0.1% (w/v) peptone water, and sets of *E. coli* suspensions at cell numbers between 101 and 104 cfu ml-1 were used for DNA extraction. Five commercial DNA extraction kits (QIAamp DNA micro kit, Dynabeads SILANE genomic DNA kit, LyseNow Food pathogen DNA extraction card, Picopure kit, DNeasy Blood and Tissue kit) and a direct boiling method were compared. DNA extractions were carried out following the manufacturer's instructions, except that the elution volume was modified to obtain DNA preparations at total volumes of 40 ul. All sets of DNA preparations were quantified by real-time PCR using the primer set URL301 and URR432 that targets the β-glucuronidase of *E. coli*. The efficiencies of DNA recovery were assessed by linear regression equations fitted to the plots of threshold cycle (Ct) values against the log numbers of *E. coli*.

Three kits, QIAamp, Dynabeads and DNeasy kits, had the lowest limit of detections (LODs) (<1 log cfu) whereas Picopure kit, LyseNow card and direct boiling method produced LOD of 3 log cfu, 2 log cfu and 1 log cfu, respectively. QIAamp, Dynabeads and DNeasy kits showed similar efficiency for DNA recovery with the slopes of -3.16, -3.18 and -3.24, the R2 values of 0.989, 0.991 and 0.997, respectively.

Assessment of DNA extraction procedures with minimum elution volumes for the recovery of *E. coli* DNA at low levels is of practical importance. The findings of this study show that DNA from 1 log cfu *E. coli* can be efficiently recovered by QIAamp, Dynabeads or DNeasy kits for quantification by real-time PCR.

Assessment of Ractopamine in Meat and Bone Meal through LC-MS/MS Using Solid Phase Extraction (SPE) **UP303**

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Introduction and Objectives: Ractopamine (RCT) used as feed additive acts metabolically to decrease fat content contributing to a lean carcass. However, RCT is not broadly accepted worldwide but limited to stringent rules imposed by some countries. To assure human consumption safety, governments are encouraging research institutions to develop sensitive methods to accurately detect and quantify RCT. Yet, little is known about RCT concentration in raw materials such as meat and bone meal (MBM). The effectiveness of a previously developed method for RCT analysis was tested to determine sources of RCT contamination in MBM and discern from potential overestimation in feed.
Methods: A method previously developed by SupelMIPTM SPE - Beta-agonists Sigma-Aldrich to quantify RCT in tissues was modified in this work to quantify RCT in MBM. Briefly, samples were hydrolyzed with protease and β-glucuronidase. Later, solvent extraction was conducted using ethyl acetate. The dried sample was ressuspended in MeOH/H2O and extracted with hexane. A final purification step was performed in SPE MIP cartridge. Samples were analyzed in a LC-MS/MS equipped with an ESI source operated in the positive-ion mode. To quantify RCT losses along the extraction processes, samples were spiked (100 ng/g) prior to hydrolysis with protease and β-glucuronidase as well as prior to ethyl acetate, hexane, and SPE.
Results: Hydrolysis reaction parameters and conditions were set to optimize enzymes activities. Ethyl acetate which has the same polarity as RCT was used as a solvent extract. Overall, RCT recovery in MBM ranged from 39.7 to 83.8%. The first three steps in extraction procedure resulted in higher RCT loss (with recoveries of 39.7, 52.5 and 59.5% respectively). The last two steps in sample

extraction showed RCT recoveries of 81.8 and 83.8%, respectively. Thus, compared to solvent extraction and SPE, considerable RCT losses were attributable to hydrolysis processes.
Conclusions: The development of an efficient methodology to accurately quantify a wide range of RCT concentration in MBM samples of high matrix complexity is very challenging. In this work, the proposed modified methodology showed recovery efficiencies as low as 39.7%. Hydrolysis seems to play a major role in RCT loss. The use of an internal standard should be considered in further experiments to account for RCT losses during each extraction procedure.

Evaluation of the Viability of Lactic Acid Bacteria in 3 Yogurts Added with Prebiotics, during Fermentation, Shelf Life as well as Their Inhibitory Effect In Vitro Towards a Strain of Escherichia Col **UP304**

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Changes in feeding habits, have come with digestive problems in the population, the milk industry has focused its interest in solving the problem developing simbiotic products. A simbiotic product must contain 10 7-10 8 UFC/g of the probiotic strain, to be considered one. The aim of the present work, was to measure the viability of the Lactic Acid Bacteria (LAB) of yogurt (L. delbrueckii subesp. bulgaricus, L. cremoris y S. thermophilus) during the fermentation and shelf life of yogurt, as well as their inhibitory effect on a strain of Escherichia coli. Yogurts were prepared using prebiotic (inulin 2.5%, polidextrose 4% or inulin:polidextrose 40:60 4%), during the fermentation period of the 3 yogurts, growths in selective agar was carried out to measure Lactobacillus (MRS agar) and Streptococcus (M17 agar), cutures were carried out each hour during the 4h fermentation period. In the 3 yogurts analyzed, Lactobacillus and Streptococcus growth, follows the classical microorganisms growth curve, Lactobacillus use better inuline during the first part the fermentation reflected as an increment in the viable Lactobacillus in the yogurt (6.9 X 10 8 CFU/mL), while Streptococcus do not seem to have a preference for any of the prebiotics used. The shelf life of the yogurts was monitored during 32 days at 5ºC, measurements carried out during this period were: pH, viability of LAB each 4 days in MRS and M17 agar. The lowest pH (3.9), was found in the yogurt added with inulin, sensory evaluation of the products showed that the products had a slight smell and acidic taste, as well as slight whey separation. After the 32 dayss of the trial the 3 yogurts kept having Lactobacillus and Streptococcus counts over 10 8 CFU/ml. The yogurt added with a mixture of prebiotics, showed a peak in Lactobacillus and Streptococcus counts on day 8 (3.2x 10 10 CFU/ml and 7.6x 10 10 CFU/ml). To see if the yogurt has biopreservative activity, a bacterial challenge towards an Escherichia coli strain coli isolated from a mastitis clinical case (Bacteriology Department of the Veterinary Faculty of the Universidad Nacional Autonoma de Mexico) was used. The yogurt was inoculated with 108 CFU/g, measured by the turdidimetric method. The contaminated samples were kept at 5ºC, during 15 days, sampling was carried during alternate days, till inhibition was found. Inhibition was reached after 12 days in the yogurt with mixed probiotics added and after 14 days for the other two.

Biopreservative Effect of Probiotic Strains (Lactobacillus Casei Shirota and Lactobacillus Johnsonii) - Towards a Strain of Eschericha Coli Isolated from a Clinical Case in Commercial Yogurts **UP305**

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