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Dedicamos este livro a todos os jovens cientistas dos Programas de Pós-Graduação da Faculdade de Zootecnia e Engenharia de Alimentos da Universidade de São Paulo.

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EDIÇÃO E DIAGRAMAÇÃO

AMANDA DA MATTA SANTOS
PÓS-GRADUANDA PELO PROGRAMA DE BIOCIÊNCIA ANIMAL (FZEA-USP).

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CAPA

AMANDA DA MATTA SANTOS
PÓS-GRADUANDA PELO PROGRAMA DE BIOCIÊNCIA ANIMAL (FZEA-USP).

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MESTRADO: *Flávia Cristina Bis*, com trabalho intitulado "Genetic, environmental and hormonal factors of sexual precocity in Nellore heifers".
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PREMIAÇÃO MELHORES TRABALHOS – CATEGORIA PÔSTER

DOUTORADO: *Jéssika Cristina Chagas Lesbon*, com trabalho intitulado "Development of an oncomirs panel for prognosis in canine tumors".
Orientador Prof. Dr. Heidge Fukumasu.

MESTRADO: *Aline Takahashi Ferrero*, com trabalho intitulado "Development of a molecular panel for precision therapy using tumor-agnostic drugs in canine cancers".
Orientador Prof. Dr. Heidge Fukumasu.

METABOLITE-BASED PREDICTION OF FEED EFFICIENCY IN BEEF CATTLE

[1]NUNES, A.T.; [1]FALEIROS, C.A.; [1]BORSATTO, L.B.; [1]XAVIER, P.L.P.; [1]FUKUMASU, H.

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Metabolomics is a promising alternative tool for the assessment and selection of feed efficiency (FE) in beef cattle in a more accurate, faster, and cost-effective manner. Nonetheless, the limited availability of studies investigating FE-related metabolites in beef cattle underscores the need for concerted efforts in the form of extensive, multicenter cohort and validation studies. Given the widespread application of the residual feed intake (RFI) parameter in feed efficiency (FE) selection, the underlying hypothesis of this project is that there is a minimum profile of metabolites highly correlated with RFI, which could be used to improve the accuracy and precocity of FE selection. To confirm this hypothesis, this study aimed to define and validate metabolites with the potential for use as RFI biomarkers. For this purpose, the prospecting stage was unfolded in two distinct steps. In the initial phase, targets were selected by analyzing data derived from a systematic review of the literature. This review was based on an initial selection of 175 articles using *Start*® software. To perform multiple correspondence analysis, the acquired data were subjected distributed in a contingency table, allowing the study of associations using the Chi-Squared test ($p < 0.05$). Then, adjusted standardized residuals (ASR) were extracted to determine the intensity of the associations and ASR higher than 1.96 were considered, indicating associations between the categories of variables. The targets selected in this step encompassed creatinine, creatine, cortisol, and formate. In the second phase, data from prior studies conducted by the Phenomics Research Group at the University of São Paulo (MARCILLI, 2019; NOVAIS et al., 2019) were analyzed using machine learning techniques. Metabolite data were subjected to attribute selection methods (Cfs Subset Eval, Correlation Attribute Eval, dendrogram and Wrapper) and based on the selected expressive attributes, preliminary tests of different computational classifier models were realized using the following algorithms: artificial neural networks, support vector machine, random forest, and k-nearest neighbors. The relevance of the results was assessed by considering the accuracy and coherence of the confusion matrix obtained in computational modeling. This step was used to identify potential metabolites, including aspartate, methionine, ornithine, proline, tyrosine, acetylornithine, carnosine, and phosphatidylcholine with a diacyl residue sum of C36:6. These results are fundamental for the following stages of the PhD project, in which the targets will be validated on a large scale to confirm their potential as effective biomarkers for feed efficiency prediction in beef cattle.

Keywords: Artificial intelligence; Correspondence analysis; Machine learning; Prospection; Residual feed intake.

Acknowledgments: This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001 and by São Paulo Research Foundation (FAPESP) grant 2022/14260-3.

CYTOTOXICITY OF PEPTIDE 44 IN CANINE MAMMARY TUMOR CELLS *IN VITRO*

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Breast cancer ranks third among the types of cancer that will cost the world the most over the next 30 years. Epidemiological studies have indicated that approximately one in every four dogs will develop cancer, which translates to 14.5 million dogs falling ill at some point in their lives. Despite substantial scientific efforts aimed at improving the available treatments, resistance and mortality rates continue to increase. Consequently, the search for new compounds and therapeutic technologies has become increasingly prominent. Several proteins are associated with mechanisms of migration, differentiation, and programmed cell death, and it has been demonstrated that some intracellular peptides participate in various signaling cascades and are linked to cell cycle control; these mechanisms are targets for cancer treatment. Therefore, we hypothesized that peptide 44 plays a regulatory role in cellular signaling and selectively acts on tumor cells rather than non-neoplastic cells. The objective of this study was to assess the cytotoxicity of peptide 44 in breast cancer and non-tumorigenic cell lines. M5, M25, and CF41 tumor cell lines, along with the Adult Canine Fibroblast (ACF) line (non-tumorigenic), were cultured at a concentration of 1×10^4 cells per well in 96-well culture plates. Cells were then treated with peptide 44, except for the control cells, at the following concentrations: 100, 50, 25, 12.5, 6.25, 3.125, 1.5625, and 0.78125 µM, for a 24-hour period. This experiment was conducted in sextuplicate, across three independent trials. The cytotoxicity of the peptide was assessed using the tetrazolium reduction assay (MTT), which allows the measurement of absorbance via spectrophotometry at a wavelength of 540 nm. The average inhibitory concentrations (IC50) of M25, CF41, ACF, and M5 cells were 11.32 ± 1.626 µM, 21.66 ± 8.608 µM, 34.33 ± 14.410 µM, and 35.04 ± 11.307 µM, respectively. No statistically significant differences in cell viability were observed between tumoral and non-tumoral cells treated with peptide 44 ($p > 0,05$). Breast cancer has a multifactorial etiology and extremely high molecular and morphological heterogeneity. Therefore, the disease responds differently to different patients and therapies. Tumor cells vary from one another, much like tumors among patients, each having a distinct molecular profile and heterogeneity. Despite varying susceptibilities to treatment among cell lines, the presumed selectivity of the peptide is not yet evident. Hence, further studies are required.

Keywords: Cancer; Cell cycle; Cell death; Neoplasia; Therapy.

Acknowledgments: This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

MOLECULAR PROSPECTION AND CHARACTERIZATION OF CANINE PNEUMOVIRUS ASSOCIATED WITH THE CANINE INFECTIOUS RESPIRATORY DISEASE COMPLEX IN DOGS

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The canine infectious respiratory disease complex (CIRDC) is a common disease in the veterinary routine, which affects dogs at any age, and is present in environments of high population density, mainly shelters and kennels. The infection can be caused by several agents like Adenovirus type 2 (CAdV-2), canine parainfluenza (CPiV), canine respiratory coronavirus (CRCoV) and Bordetella bronchiseptica. In recent years, several pathogens have been discovered and related to the morbidity and severity of respiratory infections in dogs. Among the emerging agents, canine pneumovirus stands out and has been detected in several countries. This research project has as main objectives the detection and molecular characterization of canine pneumovirus in dogs from a veterinary hospital environment and a shelter, in municipalities in the southern region of the state of Minas Gerais, aiming to contribute to a better understanding of the etiology of CIRDC in this region of Brazil. Nasal and oropharingeal swabs were collected from dogs. The participating animals were divided into three groups: symptomatics, asymptomatics and contactants (CEUA: nº7321030322). The samples were conserved with RNAlater® and freezed at -80ºC. Then, they were submitted to RNA extraction, cDNA transcription and RT-PCR to detect the agent. Up to now, 52 samples of symptomatic animals, 14 samples of asymptomatic animals and 6 samples of contactants have been tested. It was not possible to detect the canine pneumovirus at the tested samples until now. However, more samples have to be tested so that the profile of canine infectious respiratory disease complex is better defined and, therefore, new treatments and more specific vaccines or prevention methods are developed.

Keywords: Canine infectious respiratory disease complex; Dogs; Molecular diagnosis; Respiratory disease; Virus.

Acknowledgments: This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) – Finance Code 001, and by Fundação de Amparo à Pesquisa do Estado de São Paulo – Brasil (FAPESP).

EVALUATION OF THE ACTIVITY OF BENZONIDAZOLE DERIVATES AGAINST *LEISHMANIA AMAZONENSIS*

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Leishmaniasis represents a growing concern in public health, annually infecting around 12 million people and causing approximately 60,000 deaths among those affected. Currently available treatments have limited efficacy and can be toxic to patients. In this research, we investigated the potential of compounds derived from benznidazole for the development of new treatments for leishmaniasis. Benznidazole is a medication used in the chemotherapy of Chagas disease. Its mechanism of action involves in part, inducing oxidative stress within the parasite, leading to the modification of macromolecules such as the parasite's DNA. This modification compromises the parasite's ability to multiply, leading to the patient's recovery. The choice of benznidazole as a starting point for new compounds for leishmaniasis treatment was based on its effective action in other parasitic diseases, such as Chagas disease. We evaluated the activity of the new derivatives both in inhibiting the arginase enzyme of Leishmania and in inhibiting the growth of the parasite in promastigote culture. The tests were conducted over four days (96 hours) in four independent triplicates at a concentration of 100 µM for each inhibitor. We used two types of inhibitors, identified as 05 and 16. The positive control consisted of using 2 µL of DMSO solvent, the same volume as used for the inhibitor to achieve the desired final concentration in 1 mL of M199 culture medium. Screening tests were performed: the inhibition of the parasite's arginase enzyme was around 30%. In the promastigote form, the average growth inhibition was approximately 90% in about 72 hours. Based on the results obtained, there are indications of significant potential for these compounds in the development of new treatments for leishmaniasis.

Keywords: Arginase; Inhibition; Leishmaniasis; Mechanism of action; Promastigote.

Acknowledgement: This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001 and São Paulo Research Foundation (FAPESP), grant #19/23769-4.

IMMUNOHISTOCHEMICAL EVALUATION OF LOXL2 AND HIF1-α EXPRESSIONS IN CANINE MAMMARY TUMOURS

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Mammary neoplasms account for about 50% of tumors in female dogs. Even the most commonly used predictive indices, such as lymph node involvement and histological grading, are not efficient in predicting clinical behavior in this species, which justifies the search for better prognostic markers. Proteins of the LOX family have been implicated in cancer pathogenesis and are related to tumor invasion, metastasis, and prognosis in several neoplasms. LOXL2 can positively regulate HIF-α signaling pathways, which control tissue response to hypoxia. The objective of this study was to characterize the expression of LOXL2 and HIF1-α in canine mammary carcinomas, with the aim of assessing their prognostic value. A total of 105 mammary carcinomas from 91 female dogs were analyzed. Immunohistochemistry was used to detect LOXL2 and HIF1-α and the reactions were quantified using five random high-power field images for each marker. The staining intensity in epithelial cells and fibroblasts was semiquantitatively evaluated. The results were compared to histological types, mortality due to the disease, and post-surgical survival time. The most frequent histological subtype was the tubulopapillary carcinoma (34/95, 35.8%), and in 24.1% (22/91) of the cases, death was attributed to the neoplasm. Only one of the 8 highly malignant carcinomas (12.5%) showed HIF1-α positive fibroblasts, compared to 35/57 (61.4%) of the low malignancy tumors (p=0.0180). All dogs that were censored in the survival analysis were positive for LOXL2 in epithelial cells, while 66.7% (6/9) of the dogs that died due to the disease were positive for LOXL2 (p=0.0140). Our results suggest that stromal fibroblasts of highly malignant carcinomas exhibit lower expression of HIF1-α, and the absence of staining for LOXL2 is associated with a higher risk of death due to the disease.

Keywords: Dog; Hypoxia; Lysyl oxidase; Prognosis.

Acknowledgement: This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001". FAPESP (Process #2020/10582-0 e #2020/12789-1), CNPq (#303748/2021-4)

HIGH INTRAFOLLICULAR PROGESTERONE CONCENTRATIONS REGULATES ACTIN RETRACTION PROTEINS IN SMALL EXTRACELLULAR VESICLES

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Throughout the follicular development, intercellular communication among the cellular components within this milieu orchestrates critical biological processes, including the acquisition of oocyte competence. Follicular cells employ diverse communication mechanisms, one of which involves the secretion of small extracellular vesicles (sEVs) into the follicular fluid (FF). SEVs, characterized as lipid bilayer nanoparticles, exhibit the capacity to carry bioactive materials, including proteins, across various biofluids. The hypothesis of this study posits that distinct concentrations of intrafollicular progesterone (P4) induce alterations in the protein profile contained within sEVs. The study aims to investigate the effects of high and low concentrations of intrafollicular P4 on the protein content within sEVs of the FF. For that, FF was separated according to corpus luteum (CL) presence (ipsilateral and contralateral to the CL) from the cows (n=6) previously synchronized to be on day 11 of the estrous cycle. The FF was analyzed for P4 concentration (n=6; intra and interassay coefficients variations were 13.77 ng/mL and 30.02 ng/mL, respectively), and characterizing the groups (iFF: ipsilateral follicles- high P4; cFF contralateral follicles- low P4). FF was used to obtain sEVs, that were used for proteomic analysis. Total protein extraction from sEVs (iFF=6 and cFF=6) was done using RIPA (radioimmunoprecipitation assay) buffer and proteinase inhibitor cocktail. Mass spectrometry analyzes were carried out with the aid of the nanoElute nanoflow chromatographic system, from Bruker Daltonics, Bremen, Germany, coupled online to a hybrid trapped ion mobility spectrometry-quadrupole time-of-flight mass spectrometer-timsTof Pro mass spectrometer, from Bruker Daltonics. The intrafollicular P4 concentration was higher in iFF (high P4 - 188.9 ± 24.20 ng/mL) than cFF (low P4 - 27.24 ± 5.57 ng/mL; p=0.0002) group. Proteomic analysis detected 57 proteins, and two proteins were upregulated in the iFF group. Additionally, eight proteins were exclusively detected in the iFF and four exclusively detected in the cFF group. Biological processes analysis from upregulated and exclusively expressed proteins of iFF group were predicted to regulate process as actin filament severing, actin filament fragmentation, and ERK1 and ERK2 cascade. Conversely, exclusively expressed proteins from cFF group were predicted to regulate biological processes related to actin filaments maintenance as negative regulation of endopeptidase activity, intermediate filament organization. In summary, increased intrafollicular P4 concentrations regulate the protein composition within sEVs, potentially triggering actin filament retraction as actin membrane-based projections, and subsequently initiating the oocyte maturation process.

Keywords: Actin filaments; Bovine oocyte; Follicle; Nanoparticles; Proteoma.

Acknowledgement: This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001 and FAPESP 2021/06645-0.

HIGH BODY ENERGY RESERVES DO NOT AFFECT EXTRACELLULAR VESICLES PRESENT IN THE UTEROTUBAL JUNCTION OF CATTLE

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High-energy diets appear to affect later stages of follicular and early embryonic development, compromising embryo production. The first site where the embryo interacts with the maternal endometrial epithelium is the uterotubal junction (UTJ). Extracellular vesicles (EVs) are mediators of intercellular communication and modulate several reproductive processes such as the information exchange between the maternal environment and the conceptus. Also, EVs can carry molecular signals (e.g. miRNAs and proteins) in response to environmental factors as metabolism. However, little is known about the effects of the increased body energy reserves (BER) in the UTJ before the embryo presence. In this way, our hypothesis is that the increase of BER alters the size and concentration of EVs present in the UTJ fluid. The aim of this study was to evaluate the effects of increased BER in UTJ extracellular vesicles (UTJ-EVs) of cattle. For this, Nellore cows from the same herd were submitted to different nutritional plans during 67 days of feedlot, in order to maintain (MBER group) or elevate (HBER group) their body energy reserve. At the end of the feedlot period, animals were submitted to estrous synchronization, artificial insemination and were slaughtered approximately 120 hours after ovulation induction. The reproductive tracts were collected, the UTJ ipsilateral (same side) to the corpus luteum were dissected and flushed with 2 mL of phosphate-saline solution and for this study we used only the UTJ of animals in which an 8-cell embryo was found in the reproductive tract. The animals were divided in two experimental groups: 3 from the MBER group and 3 from the HBER group. The EVs were isolated from de UTJ fluid by ultracentrifugation and analyzed based on particle size and concentration by nanoparticle tracking analysis (NTA). The data obtained were normalized by Shapiro-Wilk test and compared using Student's t-test. The significance level used was 5%. No differences were identified in mean mode size (MBER: 139.2 ± 2.76 nm; HBER: 145 ± 5.66 nm) and particle concentration (MBER: 2.76 x 10^9 ± 8.17 particles/mL x 10^7; HBER: 3 x 10^9 ± 1.52 x 10^8 particles/mL) between the groups. Additionally, the influence of BER on the size of EVs from isthmus when an 8-cell embryo was present was observed by Bastos et al. (2023). However, the increase of body energy reserves appears do not affect the size and concentration of EVs present in the UTJ fluid of the cows.

Keywords: Communication; Early Embryonic Development; Environment; Feedlot; Nutrition.

Acknowledgement: This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brazil (CAPES) - Finance Code 001, FAPESP grants 2020/13075-2 and 2021/06645-0.

USING SYSTEMS BIOLOGY APPROACHES TO IDENTIFY GENE CLUSTERS IN PIGS FED WITH DIFFERENT SOURCES OF FATTY ACIDS

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Nutritional strategies have been used to promote changes and improvements in the lipid profile of meat to enhance its beneficial effects on human health. The composition of fatty acids (FA) deposited in pig tissues can be modified by dietary choices involving different types of oils. However, the effect of dietary oils such as fish and soybean on gene expression in pigs remains largely unexplored. In this context, our aim is to identify gene co-expression networks associated with the FA profile in the liver of pigs fed different oil sources. All animal procedures were approved by the Animal Care and Use Committee (CEUA 2018-28). Animals received different dietary treatments, including a basal diet during the growth-finishing phase supplemented with either 3.0% soybean oil (SOY) or 3% fish oil (FO). There were 18 animals in each treatment group. After 98 days of the experiment, all pigs were slaughtered and liver samples were collected, and stored at -80°C. RNA-Seq data was used in this study, and the quality of raw RNA-Seq reads was assessed using FastQC v.0.11.8. Reads were filtered using Trim Galore v.0.6.5 and aligned to the *Sus scrofa* 11.1 reference genome available on Ensembl. mRNA abundance for all annotated genes were determined using STAR v.2.7.6a. and transformed RNA-Seq data were normalized using the Expectation-Maximization (RSEM) v.1.3.1 software package. We then performed a signed gene co-expression analysis using the Weighted Correlation Network Analysis (WGCNA) package in R. The appropriate soft threshold was selected according to a scale-free network pattern, with 7 for SOY and 5 for SOY3.0. We subsequently identified co-expression modules associated with a wide range of FA, including myristic, palmitic, palmitoleic, stearic, oleic, linoleic, alpha-linolenic, eicosapentaenoic, docosahexaenoic (DHA), n-3, n-6, saturated (SFA), monounsaturated, polyunsaturated (PUFA), n-6:n-3 ratio, PUFA:SFA, and the atherogenic index (AI). We identified gene modules characterized by dissimilarities of less than 0.25 and a minimum module size of 30 genes. Overall, we identified several module eigengenes (ME) for the oil group and observed modules with the highest co-expression for FO (r = 0.65) and the n-6:n-3 ratio of SOY (r = -0.68). Furthermore, docosahexaenoic acid (DHA) showed the highest amount of ME in the SOY group, whereas palmitoleic acid was predominant in the fish oil group. These results improve our understanding of the basic biological mechanisms responsible for the changes in FA composition found in the livers of pigs exposed to different dietary oil sources.

Keywords: DHA, Fish oil; RNA-Seq; Soybean oil; WGCNA.

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AN ONCOLYTIC VIRUS TARGETS AND REPLICATES IN FELINE LYMPHOMA CELLS

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Cancers represent one of the leading causes of death in domestic felines, and among various types of neoplasms, lymphomas are one of the most common. Chemotherapy is the primary treatment for this disease; however, drug resistance remains the leading cause of death. Research on therapies using oncolytic viruses has gained prominence in recent years because they have the potential to selectively kill cancer cells. We hypothesized that the Newcastle Disease Virus expressing GFP (NDV-GFP) has the potential to infect and induce cell death in feline lymphoma cells without causing less harm to normal cells. Therefore, our objective was to assess the oncolytic potential of NDV in these cells by evaluating the cytotoxicity of NDV-GFP in a feline lymphoma cell line (FeLV 3281) and in healthy Peripheral Blood Mononuclear Cells (PBMCs). We also aimed to characterize oncolytic effects in terms of morphological alterations and cell death. To achieve this, we infected 1×10^4 cells (FeLV 3281 and PBMCs) for 24 h with dilutions of MOI=1, MOI=0.2, MOI=0.04, MOI=0.008, MOI=0.0016, MOI=0.0003, MOI=0.00006, and MOI=0.00001 based on the titer calculated in FeLV 3281 using the Reed-Muench method. Cytotoxicity was assessed by adding 20 µL of the CellTiter-Blue ® reagent to each well for 24 h and reading the absorbance in a spectrophotometer at wavelengths of 540 nm and 630 nm. IC_{50} determination was carried out using GraphPad Prism software (version 8.0) by nonlinear regression. Morphological changes were evaluated by optical microscopy in bright-field and fluorescent modes. For cell death analysis, neoplastic cells were treated with the calculated IC_{50} and analyzed by flow cytometry to the protocol established by Riccardi & Nicoletti, 2006. The titer and IC_{50} calculated were 3, 433 x106 $TCID_{50}$/mL and MOI = 0.3201, respectively. The most prominent cytopathic effect observed in FeLV 3281 was cell death in wells treated with higher virus concentrations, with greater GFP expression in neoplastic cells in the same wells. A lower expression of GFP was also observed in PBMCs. A higher percentage of cell death was observed in the treated (61.95% ± 2,20%) cells compared to the control cells (1.97% ± 0.54%, $p < 0.01$). Santos et al. (2021) attributed these effects to alterations in the Interferon pathway. Hence, we observed that NDV-GFP is an oncolytic agent for feline lymphoma cells. Further studies are necessary to determine whether NDV-GFP is less cytotoxic to healthy feline cells than to neoplastic cells.

Keywords: Cancer; Cat; In vitro; Lentogenic; New therapies.

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EVALUATION OF DOPPLER VELOCIMETRIC PARAMETERS OF THE CENTRAL NERVOUS SYSTEM IN HEALTHY DOGS

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Transcranial ultrasound is a diagnostic tool that brings numerous advantages, mainly because it is a non-invasive, low-cost technique that hardly requires patient sedation. Furthermore, it is capable to provide real-time information about the hemodynamics of the brain. Therefore, the purpose of this study was to evaluate Dopplervelocimetric parameters: resistivity index (RI), pulsatility index (PI), maximum velocity (Vmax) and mean velocity (Vm) of cerebral dog arteries (rostral - ACR, middle - ACM and caudal - ACC of both cerebral hemispheres), as well as basilar and vertebral arteries in order to establish normal values that can help in the diagnosis of brain diseases and hemodynamic disorders in this species. For the study, 58 healthy dogs were selected, weighing up to 10 kg and aged between 3 months and 16 years, allocated into three different age groups: Group 1 (n=10): young dogs (3 months to 1 year); Group 2 (n=38): adults (1 year to 10 years); Group 3 (n=10): old dogs (10 years to 16 years). The examination protocol included, in this order: anamnesis, physical examination, abdominal and transcranial ultrasound (Duplex Doppler) and blood collection for blood count. No sedation was performed on the dogs. It was possible to visualize the arteries in 62% of cases using Color Doppler, with basilar being the most visible (in 95% of dogs) and caudal cerebral artery the least visible (43%). The mean Dopplervelocimetric values PI, RI, Vmax (cm/s) and Vm (cm/s) obtained were, respectivaly, for the right ACR: 1.074 ± 0.248; 0.635 ± 0.099; 29.620 ± 12.419; 10.603 ± 4.856; 17.823 ± 7.340; left ACR: 1.111 ± 0.260; 0.621 ± 0.158; 28.633 ± 13.948; 9.546 ± 4.550; 17.826 ± 7.365; right ACM: 1.077 ± 0.275; 0.633 ± 0.096; 29.584 ± 11.004; 10.881 ± 4.938; 17.981 ± 7.028; left ACM: 1.125 ± 0.235; 0.656 ± 0.077; 25.722 ± 8.271; 9.058 ± 3.464; 15.156 ± 5.161; right ACC: 1.098 ± 0.349; 0.636 ± 0.098; 26.812 ± 12.729; 9.258 ± 5.360; 15.892 ± 7.365; left ACC: 1.051 ± 0.305; 0.612 ± 0.111; 26.818 ± 10.371; 9.609 ± 4.106; 15.858 ± 5.753; basilar artery: 1.726 ± 0.467; 0.756 ± 0.086; 55.106 ± 20.645; 13.767 ± 6.572; 26.111 ± 12.342; vertebral artery: 1.879 ± 0.680; 0.749 ± 0.093; 28.247 ± 11. 696; 7.120 ± 3.824 and 12.515 ± 5.868. The values between groups will later be compared. Doppler technique was best carried out on calm dogs weighing up to 5 kg.

Keywords: Brain; Canine; Cerebral arteries; Circulus arteriosus; Transcranial ultrasound.

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RESUMOS DOS TRABALHOS DOS

ESTUDANTES DO PROGRAMA DE PÓS- GRADUAÇÃO

EM ENGENHARIA DE ALIMENTOS | PPGEA

PREMIAÇÃO MELHORES TRABALHOS – CATEGORIA APRESENTAÇÃO ORAL

1º LUGAR: *Alessandra Amorim*, com trabalho intitulado "Food classification and labeling: Discussion and propose".
Orientador Prof. Dr. Paulo José do Amaral Sobral.

2º LUGAR: *Paloma Jamily Cristina Magalhães*, com trabalho intitulado "Evaluation of the functionalities of the protein fraction contained in Brazil nut press cake".
Orientadora Profa. Dra. Christianne Elisabete da Costa Rodrigues.

3º LUGAR: *Yves José de Souza Santos*, com trabalho intitulado "Rapid evaluation of cytotoxicity in CACO-2 and L929 cells by nir spectroscopy in bakery products".
Orientadora Profa. Dra. Fernanda Maria Vanin.

4º LUGAR: *Júlia Cristina José*, com trabalho intitulado "Characterization and stability study of brewer's spent yeast enriched with ascorbic acid".
Orientadora Profa.Dra. Carmen Sílvia Fávaro Trindade.

FOOD CLASSIFICATION AND LABELING: DISCUSSION AND PROPOSE

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In view of industrialization and urbanization, the human relation with time, and consequently withtheir feeding, has changed. Nowadays, food can be accessed in food services, restaurants, and supermarkets, for example, and not more exclusively at home, cooked by mothers or grand-mothers, as in the recent past. Due to scientific and technological development, a huge diversity offood can be safely consumed days or months after processing. However, these social changes in ashort period of time allowed a distrusted environment, intensified by the absence of knowledge about what we eat. Linked to culture, food consumption involves emotions and moral values, which can transform food choice in a form of activism. With societies more aware abouthealthiness, ethics and sustainability, customers are looking for products with higher nutritional value, resulted from friendly-environmental processes and morally ethical, while keeping taste andpracticality for cooking and consumption. In this context, with a goal to educate and orient peoplein a healthier way, governments from many countries developed Food Based Dietary Guidelines (FBDG). In Brazil, in turn, although there is a good intention in formulating a didactic classification, that could be easily memorized by lay people, a huge and serious mistake were doneas confusing process and formulation concepts, in addition to do not properly consider food safety,security, idiosyncrasies, and social-economic issues. In this way, this project aims to propose a new system of food classification, connected to healthiness and sustainability food trends, in alphanumeric scale, that can be used in food labels as well as instruments in public policy tools, such as FBDG. To that, three rounds of interviews with Food Scientist, Technologist and Engineers(academy and industry) will be executed to provide sufficient data to elaborate a technical- scientific food classification proposal, also easily understood by the general population. The Delphi methodology will be used. This proposal will be named TSNS, Portuguese acronym to Safety and Technology (TS), Nutrition (N), and Sustainability (S), and will be elaborated considering the characteristics and specificity of six food groups: Dairy, Meat, Cereals, Fruits andVegetables, Beverages, and Multicomponent systems. First of all, Mind Map methodology will beused to identify the most products belonging to each group and oriented the scholars during the interviews. The main objective is finding the most important topics to classify food, and a scientificrationale to score them.

Keywords: Food guidelines; Healthiness; Processed food; Sustainability; Ultra-processed food.

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OCCURRENCE OF AFLATOXINS AND FUMONISINS IN ORGANIC AND CONVENTIONAL CORN

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Data on fungal contamination, or aflatoxins (A) and fumonisins (F) in organic corn are scarce, as well as if this crop category is more susceptible to contamination compared to the conventional one. This study was conducted to evaluate both corn and corn products´ mycobiota grew in organic and conventional systems. Also, the occurrence of A and F, and the probable average daily intake (PADI) were investigated. The water activity (Aw) of 140 conventional and 60 organic samples was measured. The toxins AFB_1, AFB_2, AFG_1, AFG_2 and FB_1 and FB_2 were also determined by ultra-performance liquid chromatography. The Aw of conventional farinaceous and grains were 0.53 and 0.60, respectively. As for organics, the Aw values were 0.56 and 0.65, respectively. Fungal growth was detected in 59.29% of conventional samples, with 1.78 log CFU/g average in farinaceous and grain infection positive in 16%; and in organics 70%, 2.67 log CFU/g average in farinaceous and 12% grains infection. The most frequent genera were *Aspergillus* spp., *Penicillium* spp., *Fusarium* spp. and *Rhizopus* spp., Two or more genera were identified in 27.71% of conventional, and 61.90% of organics. AFB_1 (5.20-32.40 µg/kg), AFB_2 (4.20 µg/kg), FB_1 (up to 942.40 µg/kg) and FB_2 (up to 243.90 µg/kg). Were detected in 1.42, 0.71, 32.14 and 15,71% of conventionalsamples, respectively, in levels of, and maximum levels of and 243.90 µg FB_2/kg. In organicssamples were detected AFB_1, AFB_2, AFG_1, AFG_2, FB_1 and FB_2 in 8.33%, 6.67%, 5.00%, 1.67%, 86.66% and 18.33%, with maximum levels of 118.10 µg AFB_1/kg, 52.20 µg AFB_2/kg, 25.70 µg AFG_1/kg and 28.80 µg AFG_2 /kg, 3,462.50 µg FB_1/kg and 883.40 µg FB_2/kg. Despite fungal growth in both systems, organics showed greater co-contamination than conventional by fungi, aflatoxins and fumonisins, exceeding the legislation limits. This represents a serious hazard to human health.
Keywords: Fungi; Grain; Mycobiota; Mycotoxins; UPLC.

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BREWER'S RESIDUAL YEAST IMPREGNATED WITH VITAMINS: RELEASE PROFILE, PERMEABILITY AND EVALUATION OF ANTI-INFLAMMATORY PROPERTY

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Vitamins have been studied for decades, demonstrating their potential and importance for human health, among which are vitamins C and D. Vitamin C helps health due to its antioxidant capacity, preventing diseases such as cancer and providing an improvement in the immune system, next to it is linked to vitamin D, which provides the maintenance of calcium in the body. However, despite the benefits, these compounds are very unstable, making their ingestion or presence in food difficult. Aiming at solving this problem, processes such as microencapsulation have emerged. Linked to encapsulation, the use of brewer's spent yeast - as *Saccharomyces pastorianus* - is proposed as a promising vehicle for vitamins, adding value to this widely available by-product. After impregnation, the aim will be to carry out studies for the advancement of the project, the first of which is about the release profile of vitamins, determined in the oral, gastric, and intestinal phases, their bioavailability, and the determination of their anti-inflammatory activities. Given the above, the present project aims to study the release profile the oral, gastric, and intestinal phases (bioaccessibility), cellular transport, through assays with CaCo-2 cells, and the possible anti-inflammatory activity of pure yeast cells and those loaded with vitamins C and D_3. To prepare the particles, yeast *(Saccharomyces pastorianus* from the Fermentis brand) discarded after the production of pilsner beer and provided by the Hausen Bier brewery, located in the city of Araras – SP, and vitamins C or D3 from the Sigma-Aldrich brand will be used. subsequently going through the washing process, aiming to clean, purify and remove the malt, after which they will be exposed in the biosorption process for the possible loading of vitamins C and D. Then, following the INFOGEST protocol, the release profile will be estimated. of vitamins, followed by cell permeability using CaCo-2 cells and possible anti-inflammatory activity. With the project in question, it is expected to demonstrate the effectiveness of loaded yeasts in releasing vitamins, confirming their ability to deliver these nutrients to the body appropriately.

Keywords: Digestion; *Saccharomyces pastorianus*; Vitamin C; Vitamin D3.

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EFFECT OF EMULSIFIER TYPE OF EMULSIONS (O/W) ENCAPSULATING CRUDE RED PROPOLIS ON SOY PROTEIN ISOLATE-BASED ACTIVE FILMS PROPERTIES

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Oil-in-water (O/W) emulsions can be used for encapsulating non polar bioactive compounds. Thered propolis (RP), which presents very high antioxidant and antimicrobial activities, are not solublein water. Usually, hydroethanolic extracts of propolis have been used for active films production as source of bioactive compounds. Nevertheless, the crude RP must be richer in bioactivecompounds than its extract. Soy protein isolate (SPI) is a biopolymer derived from soy grain that has good film-forming property. Thus, interested in to develop an active film based on SPI activated by using crude RP, the aim of this work was to study the effect of the emulsifier type used in O/W emulsions encapsulating crude RP on SPI-based films properties. Emulsions were composed by the oil phase (10%, crude RP, PGPR, soy oil) and water phase (90%, distilled water, emulsifiers). Oil and water phases were firstly mixed at 60ºC/5 min and then, homogenized usingan ultraturrax (15000 rpm/2 min). After that, emulsions were sonicated at room temperature. Tween 80 (1,35%w/w, T), sodium caseinate (1.0%w/w, C) and saponin (1,0%w/w, S) were studiedas emulsifiers. SPI-based films were prepared by casting technique and characterized for determination of mechanical properties, water contact angle (WCA) and water vapor permeability(WVP). SPI control film presented tensile strength (TS) of 4.9±0.2 MPa, elastic modulus (EM) of1.5±0.1 MPa and elongation at break (EB) of 103.1±12.5%. Emulsion T caused a plasticizing effect in the biopolymer matrix, decreasing TS (3.4±0.2 MPa) and EM (0.9±0.1 MPa) andincreasing EB (189.8±11.2%). Instead, emulsion S enhanced the TS (5.6±1.3 MPa) and EM (1.9±0.4 MPa) and decreased the EB (135.7±9.5%). Emulsion C did not affect significantly TS (4.4±0.5 MPa) and EM (1.5±0.2 MPa), but increased EB (122.6±13.2%). About the surface of thefilms, SPI presented a moderate hydrophilic surface (76±4º), being that the addition of emulsion Sincreased WCA (85±3º), which could be related to the presence of oil in the film surface. However,emulsion C (78±4º) presented a proximate WCA. On the other hand, emulsion T became the surface more hydrophilic (58±4º), which could be explained by the hydrophilic nature of this emulsifier. The incorporation of the three types of emulsifiers did not affect the WVP of the activefilms. So, the emulsifier type used in the emulsion production interfered in the mechanical properties and WCA of the SPI-based films, but did not present effect in the WVP.

Keywords: Biopolymer; Emulsifiers; Red propolis.

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ESCHERICHIA COLI: EVALUATION OF SUSCEPTIBILITY TO SANITIZERS ISOLATED FROM REFRIGERATED RAW MILK AND BIOFILM FORMATION OF ISOLATES FROM CHEESES ORIGINATING FROM CONVENTIONAL AND ORGANIC PRODUCTION SYSTEMS

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A dairy industry has been adopting the organic system due to the increased global demand for products with a lower environmental impact. Both the conventional and organic systems, if not properly disinfected with appropriate sanitizers, are susceptible to contamination by bacteria, such as Escherichia coli, which can form biofilms, causing economic losses for the industry and risks to public health. Contamination can occur throughout the processing. Thus, the present work aimed to verify the susceptibility profile to sanitizers in strains of Escherichia coli isolated from refrigerated raw milk from conventional and organic production systems and to evaluate the biofilm-forming capacity induced by strains of Escherichia coli isolated from various types of cheeses from conventional and organic production systems at different times and temperatures. Twenty-five isolates of E. coli from conventional milk were used, obtained from the laboratory's biobank, and 27 samples of refrigerated raw milk from organic dairy farms located in the interior of the State of São Paulo were collected, and these isolates were evaluated for susceptibility to sanitizers sodium hypochlorite and peracetic acid by the minimum inhibitory concentration method. Regarding the capacity to form biofilms, by counting sessile cells and confirmation by scanning electron microscopy, 81 isolates of Escherichia coli from samples of various types of commercial cheeses produced in conventional (45) and organic (36) systems were used. As a result, it was found that sodium hypochlorite was more efficient in controlling E. coli isolated from milk from conventional production systems, and peracetic acid was more effective against E. coli isolated from milk from organic production systems. In addition, both production systems required concentrations above the recommended use indicated by the manufacturers of each sanitizer. Based on the results obtained, isolates from both types of cheese showed sessile cell counts indicative of biofilm formation. The longer the incubation time and temperature, the higher the sessile cell counts, with E. coli isolated from milk from conventional production systems showing higher counts and a higher percentage of cellular adhesion and possible extracellular matrix formation in coupons with 240 hours of incubation. It is concluded that proper sanitization and temperature control are precautionary factors for food industries to ensure product safety.

Keywords: Dairy products; Enterobacteriaceae; Production systems; Sanitizers; Scanning Electron Microscopy (SEM).

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ANTIOXIDANT ACTIVITY OF WHEAT-BASED COMPOSITE FLOUR WITH BY-PRODUCT OF SUNFLOWER (Helianthus annuus) OIL EXTRACTION USING EXTRUSION AND CONVENTIONAL MIXTURE

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Composite Flours (CF) usually presents higher nutritional value compared to wheat flour, used asCF basis. The by-products of oil extraction from seeds, normally discarded or used in animal feedand fertilizer, present interesting nutritional aspects. Thus, the aim of this study was to develop a CF incorporated with 30% sunflower by-product (sunflower meal) on a wheat-basis CF and evaluate the effect of the extrusion process compared to a conventional mixture on the antioxidantactivity of the CF produced. The analyses were made in wheat flour (WF) and in the CF made bythe usual mixture (CFM) and by extrusion process (CFE). The antioxidant activity was determined by DPPH (2,2-diphenyl-1-picrylhydrazyl), ORAC (Oxygen Radical Absorbance Capacity), and FRAP (Ferric reducing ability of plasma). Wheat flour antioxidant activity couldn't be quantified by DPPH and FRAP analyses, due to the lower quantification limits. WF presented the antioxidant activity of 10.10±0.53 µmol Trolox Equivalent/g flour by ORAC analyses. Both CF developed (CFM and CFE) presented higher antioxidant activity compared to wheat flour, for all methodologies studied. No significant differences were observed in the antioxidant activityaccording to DPPH and ORAC methodologies for CFM and CFE, where the CFM present EC_{50} of 0.13±0.00 mg/L and 53.58±1.73 µmol Trolox Equivalent/g flour, respectively, and CFE present EC_{50} of 0.12±0.00 mg/L and 50.47±1.24 µmol Trolox Equivalent/g flour, respectively. The extrusion process significantly reduced the antioxidant activity measured by FRAP, the CFE presented 0.62 ± 0.04 µmol Trolox Equivalent/g flour while CFM 0.71 ± 0.03 µmol Trolox Equivalent/g flour. The antioxidant activity is carried out by phenolic compounds principally, that are thermosensitive, which explains the reduction observed in FRAP methodology. However, theantioxidant activity can also be carried out by vitamins C, A, and E, carotenoids, etc. Some antioxidants, such as vitamin E and carotenoids, for example, are fat-soluble and the removal of lipids (resulting from extrusion) may have helped the extraction of these antioxidants, which can explain the non-difference visualized in DPPH and ORAC analyses. It could be concluded that CFelaboration significantly increased the antioxidant activity and the use of the extrusion process forCF production minimally reduced the antioxidant activity, in the conditions applied in this study. More studies should be realized in order to verify other possible effects on CF.

Keywords: DPPH; $EC_{50;}$ FRAP; ORAC; Wheat flour.

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CHARACTERIZATION OF PROTEIN FRACTION OF BABASSU PRESS CAKE (ORBIGNYA SÃO PAULO)

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Population growth triggers more significant food consumption, with meat products being the largest source of protein consumed by the population. This practice produces intense cattle, pigs, and poultry, requiring more physical space to raise these animals. Vegetable proteins have great potential as a source of protein for human consumption, having technological functions in addition to nutritional ones. In this context, to delineate potential protein sources, it is necessary that functional properties such as solubility, water and oil absorption, foam, and emulsion formation capacity, among others, are determined. Babassu (*Orbignya São Paulo*) is an oil palm tree, where it is possible to obtain various products and by-products from the leaves, epicarp, endocarp, mesocarp, and nuts, which are paramount for several indigenous communities. Babassu nuts contain an average of 65% oil. Therefore, they are subjected to mechanical pressing to recover the lipids. The solid residue from pressing extraction, called press cake, still contains nutrients of interest. However, this material is used for animal feed. Therefore, actions for its valorization are valid. Hence, this dissertation aims to verify the technical feasibility of extracting the proteins from babassu almond pressing cake. It is proposed to use ultrasound-assisted extraction to obtain the protein fraction and determine the functional properties of the protein material obtained. In this first semester of the master's course, the babassu almond pressing cake was purchased in a local market in the city of São Luiz (MA), and characterization. The babassu cake was analyzed using official methods (AOCS, 1998): moisture, lipids, proteins, ash and carbohydrates calculated by difference. The protein solubility curve was determined in deionized water and 0.1N NaCl with pH adjustment at 2, 4.5, and 9 (Morr et al., 1985). The composition on a wet basis was (9.0 ± 0.2) % moisture, (2.2 ± 0.1) % lipids, (13 ± 1) % proteins, (3.3 ± 0.1) % of ash and (73 ± 2) % of carbohydrates. The behavior of babassu proteins in water was U-shaped with lower solubility at pH 4. In saline solution, an increasing behavior was observed with increasing pH, with the highest solubility at pH 9.0. Through these preliminary results, it can be inferred that babassu almond cake has the potential for protein extraction.

Keywords: By-products, Food composition, Nitrogen solubility index, Protein extraction, Vegetable proteins.

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THE CONCEPTS OF FOOD DEFENSE IN A MEAT SEASONING FACTORY - A CASE STUDY

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Food defense (FD) is a comprehensive approach developed to safeguard the food safety across theentire food production chain. Originating in response to the events of September 11, 2001, in the United States of America (USA), FD encompasses a range of control and quality management strategies within the food industry. The primary objective of FD is to prevent both intentional food contamination. This study focused on the techniques of FD to elucidate potential forms ofcontamination and adulteration of ingredients used in the production of meat seasonings in a small-scale industry setting. The ultimate goal is to comply with the new regulations for exports to the United States, establish a standardized framework for the company, and serve as a model for otherenterprises engaged in similar relationships with the USA. This study was conducted at a small- scale company headquarted in the USA that distributes seasonings nationwide; its operation initiated in Brazil in 2009. Given its position in the supply chain, directly supplying exporting companies, this particular enterprise must adhere to the regulatory requirements of destination countries while simultaneously complying with Brazilian legislation. To identify the company´s vulnerabilities, a comprehensive questionnaire based on the FD Plan Builder software wasemployed. Furthermore, observation on site was carried out to assess the multiple processes employed by the target industry. The outcomes were analyzed using the aforementioned software, allowing for the identification of potential vulnerabilities and the proposal of mitigation measures.The utilization of the FD plan builder software for food manufacturing facilities, although intricate,proved to be both feasible and valuable. Comprehensive engagement with all departments within the industry enabled the identification of vulnerable points. Moreover, these findings facilitated the development of a comprehensive FD plan, outlining potential intentional ways of contamination and adulteration associated with the ingredients utilized by the company.

Keywords: Adulteration; Brazilian legislation; Food defense plan builder; Food safety; Spice export.

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IMPACT OF PASTEURIZATION ON THE MICROBIOLOGICAL QUALITY OF ORGANIC COLD BREW COFFEE

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Coffee consumption and preparation widely vary, and the cold brew method has been gaining moreand more followers worldwide. In that method, roasted and ground coffee grains are infused in cold water (~23 ºC) for up to 24 h. This study was undertaken to evaluate the impact ofpasteurization on the microbiological control of organic cold brew coffee grains subjected to light,medium and dark roasting. The cold brew coffee beverage was pasteurized at 90 °C/30 s, cooled to 10 °C, ultraclean filled into high density polyethylene bottles, and stored at 4 °C in the dark. Mesophiles, psychotrophs, molds and yeasts, and coliforms at 45 ºC counts in both raw and pasteurized beverage were carried out. Regarding the light roasting coffee, mesophiles, psychotrophs, molds and yeasts, and coliforms counts were 2.47, < 1_{est}, 1.54 and < 1_{est} logCFU/mL,respectively, in the raw beverage. As for pasteurized coffee, counts equal to 0.48 for mesophiles,and < 1_{est} logCFU/mL for psychotrophs, molds and yeasts, and coliforms were achieved. Regarding the medium roasting coffee, mesophiles, psychotrophs, molds and yeasts, and coliformscounts were 1.67, 0.18, 1.18 and < 1_{est} logCFU/mL, respectively, in the raw beverage. In the pasteurized sample, counts were 1.11 for mesophiles and < 1_{est} logCFU/mL for psychotrophs, molds and yeasts, and coliforms. Regarding the dark roasting coffee, mesophiles counts were 0.30 logCFU/mL, psychotrophs, molds and yeasts, and coliforms counts < 1_{est} logCFU/mL, respectively, in the raw beverage. As for the pasteurized one, there was no microbial growth (< 1_{est}logCFU/mL). Both raw and processed samples showed low microbial growth, and the pasteurization positively affected the microbiological control of cold brew coffee.

Keywords: Coffee processing; Food preservation; Food safety; Microbial reduction; Roasting.

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MICROBIOLOGICAL QUALITY OF MINIMALLY PROCESSED VEGETABLES IN MUNICIPALITY AGRO-INDUSTRIES UBERLÂNDIA- MG

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Changes in eating habits have led the society increase the demand for ready-to-eat foods, such as minimally processed vegetables (MPVs), due to their convenience and quick preparation while maintaining the original characteristics and nutritional quality of the products. Minimally processed vegetables must undergo appropriate sanitation processes in order to ensure adequate microbiological standards especially in terms of the absence of pathogenic microorganisms. This work has as objectives to verify the presence of *Salmonella* spp., *Listeria monocytogenes*, and *Escherichia coli* in samples of MPV collected from two agro-industries in the city of Uberlândia- MG; to use the checklist in MPV agro-industries to verify the application of good manufacturing practices and to evaluate the efficiency of sanitizers in the products. The main hypothesis to be verified is possible variation in occurrence of contamination by pathogenic microorganisms in MPVs from small rural agro-industries, compared to those from other productions. Another hypothesis is possible identification of different immersion times and different concentrations of other sanitizing products that differ from the most used product in MPVs, such as sodium hypochlorite, and which are efficient at eliminating microorganisms in vegetables without generating chemical residue. For this purpose, the vegetables: cabbage, lettuce, chicory chives and parsley were collected in 4 samples (5 samples of each type of MPVs in each sample, in each agro-industry with 10 samples in each agro-industry in the fourth sample) and subjected to Compact Dry microbiological analysis. All the samples presented *E. coli* in concentration between $1,5x10^4$ the $3,8x10^5$ colony-forming units/mL. The frequency of samples positive for *Salmonella* spp. varied between 20 and 80% in all samples, while *L. monocytogenes* was detected in all 4 types of MPV only in 2nd samples. In the next stage of the experiment, the efficiency of peracetic acid, chlorhexidine and, sodium hypochlorite as sanitizers for MPVs will be evaluated at different concentrations and for different runtimes. The data will be analyzed using descriptive statistical procedures, using the Kruskal-Wallis testto verify the existence of significant differences between the frequencies of microorganisms inthe samples, and ANOVA to evaluate the sanitizers, considering alpha = 0,05. Microbiological analyses of processed vegetables are important to demonstrate handling and marketing standards regarding to the spread of bacteria and food safety. Agro-industries need to adopt good manufacturing practices to improve the production process.

Keywords: Conventional; Efficiency; Nutritional; Pathogens; Sanitizers.

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QUANTIFICATION OF ACRYLAMIDE IN COMMERCIAL POTATO CHIPS USING VIBRATIONAL SÃO PAULOECTROSCOPY

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Acrylamide is a soluble and low molecular weight monomer formed during the Maillard reaction,that can potentially be carcinogenic. The food with highest amount of this compound are potato chips and French fries. Currently, the analysis of acrylamide in potato chips primarily involves chromatographic methods combined with spectrophotometry. Such methods require significant expenses of money, time, and training. Therefore, the development of a quantification method withan emphasis on sample preparation using Fourier-transform infrared spectroscopy (FTIR) may befaster and require relatively lower investment. The objective of this study is to develop a techniquebased on FTIR to quantify the acrylamide content in potato chips, using high-performance liquid chromatography (HPLC) to validate the technique, as well as to propose a mathematical model tocompare the amount of acrylamide present in various types of fried potatoes. For this purpose, samples of potato chips were pressed using a press die set into standardized samples. These compressed samples were analyzed using a spectrum 3 Perkin Elmer® FT-IR spectrophotometer. The spectra of the matrices were processed using GRAMS/AI software. For validation purposes, the potato chips samples will undergo through delipidization process, spiked with a known concentration of acrylamide, and undergo to a solid-phase extraction to be analyzed by HPLC. Partial Least-Square Regression (PLSR) will be used to correlate the Spectrum obtained in FTIR withthe amount of acrylamide found by HPLC. The amount of residual oil in the sample directly influences the Spectrum reading, making it the main interference to be mitigated during sample preparation. It is expected to find a mathematical model capable of determining the amount of acrylamide with a high level of reliability solely using the Spectrum obtained by FTIR, making it usable for both industrially processed and homemade fried potato chips.

Keywords: Carcinogenic compounds, Fourier Transformed Infra-Red (FTIR); High Performance Liquid Chromatography (HPLC); Method validation; Partial Least-Square Regression (PLSR).

Acknowledgement: Special acknowledgement to the Textile Research Institute (TRI Princeton) for providing all the equipment and supplies for the development of this research. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

APPLICATION OF BIOACTIVE COMPOUNDS FROM AÇAÍ AND CANIHUA IN FROZEN PORK BURGERS

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This project aims to evaluate the application of extracts of canihua (Chenopodium pallidicaule) and açaí (Euterpe precatória) in frozen pork burgers, to increase oxidative and microbiological stability while maintaining the sensory attributes and healthier appeal of this product. The extractswill be obtained from a hydroethanolic solution and then evaluated for their antioxidant power using different methods. The results of the analysis will be used to calculate the level of addition to the meat product. A Negative Control treatment (without the addition of antioxidants), a PositiveControl treatment (with the addition of sodium erythorbate, a synthetic antioxidant), and two treatments with the addition of plant extracts in different concentrations respectively will be processed. The frozen pork burgers will be characterized and evaluated every 30 days during 4 months of storage at -18°C. Tests will be carried out for physicochemical evaluation: centesimal composition, instrumental texture, objective color, pH value, cooking losses, and oxidative stability (lipid and protein oxidation and volatile compounds). For the microbiological evaluation:coliforms, aerobic mesophiles, coagulase-positive Staphylococcus aureus, Salmonella spp. and, finally, the sensory acceptance test (nine-point Hedonic Scale). All the experiments will be repeated three times.

Keywords: Acceptability test; DPPH; Lipid oxidation; Plant antioxidants; Phenolic compounds.

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ACETIC ACID CONCENTRATION IN THE AROMATIC FRACTION OF WHOLE AND DEFATTED COCOA BEAN SHELLS

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Aiming to valorize cocoa bean shells (CS), a residue from the cocoa industry, the present study evaluated the influence of the fat extraction process with solvents on the concentration of acetic acid present in the aromatic fraction of CS. The extraction process was carried out using a "Soxhlet type" solvent extraction system for 1 hour, at temperatures of 60 and 90 °C, with the solvent traditionally used by industries, hexane, or with the alternative solvents ethanol or isopropanol. Volatile compounds were isolated by headspace solid-phase microextraction (HS-SPME) and identified by gas chromatography coupled to mass spectrometry (GC-MS). The main compounds present in CS were acids, aldehydes, alcohols, pyrazines, terpenes, and ketones. The majority compound identified in the CS was acetic acid, which represented (36.8 ± 0.3%) of the relative percentage, calculated by internal normalization. The acetic acid content in cocoa samples and derivatives is variable and dependent on fermentation, drying, and roasting processes. An excessive composition of this volatile can harm the taste of the material. Given this, the concentration of acetic acid was determined using a calibration curve, which presented an angular coefficient of 3.52 x 10^6 and a coefficient of determination (R^2) of 0.99914, representing an adequate description of the data. The volatile fraction resulting from the CS presented a concentration of (22.0 ± 0.5 mg/g). In comparison, in the defatted CS, the concentrations ranged from 0 to 7.56 mg/g, representing a reduction in the acetic acid content with the extraction process. Among the defatted CS, the richest in acetic acid were those obtained with the solvent hexane and the most significant reduction occurred with the solvents ethanol and isopropanol at a temperature of 60 °C. This behavior can be related to the polarity of the solvents. Therefore, it can be observed that, in general, the fat extraction step resulted in a reduction of acetic acid in the material and that the conditions used in fat extraction, such as the type of solvent, can influence the sensorial properties of the CS.

Keywords: Ethanol; Flavor; Headspace; Solvent extraction; Volatile.

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HANSEN SOLUBILITY PARAMETERS AMONG VEGETABLE OILS AND MIXED SOLVENTS

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Due to growing concerns about the environment, government organizations and environmental entities are actively promoting the use of new solvents derived from renewable raw materials instead of traditional ones derived from fossil sources, like hexane. As a result, ethanol is emerging as an excellent alternative to traditional fossil-based solvents because it is produced through biotechnological processes and is considered safer for human use compared to hexane. However, using ethanol in the fats and oils industry presents a challenge dueto the partial solubility of this renewable solvent with lipid compounds. Some studies have indicated the need to increase the solvent-to-solid ratio in vegetable oil extraction when ethanol isused instead of hexane. To address this issue, cosolvents can be employed. Cosolvents are substances that can be solubilized in both the solute (vegetable oils) and the solvent (ethanol), thereby increasing the overall solubility of the system. Fatty compounds, such as carboxylic acidsand long-chain alcohols, can be suitable cosolvents for ethanol because they can dissolve in both substances. They exhibit good chemical stability and are considered environmentally friendly solvents but cannot be used as a solvent because they have high viscosities. Therefore, the primaryobjective of this project is to analyze potential ethanol cosolvents to enhance ethanol's solubility with vegetable oils. The suggested cosolvents include fatty compounds with carbon chains ranging from 4 to 18. Hansen's solubility parameters were utilized to identify the optimal ethanol cosolventand the mixed solvent's ideal composition. These parameters were initially obtained from the literature for pure compounds and then calculated for mixed solvents, assuming the addition of 10 % mass of cosolvent to ethanol. This calculated parameter allowed us to determine the solute-solvent distance (Ra) between the mixed solvent and various vegetable oils. The lower the Ra value, the higher the solubility between vegetable oil and the mixed solvent. The data indicate thata higher percentage of cosolvent in the mixture results in increased solubility with vegetable oils. Additionally, when longer carbon chains were employed, the solubility between the solvent and solute also increased. When analyzing the chemical functionality of the added cosolvent, it was observed that carboxylic acids led to a smaller solute-solvent distance, indicating greater solubilitybetween the mixed solvent and vegetable oils. It is essential to note that experimental data are required to validate these findings, as the high viscosity and density of fatty compounds can directly affect the extractive processes.

Keywords: Carboxylic acids; Cosolvents; Ethanol; Fatty alcohols; Vegetable oils.

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CHARACTERIZATION AND STABILITY STUDY OF BREWER'S SPENT YEAST ENRICHED WITH ASCORBIC ACID

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Ascorbic acid is an organic compound essential for the healthy functioning of the human body, mainly due to its antioxidant properties and its crucial role in maintaining health and the proper functioning of the immune system, in addition to several other functions. Despite this, ascorbic acid is not produced naturally, making it necessary to ingest it from exogenous sources. However,the biggest problem related to this nutrient is its high instability, therefore, it is interesting to lookfor ways to allow its controlled release and protect it from adverse conditions. Thus, also aiming to add value to a by-product of the brewing industry that is widely available, with low added valueand high protein content and variety of active sites, this work proposed the use of *Saccharomyces pastorianus* yeast biomass as a vehicle for ascorbic acid incorporation. *In natura* and modified biomasses were used in order to evaluate those that present the best results in preliminary biosorption tests, therefore, the materials selected for the biosorption study were *in natura* yeast (Y) and yeast after alkaline modification (YA). Thus, after optimization process, particles with ascorbic acid were produced under the best conditions and dried by lyophilization, aiming the characterization and stability studies of these *in natura* and alkaline yeast after the biosorption of ascorbic acid (Y-AA and YA-AA, respectively). For the stability test, the enriched biomasses werestored in hermetically sealed glass bottles at a temperature of 25 °C containing a saturated $MgCl_2$ solution (relative humidity of 32.3%), for a period between 0 and 35 days. Thus, every 7 days, theascorbic acid desorption process present was carried out, quantified by High Performance Liquid Chromatography. Thus, the materials were morphologically evaluated, through Optical Microscopy, Scanning Electron Microscopy, Confocal Laser Scanning Microscopy, in addition toevaluating the molecular conformations through Fourier Transform Infrared Spectroscopy, and the measurement of zeta potential, electrophoretic mobility, humidity and water activity in order to better understand the presence of the ascorbic acid in yeast cells. The particle stability study demonstrated that the concentration of incorporated ascobic acid after 35 days decreased to 38.83 ± 1.21% and 35.29 ± 0.29% for YI-AA and YA-AA, respectively, presenting values well similar. Thus, both materials presented favorable results for acting as vehicles for the incorporation of ascorbic acid, which could also be demonstrated through characterization techniques, which gave evidence of the presence of this nutrient in yeast cells.

Keywords: Biosorption; Encapsulation; Lyophilization; *Saccharomyces pastorianus*; Vitamin C.

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USE OF *Saccharomyces boulardii* IN THE PRODUCTION OF FERMENTED HONEY AND CUPUAÇU BEVERAGES

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In the production of beverages, honey is mainly used to make mead, which is a traditional alcoholic fermented drink made by diluting honey in water and adding yeast. In the literature, different fruits have been added to mead, generating new flavors and increasing the appreciation of regional fruits. The addition of fruit to mead, as well as adding organoleptic, antioxidant and nutritional properties, has given rise to a new variation of this drink, called melomel. Cupuaçu, *Theobroma grandiflorum*, is a fruit grown mainly in the Amazon region of the country. It has a yellowish color, an intense, sharp and sour taste, with a pleasant and striking aroma. The yeast S. boulardii, also known as *S. cerevisiae var. boulardii*, is a well-established probiotic yeast for the treatment of gastrointestinal diseases, that has recently been used in the production of functional foods and has achieved positive results in the production of alcoholic fermented beverages. Therefore, the aim of this study was to develop a cupuaçu melomel drink using the probiotic yeast *S. boulardii*, as well as to evaluate its microbiological and physical-chemical characteristics, phenolic compounds, antioxidant activity, gastrointestinal digestion simulated in vitro, shelf life, alcohol content and sensory characteristics. The results obtained were promising, with up to 8% alcohol content, with a viable yeast count of 10^7 CFU/mL after in vitro digestion test, as well as after 10 days of storage in the fridge at 4°C. In addition, in the sensory analysis of the acceptance type, the scores observed were satisfactory, as was the purchase intention. Thus, the yeast *S. boulardii* proved to be effective in producing a probiotic cupuaçu melomel, contributing to the probiotic food and probiotic alcoholic fermented beverage industry.

Keywords: Beneficial microorganisms; Functional beverages; Melomel; Probiotics; Unconventional yeasts.

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APPLICATION OF BIOPOLYMER-BASED COATINGS INCORPORATED WITH EMULSIONED ACTIVE PRINCIPLES IN PAPER

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Cellulosic packaging (paper) stands out for its low cost, easy degradation, and recyclability. However, the porosity of this material makes its application limited, as it can significantly influence the shelf life of the stored product. The application of coating based on biopolymers incorporated with active principles and nanoparticles can be an alternative to fix this problem, allowing the development of active nanocomposite material with improved functional and physicalproperties. Thus, the main objective of this thesis is the development of an active cellulosic material coated with cassava starch, gelatin, or chitosan-based solutions incorporated with an emulsion encapsulating active principles (alpha-tocopherol, carvacrol, and rutin) and carrying nanoparticles, one inorganic (montmorillonite) and two organics (starch and cellulose nanocrystals). For the development of nanoemulsion, Pickering and conventional emulsion, a microfluidizer or an ultraturrax has been used. Initially, preliminary tests are being carried out to establish optimal process conditions for the development of emulsions: concentration of emulsifiers: span 80 (75, 50, and 10%) and Tween 80 (25, 50, and 90%), and homogenization timein ultraturrax (5, 7 and 10 min). The emulsions were evaluated regarding particle size, polydispersity index, and stability. Subsequently, starch and cellulose nanocrystals will be produced by acid treatment. Then, the biopolymer coating-forming solutions containing only the emulsified active ingredients and the emulsified active ingredients plus nanoparticles will be prepared and applied on the paper (monolucent paper and kraft paper) using an automatic film spreader. The active films, nanocomposites, and coated cellulosic materials will be characterized regarding physical properties, microstructure, antioxidant, and antimicrobial activity. Recent preliminary test results showed that nanoemulsion produced with 50% Span 80 and 50% Tween 80 using homogenization for 5 min in the ultraturrax and three cycles in the microfluidizer at 150 MPa presented a nanometer-sized droplet (average equal to 79 nm), bimodaldistribution with a polydispersity index equal to 0.5 and a low instability index (average equal to 0.375). Furthermore, the characterization of the uncoated cellulosic material confirms the need toapply a coating due to its low water barrier properties. However, it is still necessary to establish better process conditions to obtain greater homogeneity of the emulsions for their effective application.

Keywords: Cellulosic material; Nanoemulsion; Nanoparticles; Packaging; Recyclability.

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PLANT BASED BURGUER WITH REDUCED METHYLCELLULOSE CONTENT

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The consumption of meat products has been associated with potential negative impacts on the environment, due to greenhouse gas emissions and deforestation, as well as on human health, beinglinked to the development of chronic diseases. As a result, companies have been striving to developnew plant-based products, known as "plant-based analogs." However, these products have also raised concerns due to the high number of additives and synthetic ingredients used to mimic sensory properties similar to meat products. In light of the above, the present project aims to develop a plant-based burger (plant-based analog) with a cleaner label, meaning it contains the lowest possible dosage of additives and synthetic ingredients. Four formulations of a reduced methylcellulose plant-based burger were developed, varying the levels of methylcellulose reduction (25%, 50%, 75%, and 100%), with 1g of methylcellulose added for every 100g of product in the 100% formulation. A sensory evaluation was conducted using an affective preference-ranking test with 60 consumers. The samples were presented simultaneously, coded with three-digit random numbers, and the panelists were asked to rank the burgers in their order ofpreference. The results showed that the scores obtained for the 100% methylcellulose formulationwere 159 points, for the 75% formulation were 180 points, for the 50% formulation were 178 points, and for the 25% formulation were 143 points. It was observed that the acceptance increasedfrom the 100% formulation to the 75% and 50% formulations, which remained close, and decreased in the 25% formulation. The most preferred formulations were those with 75% and 50%methylcellulose, which did not statistically differ from each other. Due to the study's objective of reducing methylcellulose, the chosen formulation was the 50% one. Therefore, based on the resultsobtained, it can be concluded that it is possible to develop a plant-based burger with a reduction ofup to 50% in methylcellulose.

Keywords: Clean label; Meat product; Sensory evaluation; Texture; Vegetable analogue.

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INCORPORATION OF NANOENCAPSULATED COENZYME Q10 IN SOY PROTEIN ISOLATE GELS

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The development of plant-based foods is currently expanding, due to the constant growth of vegetarian, vegan and flexitarian audiences. There is an increasing tendency of avoiding the consumption of animal products and making healthy food choices, leading to a huge increase of plant-based food market. Soy ingredients are the most used in the formulation of such products, including soy protein isolate (SPI). The objective of this work was to develop SPI emulsion-filled gels, incorporating nanoencapsulated CoQ10 (coenzyme Q10, a powerful antioxidant). Nanoemulsions encapsulating CoQ10 (produced with oil phase composed of medium chaintriglycerides, and phospholipids as surfactants) replaced the aqueous phase in various ratios (25 to100%) to produce heat-set emulsion-filled gels. The gels were prepared with 15 g/100 g SPI at pH7 and 0.1 M NaCl, and they were evaluated according to their visual appearance, confocal laser scanning microscopy and large deformation rheology. The results obtained demonstrated that sustainable gels were formed in all tested concentrations of droplets. According to the uniaxial compression results, the oil droplets acted as active particles because as the concentration of nanoemulsions increased, the modulus of E (Young's modulus) and σrup (rupture strain) increased. The gels produced with 90% replacement of aqueous phase by nanoemulsions showed the highestvalues of E (with no significant difference with 85%) and σrup, whereas the gels with 25% replacement showed the lowest values of E and σrup. The results of confocal laser scanning microscopy demonstrated the increasing of nanoemulsion amount in the gels did not lead to an increase in the size of the droplets or to their aggregation. According to the micrographies, the oildroplets were occupying the voids among the protein networks. In the gels produced with 100% replacement of aqueous phase by nanoemulsions, it was noticeable the presence of droplets affected the structure of protein networks, making the formation of gels more difficult. Scanning electronic microscopy (SEM) showed the nanodroplets on the external part of the microgels, as water replacement increased. According to instrumental colorimetry, the samples present look likeyellow and red, with 25% being closer to yellow and 95% to red. The data obtained in this study indicated that emulsion-filled gels produced using SPI and the CoQ10-loaded nanoemulsions are promising for the development of protein-rich food prototypes.

Keywords: Coenzyme Q10; Emulsion-filled gels; Flexitarians; Soy proteins; Vegetarians.

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CHEMICAL STABILITY, RELEASE AND IN VITRO DIGESTION OF GELATIN-BASED ACTIVE FILMS INCORPORATED WITH DOUBLE EMULSION ENCAPSULATING PITANGA LEAF HYDROETHANOLIC EXTRACT

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Active films (AF) are flexible materials which can be produced with biopolymers incorporated with active compounds (AC). These materials can be used to produced active packaging with highpotential for food preservation allowing reduction of additives into food. The Pitanga leaf hydroethanolic extract (*Eugenia uniflora* L.) (PLHE) is a substance rich in polyphenols which havebeen used in the development of AF. Considering that polyphenols are unstable under action of light or oxygen, for instance, they can be encapsulated into an emulsion protecting these compounds when exposed to environmental and gastrointestinal conditions, guaranteeing the preservation of it biological activities. Furthermore, loading AF with reinforcing fillers such as crystalline nanocellulose (CN) may be convenient in producing AF with improved physical properties. The overall objective of this work is to study the chemical stability and release of polyphenols and *in vitro* digestion of gelatin-based active films, without and with charge of CN from soybean straw, and activated by W/O/W emulsion rich in PLHE (DE). The films were analyzed in relation to their physical-chemical, mechanical and active properties, and in relation to their microstructures. They were also submitted to physical-chemical analysis before and after *in vitro* digestion. The cytotoxicity was also studied. The release of AC will be evaluated using aqueous and lipid media simulating food. The chemical stability of films and emulsions for 180 days will also be analyzed. The internal structure of the films (thickness ~80 µm) became less smooth and homogeneous with the addition of DE and/or CN. The presence of DE and CN decreased water vapor permeability and increased the tensile strength of the films, which also showed good UV/Vis light barrier properties. The addition of DE, or non-encapsulated PLHE, gave antioxidant activity to the films. Encapsulation of PLHE in DE increased the bioaccessibility of PLHE under *in vitro* digestion conditions. In the films, no cytotoxicity effects were observed before or after *in vitro* digestion. Despite the lower bioaccessibility of PLHE in the films, they stillshowed good PLHE bioavailability at the end of *in vitro* digestion. Therefore, these results were important to help elucidate the benefits of encapsulating AC in emulsified systems, the effects onthe physicochemical, mechanical and functional properties of films incorporated with thesesystems, and how interesting and safe an active packaging produced using this technology can befrom these materials. This project continues for knowing of polyphenols release and stability behavior.

Keywords: Antioxidant Activity; Barrier properties; Bioaccessibility; Cytotoxicity; Microstructure.

Acknowledgement: To FAPESP for grant (2013/07914-8) and CNPq for grant(40.3746/2021-3), Research fellowship of P.J.A.S. (30.2482/2022-9) and SWE fellowship of L.T. (40.0612/2022-4). This study was financed in part by the Coordenação deAperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

DEFATTED BLACK SOLDIER FLY LARVAE FLOUR AS A NOVEL PROTEIN INGREDIENT FOR PARTIAL REPLACEMENT OF BEEF WITHIN HOT DOG SAUSAGES

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As the global population continues to increase, the demand for food also rises, which leads to concerns about the depletion of the planet's finite resources. To address this, it is necessary to rethink current eating patterns and seek alternative protein sources that are more sustainable. In this sense, edible insects become great options due to their numerous environmental, nutritional, social, and subsistence advantages. They can be eaten whole, in parts, or ground into powder and added in an “unrecognizable” way to other types of foodstuffs, encouraging entomophagy (insectconsumption). The black soldier fly (BSF) (*Hermetia illucens* L.) is one of the species highlighted with strong potential for human consumption by the European Union and the degreasing process can be applied to reduce the lipid content and caloric density of BSF, while also improving its sensory attributes. Thus, this study aims to develop hot dog sausages with partial replacement of beef with defatted flour (DF) from BSF larvae. The wholemeal flour will be defatted through extraction with supercritical fluid and later added as a new proteic ingredient in the meat products.The treatments will be established to provide isoprotein formulations, that is, with different concentrations of DF to partially replace meat while maintaining the same protein content: a control treatment (100% beef), and two treatments using different concentrations of DF and reduced amounts of beef. The influence of the new ingredient on the physical-chemical, microbiological, and sensory acceptance characteristics and stability of the products will be evaluated. For characterization, proximate composition analyses will be carried out (moisture, proteins, lipids, and ash), and sensory acceptance. The stability will be evaluated on day 1 and after45 days of refrigerated storage. About physical-chemical stability, pH, color, texture (TPA) and lipid oxidation (TBARs) will be analyzed and, for microbiological stability, total coliforms, *Escherichia coli*, *Staphylococcus aureus*, *Clostridium perfringens*, *Salmonella spp.*, and total counts will be investigated. The entire manufacturing process will be repeated twice. Afterward, the results will undergo analysis of variance (ANOVA) and Tukey's test at a 5% significance level using SAS software version 9.4. The products are expected to have attractive sensory attributes, with reduced use of beef and minimal impact on other parameters compared to the control treatment. Furthermore, this research expects to contribute significantly to the state of the art by developing innovative products with the ultimate goal of offering ecologically sustainable proteinalternatives to assist in combating food insecurity.

Keywords: Entomophagy, *Hermetia illucens* L., Sensory acceptance, Supercritical CO_2 extraction.

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GELATIN HYDROGELS WITH CELLULOSE NANOFIBERS ISOLATED FROM SOYBEAN STRAW BY ENZYMATIC TREATMENT

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Research in biodegradable hydrogels for food packaging applications with improved mechanical, rheological, antimicrobial and absorption properties has accelerated in recent years. Nanocellulose extracted from agricultural waste can be used as a sustainable and biodegradable nanofiller to improve the physical properties of hydrogels. The objective of this study was to evaluate the effect of soybean straw cellulose nanofiber (CNF) concentration on the mechanical, viscoelastic, and swelling properties of type B gelatin hydrogels (HGB). The hypothesis is that CNF would improving the hydrogel properties prepared by physical method. Soybean straw was chemically treated with a 17.5% NaOH solution (w/v) for 15h, and bleached using solutions of 4% H_2O_2, 0.3% $MgSO_4.7H_2O$ and 2% NaOH (w/v) at 90 °C/3h. CNF were produced by enzymatic hydrolysis of 3 g soybean straw with 150 mL of sodium acetate buffer and 280 µL of an enzyme cocktail Optimash™ VR (DuPont Inc, USA) at pH 4.0 and 50 °C/ 42h, followed by mechanical treatment in ultraturrax (15000 rpm/5min) and sonication (70% amplitude/3 min). Hydrogels were prepared with 10 g of gelatin/100 g of solution and 0, 0,5, 1, 3 of CNF/100 g of gelatin. HGB were prepared in cylindrical molds (20 x 20 mm, diameter and height) at 4 °C/24h. The mechanical and viscoelastic properties, were determined by uniaxial compression at 90% and 20% deformation, respectively, using a texturometer (TAXT2, TA Instruments, UK). Swelling properties (SP) were determined gravimetrically, using freeze-dried hydrogels (20 x 5 mm, diameter and height). The increase of CNF concentration (0−3%) decreased the mechanical properties of HGB at high deformations; fracture stress varied from 282.7±12.9 to 126.5±0.3 kPa, fracture strain from 77.3±0.6 to 66.0±0.5%, whereas the elastic modulus increased from 0.28±0.01 to 0.34±0.02 kPa/%. The hydrogels presented viscoelastic behavior described by Maxwell model (R^2 >0.99), with no significant changes due to the CNF concentrations (Relaxation modulus, 28 to 32 kPa; extensional viscosity 14-16 x 10^6 $Pa.s^{-1}$) The SP decreased with the NFC presence (0-3%) from 2023.1±48.5% to 1369.9±16.4%. The mechanical behavior of HGB could be associated to the formation CNF aggregates and to a low interfacial bonding between CNF/gelatin chains influenced by large amounts of water contained in network. The formation of a more rigid network in the dry state hydrogels that makes it difficult for water molecules to penetrate, could be explained the SP. The mechanical and swelling properties of hydrogels in both states are regulated by electrostatic interactions CNF/gelatin.

Keywords: Agro-industrial waste; Gels; Nanocellulose; Nanocomposite.

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OCCURRENCE OF OCHRATOXIN A IN CONVENTIONAL, ORGANIC CORN AND DERIVED PRODUCTS IN BRAZIL

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Among the various crops that support agriculture in Brazil, corn production receives great prominence due to its versatility of use that covers both food and feed. Considering the safe consumption of corn and its derivatives, one of the main concerns is the contamination by molds, as they can produce mycotoxins. Ochratoxin A is a mycotoxin produced by species of *Aspergillus* and *Penicillium*. Therefore, corn production requires attention throughout the production chain to avoid contamination. Once fungi are usually controlled with fungicides in conventional agriculture, and the organic system does not use synthetic fungicides, it has been questioned whether there would be a higher occurrence of mycotoxins in organic corn. This study evaluated the occurrence of ochratoxin A in corn and derivatives marketed in Brazil. One hundred and forty conventional and 60 organic samples were collected: hominy, yellowhominy, canjiquinha, breakfast cereal, corn cream, cornmeal, pre-cooked cornmeal, corn flour,flocked corn flour flakes, gritz and popcorn. The samples were ground, subjected to extraction,dilution, and injection into the HPLC-MS/MS system. The conventional samples had 19 positives, being eleven samples of popcorn, three of flocked corn flour, two of hominy two of cornmeal and one of corn flour. The level of ochratoxin A detected in conventional samples ranged from 4.8 to 10.8 µg/kg, and the average of the positive samples was 7.37 µg/kg. The brazilian legislation considers 10 µg/kg of ochratoxin A in cereals and their derivatives as a maximum limit. Two popcorn samples showed levels (10.8 µg/kg) above the allowed limit. Inorganic samples, ochratoxin A was not detected. Ochratoxin A levels in conventional and organic samples were different ($p \leq 0.05$). The occurrence of ochratoxin A only in conventionalsamples suggests that some practices or inputs used in organic farming may be unfavorable to the *Aspergillus* and *Penicillium*, or to the production of toxin, since agricultural practices, transport and storage directly influence the mycotoxin production. The findings of this study indicated that the conventional system favored the occurrence of ochratoxin A in corn samplesand derivatives.

Keywords: *Aspergillus*; Fungi; Grain; Metabolites; Mycotoxin.

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FERMENTATION AND MICROBIAL VIABILITY IN THE PRODUCTION OF KOMBUCHA WITH GUARANA *(Paullinia cupana*)

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The growing consumer interest in functional foods is due to its health benefits. This includes the rising popularity of fermented beverages like kombucha, driven by their nutritional properties, which attracts both consumers and researchers. The widespread adoption of kombucha has led to the need for quality guidelines to ensure consumer safety. However, there are gaps due to the diverse range of products resulting from kombucha fermentation. Studies on kombucha productionusing substrates beyond traditional teas, like native Brazilian ingredients, including guarana, are limited and the evaluation of fermentation methods and conditions for the beverage is scarce. Guarana *(Paullinia cupana)*, a fruit native to the Amazon region, known for its stimulating and antioxidant properties, has been recognized as a potentially promising substrate. Thus, the objective of this study is to create a kombucha using guarana extract and to analyze different aspects of its fermentation, composition, and stability, including fermentation kinetics,physicochemical properties, antioxidant potential, bioactive compounds, microbial viability, and compliance with regulations. For this, we tested different factors, like acidified inoculum, solublesolids content, fermentation temperature, and fermenter proportions.

Keywords: Fermented Beverage; Fermenter; SCOBY; Starter; Tea.

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EVALUATION OF MATURATION PROCESS OF THE CREAM ON FINAL QUALITY OF THE BUTTER IN BRAZIL

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The butter consumption has growing up all over the world, as well as the demand of consumers in attribute to the product quality. Changes in the cream aging effects physico- chemical, organoleptic and performance characteristics of the butter. Among the cream components, saturated and unsaturated fatty acids are the main responsible for these characteristics. The present study aimed to compare the characteristics and composition of the cream, through the unsaturated fatty acids contents and physicochemical analysis with the aging temperature, from some Brazilians regions. Iodine values were obtained by the Wijs method (Titrando 905® – Metrohm), according to the location and seasonality of Brazil, and then the physicochemical characteristics of the butter (Foodscan®, Foss) from the two maturation heat treatments, which were evaluated and compared. After production, between the third and seventhday, the texture was evaluated through TA XT Plus® (Stable Systems), sensory properties and churning efficiency. The results of the iodine value results were all lower than 35gI_2/100 and did not have variation according to the origin and time of year. The fat results of the cream and butter, as well as the humidity did not obtain significant differences between the localities and during the months evaluated. The texture of the butter obtained a better consistency in all samples which thecream aged by the heating process, which was also confirmed by the sensory analyses for same parameter of texture/spread ability, as well as aroma and flavor. Buttermilk fat showed a better result in the Southeast region in relation to the South and Midwest. The correlations of fatty acids still need to be better studied, since individually they did not present a linear relationship as expected for the texture characteristics; and ratio between the main saturated and unsaturated fattyacids present in butter should be evaluated, as suggested by some authors recently. Thus, we can conclude that the maturation of the cream can interfere in the final quality of the butter and the heating process at this time is an important factor to enhance the main qualities of the product.

Keywords: Butter, Cream aging, Crystallization, Milk fat, Texture.

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STATIC IN VITRO DIGESTION OF MIXED PROTEIN WITH VITAMINS B12 AND D3 USING ELDERLY MODEL

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Humans enjoy longer and healthier lives, and for society this presents several challenges, but also opportunities. The older adults undergo significant changes in physiological functions that can lead to malnutrition, including lack of micronutrients and loss of muscle mass. A strategy to deal with this problem is the ingestion of food products that simultaneously deliver proteins and vitamins to assure that adequate amounts of these nutrients are consumed. Heat-set mixed protein gels (MPG) of soy protein isolate (SPI) and whey protein isolate (WPI) were produced using different ratios SPI:WPI (15 g protein/100 g gel), and it was evaluated the influence of the MPG microstructure on the digestion and bioaccessibility of proteins, and vitamins D3 and B12. SPI was previously submitted to a homogenization treatment that combined mechanical stirring (500 rpm, 20 min, 40 ºC) and high-intensity ultrasound (25,000 J, 20 kHz, 70% amplitude) to enhance its solubility. The commercial SPI powder is heterogeneous and poorly soluble. After treatment, the solubility of the SPI suspension increased by 54% and the suspension became less heterogeneous. The MPG microstructure produced with this SPI dispersion presented much more homogeneous three-dimensional networks according to CLSM and SEM. Static *in vitro* digestion adapted to elderly digestion conditions was used to obtain data about the bioaccessibility of proteins and vitamins B12 and D3. As WPI was replaced by SPI the protein released and the protein hydrolysis increased. The molecular weight distribution was estimated by SEC-HPLC and the protein composition was performed by reducing SDS-PAGE. The results showed that at the end of the gastric phase the proteins were not completely digested. However, at the end of the intestinal phase the proteins were bioaccessible for absorption. Regarding the vitamins bioaccessibility, digestion helped vitamin D3 to become more bioaccessible when reaching the intestine (higher concentration of vitamin D3 in the end of intestinal phase compared to the gastric phase). For vitamin B12, there was no difference in its concentration in the intestinal and gastric phases, indicating a good result, because vitamin B12 must be bioaccessible in the stomach to bind to the R protein, which then transports the vitamin to the duodenum, where it will be absorbed. Mixing SPI and WPI resulted in improved digestion of proteins, as well as the bioaccessibility of vitamins. Therefore, the results indicated the MPG are interesting protein matrices to be used in food products.

Keywords: Bioaccessibility; Cholecalciferol; Cobalamin; Composite gels; Plant protein.

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EFFECT OF NATURAL POMEGRANATE EXTRACT (*Punica granatuml*) AS AN INHIBITOR OF FUNGAL GROWTH IN BREAD

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The food industry's growing interest in replacing the use of synthetic additives/preservatives with natural ones has encouraged research to focus on alternative sources and work on identifying antimicrobial and antifungal agents from natural sources. Pomegranate peel is a co-product of the industry, generated by processing the fruit, which has a high content of bioactive compounds, especially phenolics and flavonoids, and a high antioxidant potential, as well as being able to act as antimicrobial and antifungal agents. The main objective of this study was to evaluate the effect of applying pomegranate peel extract as a fungal growth inhibitor in bread rolls. The effect of the concentration of pomegranate peel extract in the bread formulation was evaluated in four contractions (10% - PFER10, 12% - PFER10, 15% - PFER15 and 17% - PFER17). Control formulations were also produced (without the addition of extract and preservative - PFCN) and with the addition of a synthetic preservative (PFCP). The breads were stored for 15 days and analyzed every 3 days for fungal growth. During the period established for evaluating the breads, i.e. 15 days, the PFER10, PFER12, PFER15 and PFER17 formulations showed no fungal growth. On the other hand, the breads in the control formulation began to deteriorate on the 6th day of storage with PFCN 7.5X10 5 ±3.79ª UFC/g and 3.1X10 6 ±1.00b UFC/g. Thus, based on the results presented, it can be concluded that the use of pomegranate peel extract in bread formulations, in the range studied, allowed for the inhibition of fungal growth in loaves, promoting an increase in the shelf life of the loaves, regardless of the concentration used.

Keywords: Analysis; Fungi; Shelf life.

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OCCURRENCE OF AFLATOXINS IN ORGANIC RICE PRODUCED AND COMMERCIALIZED IN BRAZIL

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Rice, present in the daily diet of most Brazilians, provides nutrients and energy. In Brazil, there is the conventional cultivation, with agricultural inputs such as pesticides and fertilizers, which can eventually be harmful to human health and ecosystem; and the organic cultivation, which uses new planting techniques to reduce the negative effects of the conventional system. The rice grain, a nutritional medium, when subjected to inadequate practices from planting to storage, may be susceptible to contamination by fungi capable of producing mycotoxins, such as aflatoxins. In Brazilian legislation, the National Health Surveillance Agency (ANVISA) and the Ministry of Agriculture and Livestock (MAPA) are responsible for establishing the maximum tolerable limits for the presence of aflatoxins in foods in general, as well a specifically for cereals such as rice, without differentiating the type of planting. Therefore, the objective of this study was evaluate the occurrence of aflatoxins in 60 samples of organic rice collected in different regions of Brazil. Samples were analyzed for the presence of aflatoxins B1, B2, G1 and G2, using an immunoaffinity column (IAC) and high performance liquid chromatography (HPLC). Results demonstrated that, of the 60 samples analyzed, 5 samples of wholegrain rice presented levels of 0.13 to 1.24 µg/Kg for the sum of aflatoxins B_1, B_2 e G_1 and G_2, while one sample of polished rice and one of red rice with presented levels of 0.11 µg/Kg and 0.16 µg/Kg for aflatoxins B_1 and G_2, respectively. For the risk assessment regarding contaminated rice consumed by Brazilians, the average probable daily intake (IDPM) showed a value of 0.004 µg aflatoxin/Kg body a day; while the margin of exposure (MoE) was 60, what is considered of a high concern for public health; and the estimated risk index (HI) for aflatoxin B_1 was 0.41, below the recommended of HI < 1, with a low risk for developing liver cancer. The concentrations of aflatoxins obtained in this study did not exceed the maximum tolerable limit recommended by MAPA of 50 µg/Kg and by ANVISA of 5 µg/Kg of rice, regardless of planting method. However, research on this type of product is still scarce, therefore, additional studies are necessary in order to obtain a broad database for the occurrence of aflatoxins in rice from the organic planting system and, thus, to determine indeed whether there is a need for specific legislation.

Keywords: Analysis; Fungi; Shelf life.

Acknowledgement: This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

OPTIMIZATION OF THE INTERMITTENT PRESSURIZED LIQUID EXTRACTION PROCESS FOR HIGH YIELD OF BLACK SAGE LEAF EXTRACT (*Varronia curassavica* JACQ.)

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Black Sage (*Varronia curassavica* Jacq.) is a medicinal plant traditionally used by Brazilian indigenous peoples and already renowned for the use of its essential oil as an anti-inflammatory. Pressurized liquid extraction (PLE) has been used as an alternative for extraction process by eliminating or reducing the amount oftoxic organic solvents. The working principle of PLE focuses on controllingthe viscosity and diffusivity of the solvent used from variables such as temperature. Thus, it is possible to identify the effect of variables such as Temperature (T), Static time (St) (time that the solvent is in contact with the leaves), number of cycles (C) and Volume of rinsing solvent (SV) (calculated depending on the size of the extraction cell) promote extraction. In this context,this study aimed to evaluate the effect of the variables T (40°C to 80°C), St (2 min. to 10 min.),C (1cycle to 5 cycles) and SV (60% to 140%) on overall yield of the black sage extract Leaves.The extractions were carried out in order to optimize the process of obtaining the extracts by means of a rotated central composite design (RCCD). The results showed that there was an increase/decrease in the overall yield from 5,47% to 11,46% when the temperature was increased from 40°C to 70°C. The highest yield (11,46%) was identified at a temperature of 70°C, St of 8 min, VF of 120% and 4 cycles. Within the 70°C isotherm, we were able to observethat the highest yield value was 11.46%, using the conditions of St of 8 min, VF of 120% and C of 4 cycles. In the same isotherm, the condition that obtained the lowest yield was 7.93% under the conditions of St of 8 min, VF 120% and C of 2 cycles. When compared to the yield obtained via Soxlet of 14.8 ± 0,2%, the values obtained via PLE are still a little lower. This work aimed at optimizing the yield of plant extracts can have wide temperature ranges depending on the composition of the plant material, in the case of leaves such as Varronia curassavica, which contains large volumes of waxy materials and long-chain isoprenes, probably higher temperatures when using PLE with ethanol as solvent would be even more efficient, obtaining higher yields. Besides the temperature there was no interaction between the variables analyzed that influence the yield of black sage leaf extracts.

Keywords: Black sage, *Cordia verbenacea*, Erva-baleeira, Herbal extract, *Varronia curassavica*.

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GLASS TRANSITION AND STATE DIAGRAM FOR FREESE-DRIED CUPUASSU (*Theobroma grandiflorum* [Willd. ex spreng.] K. Schum) PULP

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Cupuassu pulp (CP) is highly appreciated for its characteristic acidic flavor and intense fragrance, being a good natural source of antioxidants. However, due to its high perishability, it has a very short shelf life. Various changes in physical, chemical and biological characteristics of foodstuffs occur during processing, storage and distribution. The knowledge of phase transitions of foods is important in characterizing their quality and in designing efficient processing systems. In this study, the state diagram of freeze-dried CP conditioned at various water activities at 25°C were determined using differential scanning calorimetry (DSC). Freeze-dried CP containing unfreezable and freezable water were examined to explore its state diagram. The state diagram included the freezing curve (Tm), glass transition line (Tg), and ultimate maximal-freeze-concentration condition. The freezing curve and the glass transition line were fitted according to Clausius Clapeyron model and Gordon-Taylor model, respectively. The Tg of CP decreased from 8.6 °C to -87.2 °C as the water content increased from 0.04 to 0.37 g water/g sample (w.b.), due to water plasticizing effect on the amorphous constituents of the matrix. The Gordon-Taylor parameters Tgs and k obtained for CP were 33.8 °C and 3.92 respectively. The ultimate maximal-freeze concentration conditions were found as Tg' equal to -53.5 °C and the characteristic solids content, Xs' as 0.79 g solid/g sample (w.b.) (i.e. un-freezable water, $Xw' = 0.21$ g water/g sample (w.b.)). In addition, the GAB monolayer moisture content (Xm) was observed to be 0.15 g water/g sample (d.b.). At 25° C, the critical water content (CWC) and the critical water activity (CWA) were 0.014 g water/g sample (d.b.) and 0.076 respectively. Therefore, the state diagram of freeze-dried CP can be used in determining the storage stability as a function of temperature and water content, and optimizing drying and freezing processes.

Keywords: Cupuassu; Differential scanning calorimetry; Glass transition temperature; Sorption isotherms; Water activity.

Acknowledgement: P-H, P.V. was supported by a scholarship from Programa Nacional de Becas y Crédito Educativo (PRONABEC) of the Ministerio de Educación del Perú. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

EVALUATION OF THE FUNCTIONALITIES OF THE PROTEIN FRACTION CONTAINED IN BRAZIL NUT PRESS CAKE

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The Brazil nut (*Bertholletia excelsa*), native to South America, has significant economic importance for the Amazon region. Its nutritional richness has stood out due to the high lipid content (60 – 70%) and, mainly concerning the protein content (17%) and selenium (36 µg/g) which, when associated, can form organic complexes with high bioavailability and antioxidant capacity. Few studies have evaluated the application of Brazil nut press cake (BNPC) to obtain proteins. BNPC is a solid material resulting from the extraction of lipids by pressing the nuts. Therefore, this work aimed to characterize BNPC and evaluate the protein solubility using differentsolvents and pHs, aiming at its application in obtaining protein isolates. The press cake was obtained from COOPAVAM (Cooperativa dos Agricultores do Vale do Amanhecer, Juruena/MT)and analyzed for composition in terms of moisture, lipids, proteins, and ash content using the official methods Ac 2-41, Am 5-04 and Ba 4e-93 (AOCS, 1998) and 900.02 (AOAC, 2007), respectively. The protein solubility index expressed as nitrogen solubility index (NSI, %), was determined according to the method of Morr *et al*. (1985) using deionized water or 0.1M NaCl solution using a solid/solvent ratio of 1/50 (w/w), at 25 °C, under pH values 6.0 and 9.0. After 120minutes at constant stirring at 300 rpm, the dispersions were centrifuged at 5,000 x *g* for 30 minutesat 4 °C. The supernatant was filtered through the Whatman filter, and the nitrogen content was analyzed following the Dumas method (Leco, FP-528, USA), using the factor of 5.46 (AOAC, 1995) to convert the total nitrogen content into proteins. The moisture content present in the BNPCwas 3.4 ± 0.1%. Its chemical composition, expressed as mass % on a dry basis, was 52.5 ± 0.4 lipids, 23.7 ± 0.4 proteins, and 5.4 ± 0.1 ash. The proteins presented NSI in water of 74 ± 10% at pH 6.0 and 94 ± 2% at pH 9.0, while solubility in NaCl was 78 ± 14% and 91 ± 3% at pH 6.0 and9.0, respectively. From the NSI values, it can be concluded that Brazil nut proteins have high solubility in water, indicating their possible use as a solvent in protein extraction, providing a moreeconomical and environmentally friendly process.

Keywords: Aqueous extraction; *Bertholletia excelsa;* Nitrogen solubility index.

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USE OF RESIDUES OBTAINED FROM THE FOOD TRADE TO DEVELOPMENT OF COMPOSITE FILMS REINFORCED WITH CELLULOSE NANOCRYSTALS AND WITH ANTIOXIDANT ACTIVITY

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The production of edible films from biopolymers extracted from food waste that would be discarded at open markets, such as potatoes and cassava, contributes to reducing dependence of petroleum-derived plastics while following the trend of a circular economy. However, biopolymeric films, such as starch films, have characteristics that limit their use. To solve this challenge, cellulose nanocrystals extracted from the pineapple crown can act as a reinforcing material, as they have characteristics that improve the mechanical properties of the films, increasing strength and rigidity, in addition to acting as an additional barrier, helping to reduce permeability to oxygen and moisture. These nanocomposite films can be enriched with bioactive principles, giving rise to active packaging. In this sense, the aqueous extract of the oregano leaf isan excellent option, as it will provide the films with antioxidant and antimicrobial properties, and consequently will help protect packaged foods against oxidation and deterioration caused by the action of free radicals, thus prolonging their shelf life. Therefore, the objective of this project is todevelop nanocomposite films from starch extracted from commercially discarded potato and cassava, which will be incorporated from cellulose nanocrystals extracted from the pineapple crown and with antioxidant and/or antimicrobial properties due to the addition of ethanolic extractof oregano leaf. All raw materials, with the exception of oregano, will be collected from waste discarded by traders at free fairs. The residues will be collected, the biopolymers of interest will be extracted (starch from potato and cassava and cellulose/cellulose nanocrystal from pineapple crown), and then characterized. The ethanolic extract will be produced from oregano leaves, whichwill be characterized in relation to its antioxidant and antimicrobial capacity. Subsequently, activenanocomposite films will be produced, which will be characterized in relation to their visual appearance, thickness, humidity, water solubility, microstructure, X-ray diffraction, permeability to water vapor, oxygen and carbon dioxide, contact angle, mechanical properties, optical and surface properties, UV/Visible light barrier properties, thermal properties, antioxidant and antimicrobial capacity. At the end of the project, it is intended to obtain a thin material, with antioxidant and antimicrobial activities and resistant enough to be applied as active food packaging.

Keywords: Active packaging; Circular economy; Edible films; Food waste; Herbal extract.

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SCALE UP, ECONOMIC ANALYSIS, AND IMPLEMENTATION COST ANALYSIS OF A PRESSURIZED LIQUID EXTRACTION EQUIPMENT USING ETHANOL AS SOLVENT IN OBTAINING VEGETABLE OIL

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New extraction methods are used to obtain vegetable oils with less toxic and safe extraction technologies. Pressurized liquid extraction (PLE), an intermittent process that uses a smaller amount of solvent or the complete replacement of toxic solvents, such as hexane. Developing PLE equipment on an industrial scale can help obtain vegetable oils with less environmental impact and less risk to the consumer. This work studied the scale-up and economic analysis of implementing pressurized ethanol extraction equipment using soybean flask as a matrix. Extraction kinetics, economic factors, and energy consumption were taken into consideration. In PLE scale-up, maintaining the proportion between the mass of solvent (S) and matrix (F) (S/F ratio), and maintaining the solvent residence time is important for process scheduling. This study presents an analysis of the economic viability of scaling up PLE equipment to Pilot-scale and simulation the viability for industrial scales using the SuperPro Design v8.5 software. The pilot-scale PLE consists of a jacketed stainless-steel extractor (2L) and presented an average yield of 19.28 ± 0.03% with oil recovery of 86.27 ± 0.12%. The recovery of soybean oil under the same process conditions in the PLE on laboratory scale was the same, (86.16%) or an extraction yield of 19.26%. Laminated soybean mass (S/F) of 2.5 was kept the same as well as the optimal extraction temperatures and times, 80 °C and 12 min, respectively. To simulate the feasibility of implementation on an industrial scale, 16 scenarios were simulated with 2 extractors with volumes from 2 to 5,000 L, 2 or 4 operators, 1 to 3 shifts of 8 hours per day, and the recycling of the solvent in the process. Of the 16 simulated scenarios, 7 showed a positive return on investment and the highest return on investment (133.5%) in the shortest time (0.7 years) (scenario 7). In this process simulation, two 5,000 L extractors were considered, with ethanol recycling, with high annual production of soybean oil (3,325,300 L), which would provide an oil manufacturing cost of around 1.25 US$/L. Taking into account that the selling price of soybean oil is 1.34 US$/L, the oil extracted via PLE on an industrial scale is 6.71% cheaper than soybean oil extracted using the conventional method. In addition to financial viability, oil extracted with pressurized ethanol has the advantage of using a renewable solvent, with a lower environmental impact and is safer for consumers.

Keywords: Pressurized liquid extraction; Scale up; Green solvents; Ethanol; Soybean oil.

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EFFECT OF DIFFERENT CLIMATE CONDITIONS AND CANE RIPENING STAGES FOR PRODUCTION OF NON-CENTRIFUGAL RAW CANE SUGAR

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Non-centrifugal raw cane sugar (NCRCS) is a natural sweetener from sugarcane that is easy to obtain and has a low production cost. In recent years, the demand for this product has increased. However, there are no identity standards for the NCRCS, although Codex for Sugar makes effortsto solve this issue. So far, there has been no consensus among the group, mainly due to the qualityof the raw material, which is highly variable depending on factors such as variety, environmental variations (climate), and management. The present study aims to verify the influence of two varieties of sugarcane, planted in two locations with different climatic conditions and harvested in2 cane ripening stages, on the production of NCRCS. The varieties RB966928 and RB867515 werecultivated in the city of Araras-SP, which has a subtropical climate with a dry winter, with temperatures below 18 ºC (Cwa), and in the city of Valparaíso-SP, with a tropical climate with a dry winter, an average temperature above 18 ºC (Aw). Sugarcanes with 10 and 17 months of ripening produced NCRCS. The sugarcane composition parameters were analyzed and the juice was extracted to produce sugar. The sugar cane juice was evaporated at a temperature of 120 °C, removed from heating and blended to obtain the sugars. From the results of the technological composition of the canes, it was possible to observe that canes with a sucrose/reducing sugar ratio(glycide index) lower than 12.6 do not produce NCRCS granulated style, enabling only the production of the mold style. This behavior was observed for the RB867515 variety grown in Araras and Valparaíso, with glycide indices of 8.7 and 5.9, respectively, and a ripening of 10 months. The other sugars produced had glycide indices ranging from 12.6 to 40.0. The higher thisindex, the easier it is to crystallize, improving granulated sugar production. This behavior is due to the lower content of reducing sugar, which leads to a lower hygroscopicity. Therefore, the production of granulated-style NCRCS is favored by the high sucrose/reducing sugar ratio (glycideindex), and the ripening stage had a more significant influence on the production of NCRCS thanthe different climatic conditions evaluated.

Keywords: Glycide index; Granulated style; Reducing sugar; Sucrose; Sugarcane.

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THE INFLUENCE OF CELL PRETREATMENTS ON THE BIOSORPTION OF CHOLECALCIFEROL IN YEAST BIOMASS (*Saccharomyces pastorianus*)

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Vitamin D3 (cholecalciferol) is an essential micronutrient for the maintenance of the human body,and the most important way to obtain it is through sun exposure. However, a number of factors such as lifestyle, cultural habits, and geographic location often result in insufficient sun exposure,leading to an increasing prevalence of hypovitaminosis D. Consequently, dietary supplementationis crucial to combat this deficiency, however, the incorporation of vitamin D3 into food is hamperedby its high instability. In this context, yeast biomass is an economically attractive alternative, as its porous cell structure favors the absorption of micronutrients and bioactive compounds. Although the yeast cell wall has considerable porosity, appropriate pretreatment can increase the permeability of the cell structure and consequently influence the efficiency of the biosorption process. The aim of this study was to evaluate the effect of cell pretreatments on the biosorption efficiency of cholecalciferol in *Saccharomyces pastorianus* spent yeast biomass. To conduct the study, the yeast biomass was first washed to remove residues from the brewing process. Then, thebiomass was divided into four equal parts. The first part was subjected to acid modification treatment (AYT) with sulfuric acid, the second to alkaline modification treatment (BYT) with sodium hydroxide, the third part was subjected to plasmolysis process (PYT) with sodium chloride,and the fourth part received no additional treatment, called control yeast (CYT). Both treatments were enriched with vitamin D3 at a concentration of 20 µg/mL using the biosorption method. Thebiosorbed vitamin was quantified by high-performance liquid chromatography. The biosorption efficiencies of yeast cells were 12%, 17.5%, 26.2%, and 49.8% between BYT, AYT, CYT, and PYT treatments, respectively. The yeast treatments that were acid and alkaline- and alkaline- modified had the lowest sorption capacities, even compared to the control yeast. This suggests that these treatments cause structural changes that make it difficult for the vitamin to bind to the yeastcell. PYT treatment resulted in a 45% higher sorption capacity than CYT, suggesting that of the pretreatments studied, plasmolysis causes structural changes to the cell that lead to greaterimpregnation of vitamin D3. The results highlight the importance of pretreatments in optimizing the process of biosorption of vitamin D3 by yeast biomass and provide important insights for the development of more effective strategies in the production of foods fortified with this vitamin, which is important for health.

Keywords: Brewer's spent yeasts; D vitamin; Enrichment; Plasmolysis; Sorption.

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CHARACTERIZATION OF THE EXTRACT AND MEAL OF BLACK SOLDIER FLY LARVA (*Hermetia illucens* Linnaeus) AFTER BEING OBTAINED IN INTEGRATED EXTRACTION PROCESSES WITH SUPERCRITICAL FLUID AND PRESSURIZED LIQUID

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The increase in food production is related to population growth. For this reason, there is a constant search for new food sources, including a greater interest in anthropoentomophagy, eating insects. This interest is due to the nutritional characteristics of insects, which can present high concentrations of compounds with nutritional properties, essential fatty acids, minerals, vitamins, and also because they have compounds with active properties, such as antioxidants and phenolic compounds. In view of the benefits and interest in insect consumption, this research aimed to extract oil and its minor compounds from the flour of the larvae of the black soldier fly (*Hermetia illucens* Linnaeus) using extractions with supercritical CO_2 ($scCO_2$) without and with use ethanol cosolvent, followed by extraction with pressurized liquid in an intermittent process, using ethanol as solvent. The insect meal defatted via supercritical fluid extraction (SFE) with and without cosolvent, was subjected to extraction of other compounds, mainly polar, via pressurized liquid extraction (PLE) with ethanol. The integration of these processes made it possible to obtain three products without any residue of organic solvents: an oleic extract rich in essential fatty acids and minor compounds, another rich in polar compounds and a protein powder concentrate (flour), which was used to enrich French bread. integral. The composition and physicochemical properties, the action of bioactives and minor extracts were evaluated. The nutritional, physical and microbiological compositions of defatted flour and bread were evaluated. The extracts obtained by SFE using sc-CO_2 without cosolvent, showed a higher yield when compared to conventional extraction via Soxhlet in determining the fat content, data used as a parameter. In extraction via SFE with ethanol as co-solvent, the yields were even better, showing that ethanol as co-solvent can change the polarity of sc-CO_2, extracting higher concentrations of oil and minor compounds such as phospholipids and tocopherols, for example. Extraction via PLE with ethanol from flour defatted via SFE generated an extract of polar compounds with a higher yield than those of other extractions, resulting in an ethanolic extract rich in carotenoids, phenolic compounds and antioxidants. The investigation into the composition of these extracts aims to demonstrate that they could be used in the food, pharmaceutical or even cosmetics industries. The defatted flour presented a high level of digestibility, essential amino acids and protein, and can be characterized as a protein source suitable for incorporation into the formulation of food products.

Keywords: Alpha-tocopherol; Bioactive compounds; Insect oil; Insect flour; Supercritical extraction.

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APPLICATION OF PULSED MAGNETIC FIELD IN *Saccharomyces cerevisiae* FOR BEER PRODUCTION

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Non-thermal methods are emerging technologies that have been widely used in food processing toinactivate enzymes and microorganisms, maintaining their nutritional and sensory values. Amongthem, greater emphasis can be placed on the use of magnetic fields, as this method can stimulate certain microorganisms to carry out a desired process, such as a fermentation. Beer consumption by Brazilians has been growing more and more and *Saccharomyces cerevisiae* is the most used yeast to carry out the fermentation process in the brewing industry. The hypothesis of this researchis that the magnetic field improves the fermentative activity of *Saccharomyces cerevisiae* in beer production without affecting physical-chemical aspects and the sensory quality of the product. In this context, the objective of this research will be to evaluate the effects of applying a pulsed magnetic field on the yeast *S. cerevisiae* for beer production. A factorial 2^2 was designed, varying the intensity and frequency of the magnetic field, and physical-chemical, microbiological and sensory analyzes of the beer produced will be carried out. Therefore, it is expected that the magnetic field optimize the yeast's fermentative activity and brew a beer that is sensorially accepted by consumers.

Keywords: Beverage; Non-thermal methods; Yeast.

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RAPID EVALUATION OF CYTOTOXICITY IN CACO-2 AND L929 CELLS BY NIR SPECTROSCOPY IN BAKERY PRODUCTS

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As consumers become more aware of functional products, interest in buying products that go beyond providing basic nutrients and offer additional health benefits is increasing. Among the classes of compounds that are widely studied for providing such benefits, the phenolic compound class, which is a bioactive substance found in various plant-based foods, should be highlighted. Phenolics are attributed to their cytotoxic capacity against cancer cells and various other functional aspects; therefore, the use of raw materials rich in these substances in foods has been the subject of research in the field of food science and technology. However, recent research has revealed not only their cytotoxic potential against cancer cells but also against normal cells. Pupunha fruit (Bactris gasipaes) is a matrix that has attracted great attention because, apart from having a high content of phenolic compounds, its flour has technological characteristics that permit its application in a vast range of bakery products. However, the current methods for assessing the cytotoxic potential of phenolic extracts are extremely costly, time-consuming, complex, and have a high potential for error/contamination. Few studies have assessed the effects of food matrix processing at the cellular level. Therefore, the aim of this study was to evaluate the physicochemical, nutritional, and cytotoxic effects of cookies made with pupunha flours from different processing processes and to evaluate the applicability of NIR spectroscopy and chemometrics for predicting the cytotoxic content of cookies made. For the cytotoxicity analysis, a phenolic extract will be prepared, dried, and then diluted to check its effect on CACO-2 and L929 cells. Near-infrared (NIR) spectra will be taken of the intact samples and correlated by chemometrics to the reference results. The accuracy of the proposed modeling will be evaluated using the figures of merit RMSEC, RMSECV, RMSEP, and the regression coefficients. Finally, it is hoped that a product will be developed that has cytotoxic potential against cancer cells but not against normal cells, and that the proposed model will allow for the development of a systemic approach to assist in the safe consumption of new products.

Keywords: Cookies; Function foods; Phenolic compounds; Prediction; Pupunha flour.

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RESUMOS DOS TRABALHOS DOS ESTUDANTES DO PROGRAMA DE PÓS- GRADUAÇÃO EM ZOOTECNIA | PPGZoo

PREMIAÇÃO MELHORES TRABALHOS – CATEGORIA APRESENTAÇÃO ORAL

TRABALHOS COM RESULTADOS

DOUTORADO: *Luisa Maria Ferrreira de Sousa Oliveira*, com trabalho intitulado "Effect of milk from cows with genotype A1A1 and A2A2 for B-casein, biofortified or not, in piglets after weaning: Nervous system and behavio".
Orientadora Profa. Dra. Ana Maria Centola Vidal.

MESTRADO: *Vitória Toffolo Luiz Rozin*, com trabalho intitulado "Rank of challenges on beef cow-calf production farms in Brazil".
Orientador Prof. Dr. Rodrigo Silva Goulart.

PREMIAÇÃO MELHORES TRABALHOS – CATEGORIA APRESENTAÇÃO ORAL

PROJETOS

DOUTORADO: *Mellory Martinson Martins*, com projeto intitulado "Resposta imune e desempenho de bezerros leiteiros suplementados com zinco orgânico e butirato de sódio".
Orientador Prof. Dr. Arlindo Saran Netto.

MESTRADO: *Jordana Lemos Andrade de Andrade*, com projeto intitulado "Impacto do uso de aditivo neutralizante no metabolismo ruminal de bovinos Nelore recebendo dieta com elevada concentração de amido".
Orientador Prof. Dr. Rodrigo Silva Goulart.

PURIFIED LIGNIN IN LAMB FEEDING AND ITS EFFECT ON PERFORMANCE CHARACTERISTICS, CARCASS AND MEAT QUALITY

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Currently, with globalization, new market demands have been established by consumers, generating significant changes in animal production system. With a focus on this market context, the use of industrial by-products in animal feeding has productive, economic, and environmental appeal. Purified Kraft lignin is an alternative because it is a by-product of the pulp industry that has application in animal nutrition since it is composed of phenolic fragments with antioxidant and antimicrobial action. The objective of the present work was to determine the best concentration of purified Kraft lignin in the diet of lambs in the finishing phase. The experimental design adopted was a randomized block design, with four treatments, namely: concentrations of purified Kraft lignin (0, 6, 12 and 18 g/kg of DM consumed) and eight animals per treatment. In total, 32 lambs with an average body weight of 20 kg, approximately 60 days old, from industrial crossbreeding were used. The animals were confined for 64 days and weighed on an average of every 13 days in confinement on an electronic scale, in the morning. After slaughter, carcass weight, carcass yield, pH, temperature, loin eye area, fat and meat thickness, and commercial cuts were measured. The statistical analysis performed using SAS, and means compared by the Tukey test with a probability of 5%. The inclusion of purified Kraft lignin at concentrations (6, 12, and 18 g/kg of DM) did not alter the performance parameters of body weight, average daily weight gain, feed conversion, and dry matter intake about live weight. Moreover, there were no changes in the carcass quality parameters of hot carcass weight, cold carcass weight, hot carcass yield and cold carcass yield, loin eye area, subcutaneous fat thickness, pH, and temperature (at 30 minutes and 24 hours after slaughter). Regarding the commercial meat cuts, there were no changes in the proportion of ham, loin, rib, neck, and shoulder among the treatments. This study therefore affirmed that purified Kraft lignin did not affect performance characteristics, carcass and meat quality parameters.

Keywords: Aromatic polymer; kraft; ruminant nutrition; residue; sheep.

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NUTRITION OF LAMBS WITH CHROMIUM PROPIONATE IN THE FINISHING PHASE AND ITS EFFECT ON PERFORMANCE

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Chromium is a micromineral known to improve the performance of farms animals. Its use in diets has been important for the average daily gain, dry matter consumption, increase in loin eye area and reduction on the thickness of dorsal and visceral fat, as it enhances the use of glucose that circulates in the blood, improving the effect of insulin on tissue. Therefore, its supplementation is recommended in diets with high energy value. The hypothesis of the present study is that chromium reacts positively on meat quality, carcass characteristics and performance. The objective was to evaluate the effects of two supplementary doses in the form of chromium propionate on performance, carcass characteristics and meat quality, and its interaction with minerals present in blood, muscle and bone finishing phase of lambs fed with high energy diet. To achieve the objective, 29 recently weaned lambs of the predominant Poll Dorset breed with an initial weight of 17 kg and approximately 60 days of age, were confined for 64 days. The lambs received food and water *ad libitum.* The treatments used were CTL (without addition of chromium), Cr0,5 (0,5 mg of chromium propionate/animal/day) and Cr1,5 (1,5 mg of chromium propionate/animal/day). Consumption control, weighing, blood collection, post-slaughter measurements and bone radiography were carried out, in addition to measuring the concentrations of minerals such as calcium and phosphorus in blood, muscle and bone. The data were analyzed using the MIXED procedure of the computer program 'Statistical Analysis System' (SAS, ver. 9.4, SAS Inst., Inc., Cary, NC, 2018). A completely randomized design was used, and the effects of chromium concentration on diets were evaluated using linear and quadratic orthogonal polynomials. Regarding the performance of the lambs, it was observed that dry matter consumption increased linearly with the chromium dosage ($P < 0,05$), consequently, it was also significant in relation to feed conversion, with values of 3,34 for CTL, 3,27 for Cr0,5 and 4,08 for Cr1,5 ($P < 0,05$), and feed efficiency with values of 0,31 for CTL and Cr0,5, and 0,26 for Cr1,5 ($P < 0,05$). Regarding carcass and meat characteristics, there was no significant effect related to chromium dosage ($P > 0,05$). We concluded that chromium has relevant results in relation to performance parameters, however further investigation on chromium dosage and sources are required.

Keywords: Bone density; carcass; glucose; insulin; micromineral.

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IDENTIFYING MOBILE GENETIC ELEMENTS IN THE RUMEN OF NELLORE CATTLE

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With the advent of metagenomics, it has become possible to understand the relevance of dynamics associated with the ruminal microbiome in the variation in phenotypes of interest in animal production. Among the applications of this technique, the prospecting of mobile genetic elements (EGM) stands out, which allows the movement of DNA between genomes, in addition to controlling populations through lytic bacteriophages, which maintain the balance of the environment and allow the evolution of host microorganisms. Therefore, the objective of this study was to associate ruminal bacteria through physical chromosomal links with their EGMs to establish the relationship between viruses and bacteria in the ruminal environment. For this, shotgun metagenomic sequencing was carried out with DNA extracted from a pool of rumen content samples from four Nellore cows kept on pasture using ProxiMeta™, a proximity ligation method based on Hi-C. A total of 107 assembled bacterial genomes were obtained, which were mainly attributed to the families *Lachnospiraceae*, *Bacteroidaceae*, *P3*, *Ruminococcaceae*, *Saccharofermentanaceae*, and *Treponemataceae*. We identified 31 associations between host bacteria and EGM, of which 17 were linked to viruses and 14 to plasmids. Co-infection events were also identified in which a host harbored more than one EMG or the same EGM had more than one host. The viral clusters were assigned to the families Myoviridae and Siphoviridae, but their genomes have not yet been classified. The presence of antibiotic resistance genes was also analyzed, and 12 resistance genes were identified, of which six were present in the plasmid contigs integrated into the bacterial host genome. Among them, seven conferred resistance to tetracycline by *tet32*, *tet40*, *tet44*, *tetO*, *tetQ*, *and tetW*, and the others conferred resistance to the nitroimidazole *nimJ* classes: macrolide *mefA*, lincosamide *lnuC*, beta-lactam *blaACI-1*, and aminoglycoside *aadE*. This study successfully characterized the relationship between EGM and its microbial hosts in Brazilian cattle, thereby allowing for characterization of the viruses present in the rumen. This study also examined the association between the abundance of bacterial hosts and their role in shaping a comprehensive understanding of the ruminal environment of Nellore cattle maintained on pasture.

Keywords: Hi-C; metagenomics; plasmids; rumen content; viruses.

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COMPUTER VISION APPLIED TO MONITORING INDIVIDUAL BODY MASS OF FINISHING PIGS

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Recent studies in animal production demonstrate that performance measures related to body mass serve as assistance in assessing animal health and quality control of the animal protein produced. In recent years, significant advances have been made in estimating pig mass through the use of computer vision and machine learning techniques with models for estimating pig body mass by indirect measurements that correlate physical characteristics extracted from point clouds of the animal. , collected using RGB-D depth cameras. This study seeks to develop a computer vision system based on machine learning to identify the mass of finishing pigs through 3D images and individualized by an RFID system. For this purpose, physical characteristics extracted from RGB-D images collected with a depth camera will be used in an automated system with individualization of animals using RFID. Computational models for predicting body mass will be developed and evaluated. The artificial intelligence techniques will be based on machine learning algorithms to build prediction models that will be integrated into an RFID system to identify animals in the drinking area. The models will be evaluated and compared with real benchmark measurements using performance metrics such as those obtained by regression analysis. To date, the data collection system is running and machine learning models are being developed to process the data at the end of collection. So far, the results obtained show the possibility of collecting images with the depth camera to predict the mass of pigs. Through the results of the project, we seek to highlight not only the best computational techniques for building the models, but also to highlight the potential of using the RGB-D camera associated with RFID identification.

Keywords: Animal performance; swine farming; non-invasive measurement; imaging depth; artificial intelligence.

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SUGARCANE/LIVESTOCK INTEGRATION: COMPARISON OF A CONVENTIONAL FEEDLOT DIET AND A DIET WITH HIGH LEVEL OF ETHANOL BY-PRODUCTS ON ENVIRONMENTAL IMPACT AND ANIMAL PERFORMANCE

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Agricultural areas are losing space to crops destined for biofuels production. To avoid deforestation for livestock production, livestock-sugarcane integration is an alternative. The inclusion of co-products from sugar and ethanol producing plants is commonly used in feedlot cattle diets. The objective of this work is to compare a diet with the maximum inclusion of co-products from biofuel production, with a diet optimized with conventional ingredients. The experimental confinement and feed analyzes of foods are being carried out at Embrapa Pecuária Sudeste, São Carlos, SP. The confinement has four paddocks, with Intergado® equipment (Contagem-MG, Brazil), which allows individual and daily measurement of food, water consumption and body weight and an equipment for measuring enteric methane emission (GreenFeed®, C- Lock Inc., Rapid City, South Dakota, USA). The treatments are: (i) conventional optimized diet (CTRL) containing corn silage, ground corn, soybean hulls, soybean meal, urea and mineral premix; and (ii) diet with high inclusion of co-products (CANAP), containing sugarcane bagasse *in natura*, ground corn, dry distiller's grains with solubles (DDGS), peanut oil, potassium chloride, calcite, urea and mineral premix. Forty intact male Nellore cattle, aged 24 ± 0.4 months, and 374 ± 37 kg of initial average live weight (LW), were allocated to four paddocks (10 animals/paddock) in an experimental design in randomized blocks (body weight used as the blocking factor) and two paddocks for each treatment. After the period of adaptation to the facilities and diets, a 90-day trial period is being carried out. The variables analyzed will be dry matter intake (DMI), daily weight gain (ADG), feed efficiency (FE), water intake and enteric methane emission. Data will be analyzed using the SAS statistical software (SAS Inst. Inc. Cary, NY). The results will be subjected to analysis of variance with a 5% significance comparison test. Previous data from the first 25 days of feeding, after the adaptation period, demonstrated a 16% less DMI for animals fed with the CANAP treatment. On the other hand, ADG, FE and water intake were 16%, 38% and 22% higher, respectively, for the CANAP diet. At the end of the experiment, it is expected to confirm that the CANAP diet can present improvements in environmental aspects and be at least as efficient as a conventional diet.

Keywords: biofuels; co-products; enteric methane; GreenFeed; Intergado

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RANK OF CHALLENGES ON BEEF COW-CALF PRODUCTION FARMS IN BRAZIL

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The lack of information on the perception of producers on beef cow-calf production farms in Brazil is notable. This study aimed to classify, in order of importance, the main challenges faced on beef cow-calf producers in Brazil. Data were collected from 280 beef cow-calf production farms through an online questionnaire using the SurveyMonkey® platform. Each producer assigned scores to various categories on an increasing scale of 1 to 10, with 10 representing the highest level of difficulty. The farms used in this study had the following distribution across the national territory: 95 farms in the Central-West region, 36 in the Northeast, 36 in the North, 72 in the Southeast and 41 in the South. All data collected refers to the year of production 2021. Date was analyzed using the Means and Freq procedure of SAS 9.3 (SAS Institute Inc., Cary, NC, USA). The ranking was established based on the general averages obtained. The cost of nutritional inputs recorded the highest score, reaching 7.48, followed by employee training, evaluated at 6.15, and the maintenance of a good body condition score of cows, which reached 6.10. Labor laws received a rating of 5.8, while collection, analysis of zootechnical data and interpretation received a rating of 5.25. Next, logistics and distribution of supplements to animals were rated 5.12. The lowest scores were observed in the choice of semen, with 2.87, in obtaining financing, with 3.51, in the marketing of calves, with 3.37, and in the selection of bulls for replacement, with 3.00. In relation to states, the highest averages were also found for the cost of nutritional inputs. The results indicate that many producers face financial challenges related to adequate animal nutrition, which can directly impact the reproduction of beef cows and, consequently, the farm's productivity. Furthermore, employee training deserves attention, as beef cow-calf production farms depend significantly on effective daily animal management and competent managers. In summary, this data offers precious information for improving the services provided by the beef cattle industry to reduce such challenges and support beef cow-calf production farms in Brazil.

Keywords: Difficulty; nutrition; perception; producers; questionnaire.

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