Valorization of microalgal extracts obtained by pulsed electric field in lactic acid fermentation

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Microalgae have been recognized as a fast growing biomass sources rich in proteins, poly unsaturated fatty acids, carbohydrates and other molecules with antioxidant, antibacterial, antifungal and anti-glycation properties. Most often microalgae are studied as sources of lipids for biodiesel production; however, water soluble fraction of biomass remains to be valorized. If pulsed electric field (PEF) is applied on cells and transmembrane potential surpasses certain threshold value, the cell membrane's permeability increases and this phenomenon is called electroporation. Depending on the conditions of applied PEF, this process is reversible or irreversible leading to the cell death. Increased permeability of cell membrane causes transport of molecules through the membrane. Therefore, PEF treatment can be used for extractions, drying, downstream processing, gene transfer or microbial inactivation (Djukic-Vukovic et al., 2017; Gusbeth et al., 2009; Mahnič-Kalamiza et al., 2014).

In our work, we used PEF treatment to obtain water extracts of *Chlorella vulgaris* microalgae biomass. These were used for growth of *Lactobacillus rhamnosus* and *Lactobacillus paracasei*, bacteria with probiotic characteristics (Djukic-Vukovic et al., 2015) and their capability to produce lactic acid on extracts was examined. Studied bacteria are fastidious microorganisms which require nitrogen sources in media for growth and lactic acid production. It is estimated that 38 % of costs in conventional fermentative lactic acid production goes on the expensive nitrogen sources (Tejayadi and Cheryan, 1995). *Chlorella vulgaris* biomass is predominantly rich in proteins while it is very limited in fermentable sugars. Thus, main criterion for PEF treatment optimization was to obtain as high as possible concentration of proteins in final extract and bead milling was used as reference method for protein extraction from microalgae. After the optimization of PEF treatment, extracts were supplemented with up to 25 g/L of glucose to obtain optimal C/N ration for lactic acid fermentation. Lactic acid concentration and viable cell number were determined during the fermentation.

In 24 h of lactic acid fermentation, viable cell number increased for around 2-3 log units, being 5.2×10^8 CFU/ml of fermented extract for *Lactobacillus rhamnosus* and 1.1×10^9 CFU/ml for *Lactobacillus paracasei*. Lactic acid yield amounted up to 85 % for *Lactobacillus paracasei* and up to 91 % for *Lactobacillus rhamnosus*, with optical purity of obtained L-lactic acid above 97 % for both studied strains. High lactic acid yield and high number of viable probiotic biomass after fermentation suggest potential of PEF for extraction of valuable proteins from microalgal biomass for this application. Remains after lactic acid fermentation performed on these extracts can be dried and used as valuable probiotic additive for food or feed, since both studied bacteria have GRAS status according to FDA (U.S. Food and Drug Administration, 2015) and *Chlorella* sp. are also accepted for human intake. This way, water soluble fraction of microalgal biomass could be revalorized as effective substitute for expensive nitrogen sources in lactic acid fermentation as well as for probiotic biomass production.

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