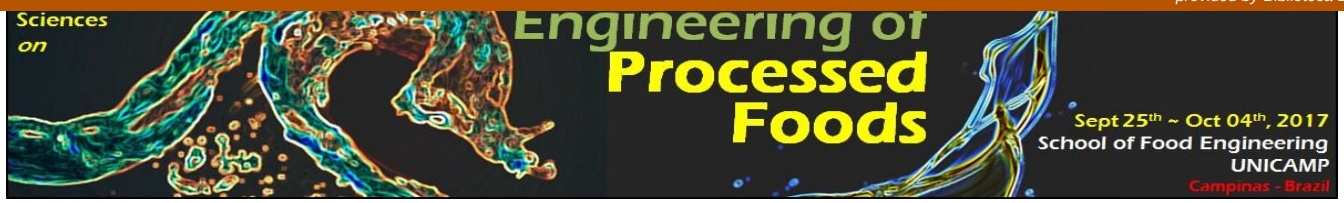

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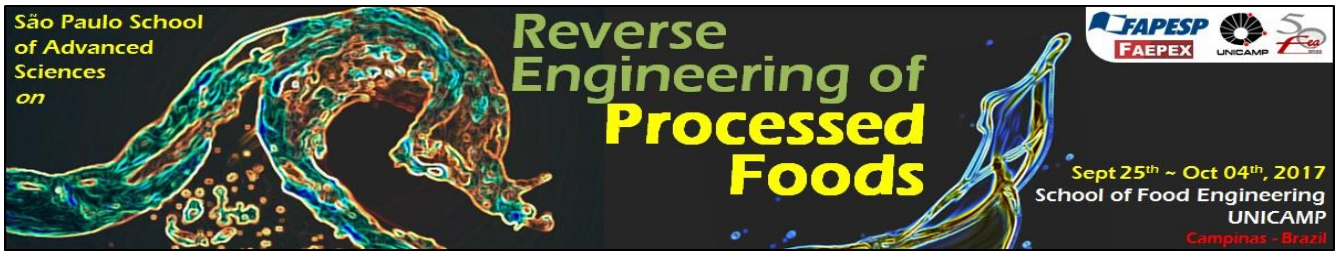

SURVIVAL *LACTOBACILLUS RHAMNOSUS* MICROENCAPSULATED BY SPRAY DRYING IN PRESENCE OF TREHALOE AND SUCROSE DURING STORAGE

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The consumer's demand of probiotic functional food boosts the development of newly dried formulations to protect and increase the viability of these bacteria, such as *Lactobacillus rhamnosus*. Spray drying is a widely used technique of microencapsulation, giving rise to high cell survival rates if the optimal drying conditions and the appropriate protectant agents are used. In this sense, whey proteins (WPI) and maltodextrin (MD) have been used as coating and protectant agents, respectively. In addition, the protective effect of low molecular weight sugars such as glucose, fructose and lactose together with whey proteins have been shown to exert positive effects on the survival of *L. rhamnosus*. Nevertheless, the protective effect of other sugars such as sucrose (S) or trehalose (T) have not been studied. To this end, the viability of encapsulated *L.rhamnosus* by spray drying using different formulations based on WPI:MD with and without sucrose or trehalose as protective agents, was studied as a function of the storage time and the equilibrium water activity (aw). Formulations of probiotics products, based on different blends of WPI:MD (1:2), WPI:MD:S (1:1:1) and WPI:MD:T (1:1:1) containing a proper concentration (109 CFU/mL) of *L. Rhamnosus* were obtained by sray drying. The dried products were stored at 20 °C under different water activity (aw) conditions (0.11 to 0.75) and the viability of the cells was determined over time (till 7 months). As a results, at t=0, *L. Rhamnosus* counts were closed to 109 CFU/g, exhibiting a great cell survival after spray drying process in all formulations. This viability throughout time depended on the water activity and composition of the powders. The cell viability decreased in line with the rise of the aw and the storage time, especially after 154 days of storage at 20 C. Thus, all formulations stored at the lowest aw (0.11) exhibited the greatest viability, around 80 % up to 84 days of storage. At intermediate aw values (up to 0.43), the viability decreased to 65% when storing the samples up to 84 days. For longer storage periods and higher aw, the survival of the bacteria was markedely affected (survival lower than 40%). The presence of sucrose or trehalose significantly ($p<0.05$) enhanced the cell survival ratio at every aw studied. Non-significant differences were found between formulations with sucrose or trehalose. So, to preserve the viability of encapsulated *L. Rhamnosus* for almost 3 months is recommended to incorporate sucrose or trehalose as protective agents into the WPI:MD formulation and to store the powders at low water activity and 20 C.

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TAILLORING DEEP EUTECTIC SOLVENTS FOR THE EXTRACTION OF VALUABLE COMPOUNDS FROM NATURAL SOURCES USING CHOLINE CHLORIDE AND CARBOXYLIC ACIDS MIXTURES: OPTIMIZATION OF THE EXTRACTION OF PHENOLIC COMPOUNDS FROM JUGLANS REGIA L. LEAVES

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Phenolic compounds are a wide group of plant secondary metabolites with increasing interest due to their antioxidant ability and marked effects on oxidative processes related to several chronic diseases. Nowadays, they find applications in pharmaceutical products, functional foods and natural-based cosmetics. Therefore, the extraction and identification of these valuable compounds from different plants have become a major research area. Nevertheless, conventional solid-liquid extraction of phenolics usually involves the use of flammable, toxic and volatile organic solvents. Deep eutectic solvents (DES) are gaining much interest as alternative solvents to extract valuable compounds from natural matrices. DES can be considered "designer solvents" due to the possibility of combining different HBA (hydrogen bond acceptor) and HBD (hydrogen bond donor) to obtain solvents with specific properties to the target application. In this study, DES composed of choline chloride (CC) and different groups of organic acids were prepared, in order to extract phenolic compounds from leaves of *Juglans regia* L. (walnut leaves). The initial screening involved monocarboxylic (acetic, propionic, butyric, valeric, lactic and glycolic acids), dicarboxylic (malonic, glutaric and malic acids), tricarboxylic (citric) and aromatic acids (phenylacetic acid, 3-phenylpropanoic acid). The initial extraction conditions were: 50 ºC, 60 min extraction time and 20% water content. The main phenolic

compounds (neochlorogenic acid, quercetin 3-*O*-glucoside and quercetin *O*-pentoside) were quantified by high performance liquid chromatography coupled to a diode de array detector (HPLC-DAD at 280 and 370 nm). Higher extraction yields were obtained using CC:butyric acid and CC:3-phenylpropanoic acid. For these systems, the stoichiometric ratio of HBA and HBD as well as the water content in the DES solutions were further evaluated. Better results were obtained using DES compared to the conventional water + ethanol mixed solvent. The present work contributes to the valorization of walnut leaves extracts using alternative solvents that could be tailored for potential applications in the food and pharmaceutical areas.

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ULTRASOUND ASSISTED MOLECULAR ENCAPSULATION OF ANTI-GLICATION AND ANTIOXIDANT THYME COMPONENTS IN β-CICLODEXTRIN

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Nowadays the demand for active natural compounds to replace synthetic additives in formulated foods has increased. Thyme (*Thymus vulgaris* L.) is a natural source of antioxidant and anti-glycation activities. The advanced glycation end products (AGEs) are generated by incorporation of sugar or aldehyde residues derived from oxidized lipids to the protein structure. Polyphenols and other antioxidants are protective agents of the effect caused by free radicals have been proposed as glycation regulator agents. The aim of this work was to obtain thyme extracts (ThE), as bioactive compounds sources to evaluate their potential antioxidant, anti-glycation and formation inhibitor for AGEs in a bovine serum albumin model system (BSA/glucose). The combination of β-cyclodextrin (BCD) aqueous solution and ultrasound was found to be very effective, it was proposed as a novel strategy to extract both hydrophobic and hydrophilic compounds in aqueous medium, in one step and avoiding use of organic solvents. The extraction was carried out employing ultrasound and different solutions (BCD 5 and 15 mM, water, and water:Ethanol 1:1) that were shaken (1-10h) at 25°C. The model system was incubated at 55°C with or without ThE 5% and polyphenol content (TPC) was determined for Folin-Ciocalteu, the antioxidant capacity by DPPH•, by FRAP and anti-glycation activity evaluating the protein mobility by electrophoresis in 10% polyacrylamide gel (SDS-PAGE) that were stained with Coomassie Blue and the Schiff reagent PAS to reveal glycoproteins. Initial stages of protein glycation were determined using furosine as a reaction marker, applying high resolution liquid chromatography (HPLC). Independently to the employed solution, the thyme bioactive compounds extraction was increased as increasing the treatment time. Extraction was more efficient in BCD solutions. It was observed that 5 and 15 mM BCD solutions allow to duplicate and triplicate, respectively, the TPC and the antioxidant capacity respect to the treatment without BCD. The presence of high molecular weight bands in ThE absence proved the AGEs formation for BSA/glucose model system. PAS stained revealed that the glycation in ThE presence was inhibited about 93% with extracts incubated 24 h and about 84% for those incubated during 72 h. In ThE presence, the furosine development was inhibited about 3% for extracts incubated during 24 h and 23% for those incubated 72 h. From these results, it is proposed the combined use of BCD and ultrasound as strategy to extract bioactive compounds from vegetables in aqueous medium, in one step and without organic solvents. The ThE may has an interesting role as antioxidant and as potential inhibitor and/or natural regulator for the protein glycation process, which results promising for its employing in functional foods and/or nutraceuticals.

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ULTRASOUND-ASSISTED FORMATION OF O/W PICKERING-EMULSIONS STABILIZED BY CHITOSAN PARTICLES

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Deprotonated chitosan nanoparticles showed a potential to be a food-grade particle stabilizer of oil-in-water (O/W) Pickering