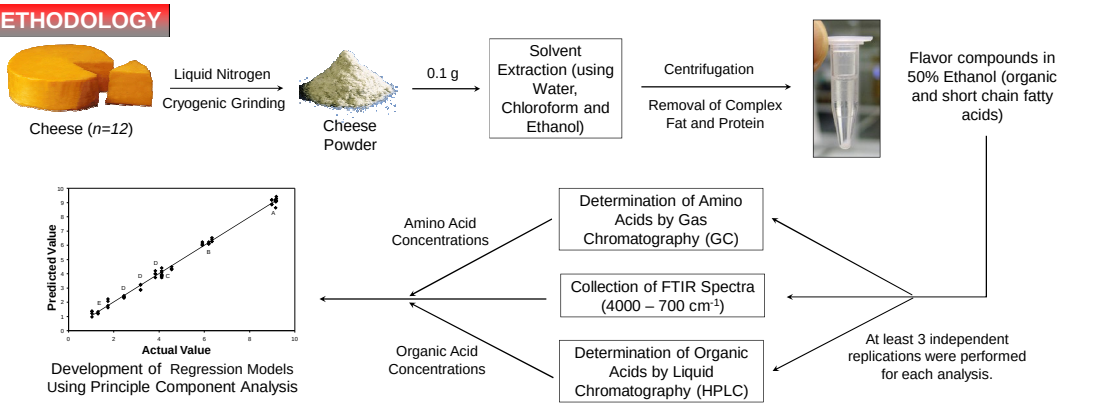
The objective of this research was to investigate the potential of using Fourier transform infrared (FTIR) spectroscopy combined with a simple extraction method and multivariate statistical analysis to rapidly and simultaneously measure organic acids and amino acids in Cheddar cheese.

SIGNIFICANCE

This rapid and simultaneous instrumental method for amino acid and organic acid analysis in cheese can:

- Speed up amino acid and organic acid analysis.
- Serve as a new tool for studying the ripening process.
- Reduce usage of hazardous solvents commonly associated with chromatographic methods.
- Be a cost-effective and time-saving tool to the cheese industry as well as research institutions.

METHODOLOGY



RESULTS

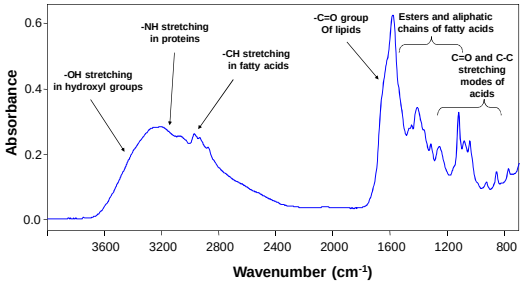


Fig 1. Typical FTIR Spectrum of Cheddar Cheese Extract

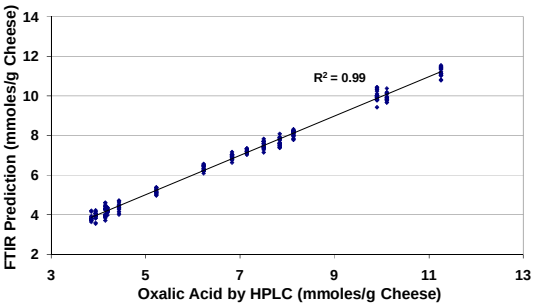


Fig 2. PLS regression model for FTIR-prediction of Oxalic Acid

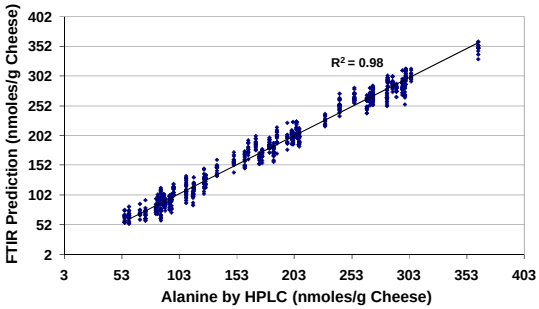


Fig 3. PLS regression model for FTIR-prediction of Alanine

Table 1. Coefficient of Determination and Standard Error of Prediction Values for Prediction of Organic Acids and Amino Acids by FTIR.

Organic Acid	R^2	Standard Error of Prediction (mmoles/g Cheese)
Oxalic Acid	0.99	0.2
Formic Acid	0.96	10.5
Lactic Acid	0.98	13.5
Amino Acid	**R^2**	**Standard Error of Prediction (nmoles/g Cheese**
Alanine	0.98	14.1
Glycine	0.98	15.1
Valine	0.97	33.0
Leucine	0.99	64.0
Isoleucine	0.97	7.0
Threonine	0.96	14.2
Serine	0.96	22.1
Proline	0.90	16.8
Asparagine	0.98	51.0
Aspartic Acid	0.94	13.9
Methionine	0.98	8.9
Hydroxyproline	0.95	9.3
Glutamic Acid	0.97	85.1
Phenylalanine	0.98	32.7
Glutamine	0.97	27.1
Arginine	0.98	23.6
Lysine	0.97	24.7
Histidine	0.90	12.6
Tyrosine	0.97	11.6
Tryptophan	0.90	4.7

*Standard Error of Prediction is an estimate of the error expected while predicting unknown samples.

DISCUSSION

- The novel extraction method reduced the effect of cheese matrix and yielded well-defined and consistent spectra (**Fig. 1**). Important functional group absorptions between 1800 and 900 cm^{-1} are marked.
- The standard curves developed for determination of 3 organic acids and 20 amino acids using HPLC and GC, respectively, had excellent fit with coefficient of determination (R^2) for all the standard curves greater than 0.99 (data not shown).
- The partial least squares (PLS) models developed by correlating FTIR spectra with the organic acid and amino acid content showed excellent predictive capability. Models developed for Oxalic Acid (**Fig. 2**) and Alanine (**Fig. 3**) are shown.
- The R^2 and standard errors of prediction (estimate of the error expected while predicting unknown samples), shown in **Table 1**, clearly indicate the potential of this technique as a accurate, quick and convenient alternative.
- Lactic acid, glutamic acid, leucine, asparagine, phenylalanine, and valine showed the greatest amount of change, with their concentrations increasing by at least 4 times by the end 73 days of ripening (data not shown).
- Total analysis time for the determination of 3 organic acids and 20 amino acids by FTIR was less than 20 min per sample and required just 1 mL of organic solvents.

CONCLUSIONS

- Previous research with Swiss cheese have shown potential for accurate determination of 18 cheese flavor characteristics. Furthermore, the capability of this method to predict the flavor quality of cheese and the composition (moisture, salt, and fat) has been established previously.
- With this research extending the applications to organic acid and amino acid analysis, a great potential exists for this technique as a rapid, cost-effective, nearly reagent-free, accurate, and simple method for simultaneous determination of cheese characteristics.

CONCLUSIONS

- Subramanian, A., Harper, W.J., and L.E. Rodriguez-Saona. 2009. Cheddar cheese classification based on flavor quality using a novel extraction method and Fourier transform infrared spectroscopy. *Journal of Dairy Science*, 92: 87-94.
- Subramanian, A., and L.E. Rodriguez-Saona, inventors. 2008. Rapid Extraction Method for Analysis of Cheese Flavor Using Infrared Spectroscopy. The Ohio State University, assignee. Provisional Patent Appln. Ser. No. 61/059,890.

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