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### **XVIII 18-04 HYDROLYSIS OF LACTOSE IN WHEY PERMEATE FOR SUBSEQUENT FERMENTATION TO ETHANOL**

A. Coté, W. A. Brown, D. Cameron and G. P. van Walsum  
J. Dairy Sci. 87(6): 1608. 2004.

This study proposes an alternative strategy: treat the permeate with acid to liberate monomeric sugars that are readily fermented into ethanol. They identified optimum hydrolysis conditions that yield mostly monomeric sugars and limit formation of fermentation inhibitors such as hydroxymethyl furfural by caramelization reactions. Both lactose solutions and commercial whey permeates were hydrolyzed using inorganic acids and carbonic acid. In all cases, more glucose was consumed by secondary reactions than galactose. Galactose was recovered in approximately stoichiometric proportions. Whey permeate has substantial buffering capacity—even at high partial pressures (>5500 kPa[g]), carbon dioxide had little effect on the pH in whey permeate solutions. The elevated temperatures required for hydrolysis with CO<sub>2</sub>-generated inhibitory compounds through caramelization reactions. For these reasons, carbon dioxide was not a feasible acidulant. With mineral acids reversion reactions dominated, resulting in a stable amount of glucose released. However, the Maillard browning reactions also appeared to be involved. By applying Hammett's acidity function, kinetic data from all experiments were described by a single line. With concentrated inorganic acids, low reaction temperatures allowed lactose hydrolysis with minimal by-product formation and generated a hexose-rich solution amenable to fermentation.

### **XVIII 19-04 STRUCTURAL ANALYSIS OF A NEW ANTI-HYPERTENSIVE PEPTIDE (β-LACTOSIN B) ISOLATED FROM A COMMERCIAL WHEY PRODUCT**

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J. Dairy Sci. 87(7): 1967. 2004.

Angiotensin-converting enzyme (ACE) inhibitory activities and anti-hypertensive activities in spontaneously hypertensive rats (SHR) of 12 kinds of commercial peptides of food additive grade were measured. Four peptide products derived from milk proteins showed strong anti-hypertensive activities (>−18.0 mm Hg). A sample of WE80BG derived from whey proteins showed the strongest anti-hypertensive activity (−21.2 ± 16.9 mm Hg) with a medium level of ACE inhibitory activity (53.6%), and it was subjected to hydrophobic and gel filtration chromatography. From the low molecular weight fraction,

an anti-hypertensive peptide was isolated by using reversed-phase HPLC, and it was found to be a tetrapeptide, alanine-leucine-proline-methionine (Ala-Leu-Pro-Met, ALPM), the origin of which was estimated to be  $\beta$ -lactoglobulin f 142 to 145. At 8 h after oral administration of ALPM in SHR, systolic blood pressure was significantly decreased ( $-21.4 \pm 7.8$  mm Hg), but the IC50 value (concentration of peptide needed to inhibit 50% of the ACE activity) of ALPM was not so high. We named the Ala-Leu-Pro-Met “ $\beta$ -lactosin B.” This peptide is the second anti-hypertensive peptide found from  $\beta$ -lactoglobulin. Because WE80BG containing ALPM was also found to show the strongest anti-hypertensive activity ( $-24.5 \pm 10$  mm Hg) at 8 h after oral administration in SHR, WE80BG would be suitable for application to the development of a new food expected to have anti-hypertensive effects.

**XVIII 20-04 QUALITY AT TIME OF PURCHASE OF DRIED MILK PRODUCTS COMMERCIALLY PACKAGED IN REDUCED OXYGEN ATMOSPHERE**

M. A. Lloyd, J. Zou, H. Farnsworth, L. V. Ogden and O. A. Pike  
J. Dairy Sci. 87(8): 2337. 2004.

The objective of this research was to determine the sensory and nutritional quality of these dried milk products at the time of purchase. In the 10 brands tested, wide variation existed in headspace oxygen, can seam quality, sensory quality, and vitamin A (with 6 of 10 brands entirely lacking the vitamin). Manufacturers of dried milk products packaged in cans for long-term storage need to give careful attention to can seam quality, product labeling, and vitamin fortification.

**XVIII 21-04 EFFECTS OF NATIVE AND DENATURED WHEY PROTEINS ON PLASMINOGEN ACTIVATOR ACTIVITY**

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J. Dairy Sci. 87(8): 2344. 2004.

The objective of this research was to determine the effect of both unheated and heat-denatured  $\beta$ -lactoglobulin ( $\beta$ -LG),  $\alpha$ -lactalbumin ( $\alpha$ -LA), and BSA on plasminogen activators. Plasminogen activator activity was significantly stimulated by non-heat treated and denatured  $\alpha$ -LA as well as by denatured  $\beta$ -LG. The stimulation effect by these whey proteins was kinetically characterized, which showed that all 3 significantly increased the rate of plasminogen activation. The stimulation effect was shown to be independent of any effect of the whey proteins on plasmin activity by testing 2 different substrates, D-Val-Leu-Lys *p*-nitroanilide (S-2251) and Spectrozyme PL (Spec PL), in a plasmin assay. Results confirmed the inhibitory effect of whey proteins on plasmin observed by several researchers. However, use of SpecPL did not suggest inhibition. Ligand binding studies showed this discrepancy to be due to significant interaction between S-2251 and the whey proteins. Overall, this study indicates that whey protein incorporation into cheese may not hinder plasmin activity and may stimulate plasminogen activation. Furthermore, the results indicate the need for careful consideration of the type of synthetic substrate chosen for model work involving whey proteins and the plasmin system.

**XVIII 22-04 DISTINCTION BETWEEN DRY AND RAW MILK USING MONOCLONAL ANTIBODIES PREPARED AGAINST DRY MILK PROTEINS**

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J. Dairy Sci. 87(8): 2720. 2004.

This study attempted to develop a sensitive mAb that might distinguish the DMLK from freshly prepared raw milk. To test this possibility, we immunized mice with commercially prepared DMLK and produced a panel of mAb. From 900 hybridomas screened using an ELISA, 4 clones were found to be specific to DMLK; the other 68 clones recognized both DMLK and raw milk. In contrast to polyclonal antibodies, only the specific mAb could detect the DMLK spiked into the raw milk at as low as 5% in concentration (vol/vol). Western blot analysis shows that these specific mAb were all directed against  $\beta$ -lactoglobulin (LG) and LG-milk protein conjugates. These mAb reacted with raw milk heated at 95° for 15 min; the reaction with LG-conjugates, however, was abolished when treated with reducing reagent. Thus, results suggests that a new antigenic epitope was exposed in a heating process, and the thio group of LG cross linked with other protein moiety played a provocative role in mAb recognition. A hypothetical model with respect to the interaction between the mAb and DMLK is proposed and discussed.

**XVIII 23-04 ANTIBOTULINAL ACTIVITY OF PROCESS CHEESE INGREDIENTS**

K. A. Glass and E. A. Johnson  
J. of Food Protection 67(8): 1765. 2004.

Ingredients used in the manufacture of reduced-fat process cheese products were screened for their ability to inhibit growth of *Clostridium botulinum* serotypes A and B in media. Reinforced clostridial medium (RCM) supplemented with 0, 0.5, 1, 2, 3, 5, or 10% (wt/vol) of various ingredients, including a carbohydrate-based fat replacer, an enzyme-modified cheese (EMC) derived from a Blue cheese, sweet whey, modified whey protein, or whey protein concentrate, did not inhibit botulinal growth and toxin production when stored at 30°C for 1 week. In contrast, RCM supplemented with 10% soy-based flavor enhancer, 10% Parmesan EMC, or 5 or 10% Cheddar EMC inhibited botulinal toxin production in media for at least 6 weeks of storage at 30°C. Subsequent trials revealed that the antibotulinal effect varied significantly among 13 lots of EMC and that the antimicrobial effect was not correlated with the pH or water activity of the EMC.

**XVIII 24-04 FACTORS THAT CONTRIBUTE TO THE BOTULINAL SAFETY OF REDUCED-FAT AND FAT-FREE PROCESS CHEESE PRODUCTS**

K. A. Glass and E. A. Johnson  
J. of Food Protection 67(8): 1687. 2004.

The effects of fat, type of natural cheese, and adjunct process cheese ingredients were evaluated to determine factors that contribute to the botulinal safety of reduced-fat (RF) process cheese products stored at 30°C. In the first set of experiments, pasteurized process cheese products (PPCPs) were formulated using full-fat (FF) Cheddar, 30% RF

Cheddar, or skim milk (SM) cheese as cheese-base types and were standardized to 59% moisture, pH 5.75, 2.8 or 3.2% total salts, and 15 to 19% fat. Subsequent trials evaluated the effect of fat levels and adjunct ingredients in PPCPs made with SM, RF, and FF cheese (final fat levels, less than 1, 13, and 24%, respectively). When fat levels of PPCPs were comparable (15.1, 19.1, and 16.2 for product manufactured with SC, RF, and FF cheese, respectively), botulinal toxin production was delayed for up to 2 days in PPCPs formulated with SM compared with RF or FF cheese; however, the effect was not statistically significant. When fat levels were reduced to less than 1% in SM PPCPs, toxin production was delayed 2 weeks in products made with SM compared with RF or FF cheese manufactured with 13 or 24% fat, respectively. The antibotulinal effect of adjunct ingredients varied among the products manufactured with different fat levels. Sodium lactate significantly delayed toxin production ( $P < 0.05$ ) for all fat levels tested, whereas  $\alpha$ -glucan fat replacer did not delay toxin production. An enzyme-modified cheese used as a flavor enhancer significantly delayed toxin production ( $P < 0.05$ ) in SM (less than 1% fat) products but had little to no inhibitory effect in RF (13% fat) and FF (24% fat) cheese products. Similarly, monolaurin increased the time to detectable toxin in SM products but was ineffective in RF or FF cheese products. These results verify that RF PPCPs exhibit greater safety than FF products and that safety may be enhanced by using certain adjunct ingredients as antimicrobials.

**XVIII 25-04 A VALIDATED PCR-BASED METHOD TO DETECT LISTERIA MONOCYTOGENES USING RAW MILK AS A FOOD MODEL—TOWARDS AN INTERNATIONAL STANDARD**

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*J. of Food Protection* 67(8): 1646. 2004.

A PCR assay with an internal amplification control was developed for *Listeria monocytogenes*. The assay has a 99% detection probability of seven cells per reaction. When tested against 38 *L. monocytogenes* strains and 52 nontarget strains, the PCR assay was 100% inclusive (positive signal from target) and 100% exclusive (no positive signal from nontarget). The assay was then evaluated in a collaborative trial involving 12 European laboratories, where it was tested against an additional 14 target and 14 nontarget strains. In that trial, the inclusivity was 100% and the exclusivity was 99.4%, and both the accordance (repeatability) and the concordance (reproducibility) were 99.4%. The assay was incorporated within a method for the detection of *L. monocytogenes* in raw milk, which involves 24 h of enrichment in half-Fraser broth followed by 16 h of enrichment in a medium that can be added directly into the PCR. The performance characteristics of the PCR-based method were evaluated in a collaborative trial involving 13 laboratories. This method provides a basis for the application of routine PCR-based analysis to dairy products and other foodstuffs and should be appropriate for international standardization.