



Global Cheese TECHNOLOGY FORUM

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Student Research Poster Abstracts

Growth Characteristics — Salt, pH, and Temperature Tolerance of *Lactobacillus wasatchensis*

Isaac B. Bowen¹, Donald J. McMahon¹, Craig J. Oberg², and Michele D. Culumber²

¹Department of Nutrition, Dietetics and Food Sciences, Utah State University, Logan, Utah

²Department of Microbiology, Weber State University, Ogden, Utah

Growth characteristics of *Lactobacillus wasatchensis*, a nonstarter lactic acid bacteria that can produce unwanted gas formation during the storage of cheese, was studied. Previous research had shown that *Lb. wasatchensis* can grow at pH 5.2 in up to at least 5% salt concentration. There was also some preliminary evidence that it has some survival under high temperature short time (HTST) pasteurization conditions. In this study, growth characteristics were studied by inoculating *Lb. wasatchensis* WDC04 into 24 micro-well plates in MRS-ribose broth at various salt concentrations and pH. Growth was monitored by measuring absorbance at 600 nm. The upper limit of salt tolerance of *Lb. wasatchensis* was determined to be 5.8% salt. At a standard cheddar cheese pH range (pH 5 to 6), *Lb. wasatchensis* was shown to grow well, with growth limited above pH 7 and at pH 4 and below. Whether *Lb. wasatchensis* can survive pasteurization was studied by processing 30-liter batches of milk inoculated with 10^8 cfu/ml of *Lb. wasatchensis* WDC04 through an HTST pasteurizer with set temperatures of 69, 72, 74, and 76°C and a holding time of 15 seconds. Under these conditions, no survival of *Lb. wasatchensis* was observed at any of the temperatures. In conclusion, we found that *Lb. wasatchensis* is well suited for growing in cheddar cheese during storage as it can grow at the salt and pH conditions typical of cheddar cheese, and would not be inhibited by salt in a 37% moisture cheese until the salt content was 2.2% or higher. Since there was at least an 8-log destruction of *Lb. wasatchensis* during normal pasteurization, or even sub-pasteurization heat treatment of milk 69°C (156°F), its presence as part of the NSLAB microbiota of cheese appears to be through other environmental contamination.

Utilization of Acid Whey in Extruded Products

Ashton Yoon & Syed Rizvi, Department of Food Science, Cornell University, Ithaca, New York

Acid whey, a low-value byproduct, is a problem-child of the dairy industry and presents a substantial challenge to convert it into value-added products. Addition of 'as is' and concentrated acid whey in lieu of water during manufacture of extruded products represents a unique opportunity to convert it into value added snacks and other related products. Commercial acid whey was concentrated to different levels using a vacuum evaporator. Formulations containing nonfat dry milk (NFDM) and milk protein concentrate (MPC-80) were converted into extrudates with supercritical fluid extrusion (SCFX) by injecting acid whey at concentrations up to 3.5x. The process protocols were based on previously

established operating conditions. The product responses (bulk density, piece density, expansion ratio, water soluble index, water absorption index, color and texture) were analyzed statistically. It was observed that while acceptable, the high acid whey concentration significantly affected product characteristics due high lactose content. Based on the results to date, new strategies we are currently pursuing to enhance the potential for acid whey utilization in extruded products via SCFX will be presented and discussed.

Comparing Composition and Maturation of Camembert Cheese Variants

*Danton Batty, Joy Waite-Cusic, Lisbeth Meunier-Goddik
Department of Food Science and Technology, Oregon State University, Corvallis, Oregon*

Camembert cheese can be produced by several different processes. From these different make procedures, multiple styles are achieved. These include the traditional lactic curd, rennet curd types, and extended shelf life stabilized curd. The objective of this study was to compare the difference in physiochemical properties and maturation of multiple Camembert cheese styles. Having a better understanding of how these recipes perform allows the cheese maker to better meet their customer needs. Five varieties of Camembert cheese were manufactured and analyzed for protein, fat, moisture, sodium and minerals. The firmness, pH and color were also evaluated throughout the maturation period. Between the different varieties there were significant differences across most compositional components. The most notable being moisture content, ranging from 52.8% to 59.5% for the solubilized curd and lactic curd, respectively. Another notable difference in composition is %Ca/SNF, with the lactic curd variety being the lowest and stabilized variety being highest at 0.73% and 1.12%, respectively. The decrease in firmness during maturation was also significantly different with the sweet curd low pH variety decreasing the most (4.25 N) and the stabilized variety decreasing the least (0.44 N) at day 35 into maturation. The pH during maturation followed the same trend for all cheeses. By the 50th day of maturation all cheeses had a paste pH greater than 7 even though the initial pH varied greatly (4.2 to 5.2). Understanding how these different recipes influence the composition and maturation could be of useful to any bloomy rind cheese manufacturer when deciding on a make procedure.

Impact of Milk Hauling Practices on Microbiological Quality

Eva Kuhn, Lisbeth Goddik, Joy Waite-Cusic
Department of Food Science and Technology, Oregon State University, Corvallis, Oregon*

The Pasteurized Milk Ordinance (PMO) allows for milk tanker trucks to be used repeatedly for 24 hours before mandatory clean-in-place (CIP) cleaning. There are no specifications for length of time a tanker can be empty between loads. We partnered with a Pacific Northwest dairy company to investigate if extended idle time between loads influences microbiological populations in subsequent loads of milk. This processor does not allow tanker trucks to sit idle between loads for more than 6 hours. Two farms were selected to participate in the study based historical microbiological data from January 2014 through December 2015, quantified using Foss Bactoscan and reported as individual bacteria count (IBC) and preliminary incubation count (PIC). Historically, Farm A IBC and PIC (n=729) averaged 47.8 and 432.3, and

Farm B (N=982) had substantially lower average IBC and PIC (8.8 and 13.2). The study occurred over six consecutive days; for three days Farm B milk was collected immediately after unloading farm A, and the other three days Farm B milk was collected 6 hours after unloading. For each day milk samples were obtained each farm bulk tank and from the tanker prior to unloading. Each sample was microbiologically assessed in duplicate for standard plate count (SPC), lactic acid bacteria (LAB), coliforms. Colony isolates were assessed for lipolytic and proteolytic activity using spirit blue agar (SBA) and skim milk agar (SMA), respectively. There was not a significant difference in microbiological counts and enzyme activity in farm B's tanker sample where comparing 0 and 6 hours between hauling. We have demonstrated that 6 hours between loads does not negatively impact subsequent loads of milk, and that the processors parameters are adequate.

Accurate Monitoring of Living and Total Bacterial Populations in Milk to Predict Cheese Defects

Zhengyao Xue¹, Zachary Quart¹, Mary Kable², Jessie Heidenreich³, Jeremy McLeod³ and Maria L. Marco¹

¹Department of Food Science and Technology, University of California Davis, Davis, California

²US Department of Agriculture, Western Human Nutrition Research Center, Davis, California

³Hilmar Cheese Company, Hilmar, California

Milk and cheese are ecosystems that can harbor diverse bacterial communities and are generally vulnerable to spoilage and other defects. Cheese defects constitute a considerable burden to the dairy industry, among which slits are one of the most important concerns for Cheddar cheese. To advance the goal of producing cheese free of slit defects, we employed modern, high-throughput DNA sequencing methods to understand how the bacterial diversities of raw and pasteurized milk affect the quality attributes of the final cheese product. Thus far, we have shown that the bacterial communities in raw and pasteurized milk undergo significant alterations at each heating and mixing step. More importantly, we found that pasteurized milk resulting in either high quality or slit-forming cheeses contain distinct microbiomes. Specifically, endospore-forming and thermophilic bacterial taxa are correlated with slit development in cheese and the abundance of these taxa in pasteurized milk used directly for cheese making are highly dependent on equipment-cleaning schedules. Our findings show how this innovative approach can be used by the dairy industry to identify, manage, and prevent detrimental bacterial contamination during production and processing to ultimately result in consistent cheese products with optimal sensory profiles and nutritive benefits.

Application of Qualitative Multivariate Analysis for Consumer Insights on Cheddar Cheese Shreds

Katherine Speight, Angelina Schiano, William Harwood and MaryAnne Drake

Department of Food, Bioprocessing, and Nutrition Sciences

North Carolina State University, Raleigh, North Carolina

Pre-packaged Cheddar cheese shreds are a ubiquitous item. Knowledge of key consumer attributes for purchase decision and satisfaction are crucial for product success and innovation. Recent studies have not established attributes that consumers prefer when purchasing pre-packaged cheese shreds. Qualitative multivariate analysis (QMA) provides

a unique approach to define consumer desires. The objective of this study was to determine the attributes of Cheddar cheese shreds that impact consumer liking and satisfaction. Nine representative Cheddar cheese shreds were chosen for QMA with consumer in-home testing. Thirteen consumers were selected to evaluate each product for 4 weeks and record their thoughts in an online diary. Likes, dislikes and preferences were tabulated. Subsequently, consumers participated in a 2.5 h follow up discussion to summarize likes and dislikes. Meltability, orange color, lack of clumps, ability to re-seal and “Cheddar” flavor were preferred Cheddar shred qualities by consumers. These results can be used by processors for product optimization.

Sugar Reduction Design Rules: Effect of Food Matrix on Sweetness Perception

*Ty B. Wagoner, Heather R. McCain, E. Allen Foegeding and MaryAnne Drake
Department of Food, Bioprocessing and Nutrition Sciences,
Southeast Dairy Foods Research Center, North Carolina State University, Raleigh, North Carolina*

In an effort to formulate healthier food products, sugar is often reduced or replaced with artificial or natural non-nutritive sweeteners. However, the removal of sucrose often modifies food texture, and non-nutritive sweeteners inherently exhibit differences in sweetness quality, onset, and overall intensity. Therefore, the objectives of this study were to 1) evaluate the effects of texture on sweetness dose-response profiles of whey protein solutions sweetened with sucrose, sucralose, stevia, or monk fruit extracts, 2) determine sweetener concentrations required for iso-sweetness with 6% w/w sucrose, and 3) assess temporal progression of dominant attributes. Whey protein-based model foods were developed without compositional changes by varying heating time to generate thin fluid, thick fluid, and semi-solid structures with different textural properties. Dose-response power functions and iso-sweet equivalencies were determined using magnitude estimation scaling. Temporal profiles of dominant texture, taste, and flavor attributes were evaluated using temporal dominance of sensations (TDS). A significant texture effect was observed ($p < 0.05$), where more viscous or semisolid textures required greater amounts of sweetener for iso-sweetness. Moreover, the dose-response slope decreased with increasing texture for sucralose, stevia, and monk fruit-sweetened textures. Results of TDS indicated that increased texture prolonged the dominance of several attributes. The results of this study indicate that food matrix, sweetener type, and usage level need to be considered when attempting to modify texture or reduce sucrose.

Identification of the Source of Volatile Sulfur Compounds in Milks During Thermal Processing

*Yejin Jo¹, Brandon G. Carter¹, David M. Barbano² and MaryAnne Drake¹
¹Department of Food, Bioprocessing, and Nutrition Sciences
North Carolina State University Raleigh, North Carolina.*

²Northeast Dairy Foods Research Center, Department of Food Science, Cornell University, Ithaca, New York

Flavor of milk is a key quality attribute discerned by consumers. The presence of volatile sulfur compound flavors in milk decrease consumer liking. These sulfurous off flavors are presumably formed by whey protein denaturation and

increase with increased heat load and rate of heating. Previous research has established distinct differences in flavor profiles of fluid milk processed by high temperature short time pasteurization (HTST) and ultrapasteurization (UP) by indirect heat (IND-UP) or direct steam injection (DSI-UP). Studies have not addressed the source of sulfur flavor compounds or how they are formed during milk heat treatment. The objective of this study was to elucidate the source and formation of volatile sulfur compounds in fluid milk with a specific focus on the comparison of protein source and temperature effects on milks by HTST and UP. Raw skim milk was obtained and served as a control. Reformulated skim milks (RSM) were manufactured by blending freshly processed micellar casein concentrate (MCC) 95% serum protein reduced or serum protein isolate (SPI) at equivalent protein content as skim milk (3.3% w/v) with milk permeate. Skim milk and RSM were pasteurized at 78°C for 15s (HTST) or 140°C for 2.3s by IND-UP or DSI-UP. Sensory properties of milks were documented by a trained descriptive sensory panel. Volatile sulfur compounds in milks were evaluated using solid phase micro-extraction (SPME) followed by gas chromatography-triple quadrupole mass spectrometry (GC-MS/MS) combined with a sulfur selective flame photometric detector. Sensory panelists confirmed increased cooked and sulfur/eggy flavors in skim and RSM processed by DSI-UP followed by IND-UP and HTST. RSM with SPI had higher sulfur/eggy flavors compared to skim milk or MCC RSM at any temperature ($p < 0.05$). RSM with SPI contained higher concentrations of hydrogen sulfide, carbon disulfide, dimethyl sulfide, dimethyl disulfide, dimethyl trisulfide, and methional compared to MCC RSM or skim milk following DSI or IND UP ($p < 0.05$). The results demonstrate that off flavor sulfur compounds are primarily sourced from the serum protein fraction of milk proteins and provide baseline information for development of milk based beverages with decreased undesirable sulfur flavors from high heat treatments.

Comparison of Central Location and Home Use Tests for Ready-to-Mix Whey Protein Beverages

*M.T. Zhang, Y. Jo, Stephanie Meals Koletsos and MaryAnne Drake
Department of Food, Bioprocessing, and Nutrition Sciences
North Carolina State University Raleigh, North Carolina*

Ready-to-Mix whey protein beverages (RTMBEVS) are an expanding product category and consumer acceptance of these products is important. Previous research has indicated that results obtained from Central Location Testing (CLT) and Home Use Testing (HUT) can vary depending on product type. CLTs are less expensive and since RTMBEVS are prepared at-home by consumers, understanding possible gaps between CLT and HUT results are critical for this category. The objective of this study was to compare results obtained from a CLT and a HUT. Ten representative RTMBEVS were rehydrated with spring water and evaluated by protein beverage consumers ($n=160$) for a CLT. Testing was conducted across two days with five beverages evaluated per day. Nine representative RTMBEVS were selected for a HUT ($n=122$) where consumers were allowed to choose their mixing method (shaker, blender, stirring) and liquid base (water or skim milk). Consumers evaluated beverages over three weeks, three samples per week. Overall liking and other attributes were scored by both consumer groups. Data were evaluated by univariate and multivariate analyses. Overall liking scores from the HUT were higher than scores from the CLT ($p < 0.05$). The products with the highest and lowest overall liking scores were consistent between CLT and HUT. More differences ($p < 0.05$) were observed among samples by CLT compared to HUT, when liking was averaged across all consumers. However, similar differentiation of overall liking among beverages were obtained by both methods. Following cluster analysis, more beverages obtained differences ($p < 0.05$) in overall liking among clusters by HUT compared to CLT. Similar drivers of liking (fruity flavor and

sweet taste) were identified for one cluster by both methods. Key drivers of liking for cluster 2 were fruity and buttery flavor by the CLT and viscosity by the HUT. These results indicate that a CLT can be utilized to differentiate consumer acceptance of taste and flavor among RTMBEVS, with some potential shortcomings on mouthfeel and mixing experience related attributes.

Use of Acid Whey Protein as an Ingredient in Nonfat Set-Style Yogurt

Bryan Wherry¹, David M. Barbano² and MaryAnne Drake¹

*¹Department of Food, Bioprocessing, and Nutrition Sciences
North Carolina State University Raleigh, North Carolina.*

²Northeast Dairy Foods Research Center, Department of Food Science, Cornell University, Ithaca, New York

Acid whey, resulting from the production of soft cheeses, is a disposal problem for the dairy industry. Few uses have been found for acid whey because of its high ash content, low pH, and high organic content, which requires costly waste treatment. Acid whey disposal results in losses of over 100 million pounds of whey protein. The objective of this study was to explore the potential of whey protein from acid whey in yogurt applications. Cottage cheese acid whey and Cheddar cheese whey were produced from standard Cottage cheese and Cheddar cheese make procedures, respectively. Food grade ammonium hydroxide was used to neutralize the acid whey. The whey and skim milk were concentrated using an ultrafiltration system equipped with 11 polyethersulfone cartridge membrane filters. Nonfat, unflavored set-style yogurt made with acid whey protein (AWP) was compared to yogurt made with more sweet whey protein (SWP) and yogurt made from skim milk concentrate (SM). Yogurt mix was standardized to protein, lactose, and fat of 6.00%, 6.50% and 0.10% respectively. Yogurt was fermented to pH 4.6 and stored at 4°C. The experiment was replicated in triplicate. The yogurts were measured over a period of 8 weeks for pH, titratable acidity, whey separation, color, and gel strength. Trained panel profiling was conducted on days 0, 14, 28, and 56. Yogurt made with AWP had similar color and titratable acidity to those made with SWP ($p>0.05$). Yogurt with AWP had higher values of syneresis, lower gel strength, higher sour taste and lower firmness and viscosity compared to yogurts made with SWP ($p<0.05$). Both yogurts with AWP and SWP were distinct in sensory character from the control yogurt made from SM. These results indicate that AWP can be used as an ingredient in yogurt.

Comparison of Liquid Chromatography Methods for Specialty Free Amino Acid Analysis from Dairy Foods

*Kenneth George Vogel III, D.M. Benoist, MaryAnne Drake
Department of Food, Bioprocessing, and Nutrition Sciences
North Carolina State University Raleigh, North Carolina*

Free amino acids are increasingly popular food ingredients as consumers seek to increase their intake of specific amino acids and amino acid analogs. Branched chain amino acids are perceived to increase muscle mass, other free amino acids such as theanine can reduce stress and improve cognition. Protein beverages, bars and gels are all popular vehicles for these functional ingredients. Separation and detection of these compounds is necessary for appropriate dosage into foods, but can be challenging.

The objective of this study was to compare multiple liquid chromatography methods for the quantification of free amino acids. Whey protein beverages (n=6) and gels (n=2) were the foods evaluated. Three representative amino acids were selected for evaluation: theanine, isoleucine and valine. Free amino acids were extracted by warm water, methanol and sonication/agitation followed by solid phase extraction (SPE) with a C18 SPE column. Following extraction, free amino acids, L-theanine, L-isoleucine, and L-valine were detected and quantified by HPLC-PDA with precolumn derivitization by OPA (orthophthalaldehyde) and 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC), UPLC-FLR (fluorescence) with precolumn derivitization by OPA and AQC, and by UPLC-MS (mass spectrometry) with simultaneous UPLC-PDA (photodiode array). Methods were analyzed for speed, limits of quantification (LOQ), limits of detection (LOD) and separation (resolution) of compounds. Precolumn derivitization allowed for clear detection of the selected amino acids by both HPLC-PDA and UPLC-FLR. The isoindole compound formed from the OPA derivitization was, however, prone to degradation making the timing of the reagent addition and injection critical. OPA derivitization therefore required manual input for each injection. Both UPLC-FLR and UPLC-PDA with AQC derivitization produced good separation and quantification of the selected free amino acids without the time sensitivity of OPA derivitization. UPLC-MS with simultaneous PDA provided an LoD 13 femtomoles and a LoQ of 130 femtomoles as well as good selectivity. These results define the benefits and detriments to each method for amino acid analysis in dairy matrices, allowing product developers informed choices when selecting a method to quantify free amino acids.