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Phenolic acid degradation potential and growth behavior of lactic acid bacteria in sunflower substrates

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ABSTRACT

Sunflower flour provides a high content of protein with a well-balanced amino acid composition and is therefore regarded as an attractive source for protein. The use for human nutrition is hindered by phenolic compounds, mainly chlorogenic acid, which can lead under specific circumstances to undesirable discolorations. In this study, growth behavior and degradation ability of chlorogenic acid of four lactic acid bacteria were explored. Data suggested that significant higher fermentation performances on sunflower flour as compared to sunflower protein concentrate were reached by *Lactobacillus plantarum, Pediococcus pentosaceus, Lactobacillus gasseri* and *Bifidobacterium animalis* subsp. *lactis*. In fermentation with the latter two strains reduced amounts of chlorogenic acid were observed in sunflower flour (−11.4% and −19.8%, respectively), which were more pronounced in the protein concentrate (−50.7% and −95.6%, respectively). High tolerances against chlorogenic acid and the cleavage product quinic acid with a minimum inhibitory concentration (MIC) of ≥20.48 mg/ml after 48 h were recorded for all strains except *Bifidobacterium animalis* subsp. *lactis*, which was more sensitive. The second cleavage compound, caffeic acid revealed a higher antimicrobial potential with MIC values of 0.64–5.12 mg/ml. In this proof of concept study, degradation *versus* inhibitory effect suggest the existence of basic mechanisms of interaction between phenolic acids in sunflower and lactic acid bacteria and a feasible way to reduce the chlorogenic acid content, which may help to avoid undesired color changes.

1. Introduction

Sunflower seeds (*Helianthus annuus* L.) constitute a basic plant material for oil products, food stuff and pet food. The main use of cultivated sunflower seeds is for oil production, leading to high amounts of by-products like sunflower flour. Due to the high protein content, which varies from 40 to 66%, the sunflower flour is regarded as a promising alternative protein source for animal protein (Bau et al., 1983; González-Pérez and Vereijken, 2007; Kausar et al., 2004). Moreover, sunflower seeds exhibit a widespread availability, low concentrations of antinutritional factors and valuable amino acid composition (González-Pérez and Vereijken, 2007). However, the direct application of flours and protein concentrates as food ingredients has been limited up to now, because sunflower by-products are rich in phenolic compounds, which amount up to 4% (Leung et al., 1981; Pedrosa et al., 2000; Weisz et al., 2009). The predominant compound with 70% of the total phenolic compounds is chlorogenic acid (5-*O*-caffeoylquinic acid) an ester of caffeic and quinic acid, which has a widespread occurrence in fruits and vegetables. The concentration of chlorogenic acid with 23–33 g/kg dry matter of sunflower kernels is distinctly higher than the amounts in apple (0.2 g/kg), potato (1.2 g/kg) or other vegetables (Clifford, 1999; Sabir et al., 1974; Weisz et al., 2009). This high content can negatively affect the protein solubility and color of the sunflower substrates, due to the oxidation into orthoquinones, which are converted into brown polymers under alkaline conditions, e.g. when added to bakery products. Besides, the irreversible linkage of chlorogenic acid to proteins can lead to dark-colored products (Bau et al., 1983; Cater et al., 1972; Gassmann, 1983; Prigent, 2005; Rawel et al., 2002; Saeed and Cheryan, 1989; Shamanthaka Sastry and Subramanian, 1984). As a light color is desirable for protein products several procedures were tested to remove the phenolic components of sunflower flour before protein extraction. These attempts are time

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consuming and often increase the protein loss and protein denaturation (Prasad, 1990; Tranchino et al., 1983; Vermeersch et al., 1987). Therefore it is still a challenging task to reduce the phenolic compounds in a sustainable and cost-effective way. A promising approach could be a fermentation process, which may lead to a reduced amount of phenolic compounds due to the ability of some microorganisms to degrade phenolic acids. Several species of *Lactobacillus* were identified as competent strains to metabolize phenolic acids (e.g. chlorogenic acid, caffeic acid, *p*-coumaric acid, protocatechuic acid) in nutrient media, fruit juices, vegetable puree or sorghum (Filannino et al., 2015; Sanchez-Maldonado et al., 2011; Svensson et al., 2010). Microbial enzymes that hydrolyze the ester bond in chlorogenic acid, like cinnamoyl esterases were found in different lactobacilli (Couteau et al., 2001; Esteban-Torres et al., 2013; Guglielmetti et al., 2008; Lai et al., 2009; Plumb et al., 1999; Raimondi et al., 2015). However, an inhibition of microbial growth by the degradation products is also known. Stead (1993) described that *Lactobacillus brevis* was inhibited by hydroxycinnamic acids (caffeic, coumaric and ferulic acid) in concentration of 500 and 1000 mg/l and Parkar et al. (2008) measured a minimum inhibitory concentration of chlorogenic and caffeic acid of ≤250 mg/l against *Lactobacillus rhamnosus*. A relationship between the antimicrobial potential of phenolic acids and their metabolism by lactobacilli was elucidated by Sanchez-Maldonado et al. (2011). The researchers suggested that the metabolism of phenolic compounds contributes to the detoxification of noxious substances because the cleavage products of caffeic acid, *p*-coumaric acid and protocatechuic acid had lower antimicrobial activities than the original substances. Moreover, they observed that the sensitivity to phenolic substances correlated with the degradation ability. In conclusion, the tolerance of microorganisms to phenolic acids as well as their degradation ability seems to be strain or species specific (van Beek and Priest, 2000; Curiel et al., 2010).

Currently, only little information is available about the spontaneous fermentation of sunflower substrates (Canella et al., 1984). Therefore it was the general aim of the study to investigate the fermentation performance of lactic acid bacteria in sunflower substrates and to evaluate interactions of the phenolic compounds with microorganisms. Growth and metabolism of four different lactic acid bacteria was measured in sunflower flour and sunflower protein concentrate, which contains only trace amounts of secondary plant metabolites. In order to contribute to the scarce and partially contradictory research information, the antimicrobial potential of chlorogenic, caffeic and quinic acid against the selected bacteria was determined. To evaluate the microbial degradation ability of phenolic acids, chlorogenic and caffeic acid were quantified during fermentation of sunflower substrates.

2. Material and methods

2.1. Raw materials and chemicals

Dehulled sunflower seeds were purchased from Goldene Mühle (Garrel, Germany) and processed in a 1.5 m^3 pilot plant percolator (e&e Verfahrenstechnik, Warendorf, Germany) at a maximum temperature of 60 °C according to Pickardt et al. (2009) to obtain a defatted sunflower flour. For the production of the protein concentrate, press cake from dehulled sunflower seeds was gently defatted using hexane according to the same procedure. The derived defatted flour was extracted with an aqueous-alcoholic solution (70% ethanol) in a 300 L percolator.

All chemicals used in this study were analytical grade. Sulfuric acid, formic acid, acetic acid, hydrochloric acid, perchloric acid, methanol, ethanol and acetonitrile were purchased from Th. Geyer (Renningen, Germany). Sodium hydroxide (50% w/w) was obtained from Acros Organics (Thermo Fisher Scientific, Waltham, MA). For quantification the following substances were used as external standards: chlorogenic acid, caffeic acid, sodium DL-lactate, sucrose (Sigma–Aldrich Inc., St. Louis, Missouri, USA), galactose, raffinose, sodium acetate (Roth, Karlsruhe, Germany) and stachyose (TCI Europe, Zwijndrecht, Belgium).

2.2. Chemical analyses

The chemical composition of sunflower substrates in terms of dry matter and protein content was assayed in duplicate according to AOAC methods (AOAC, 2005a, 2005b). For dry matter determination a thermogravimetric system (TGA 601, Leco Instrumente, Mönchengladbach, Germany) and for protein quantification a Protein/Nitrogen Analyzer FP 528 (Leco, St. Joseph, USA) was used. Quantification of lipids was performed by gas chromatography (DGF, 2004).

2.3. Bacterial strains and growth conditions

Four lactic acid bacterial strains were used in this study: *Bifidobacterium* (*B.*) *animalis* subsp. *lactis* DSM 10140, *Pediococcus* (*Ped.*) *pentosaceus* DSM 20336^T, *Lactobacillus* (*Lb.*) *gasseri* DSM 20243^T and *Lb. plantarum* TMW 1.460. Strains were purchased from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany) or were taken from the collection of the Lehrstuhl für Technische Mikrobiologie Weihenstephan (TMW), Technische Universität München, Germany. *B. animalis* subsp. *lactis* and *Lb. gasseri* were grown anaerobically in de Man Rogosa Sharpe medium (MRS, Merck, Darmstadt, Germany) with 0.05% cysteine at 37 °C. *Lb. plantarum* was incubated anaerobically at 30 °C in MRS medium and *Ped. pentosaceus* was cultured anaerobically in MRS broth at 37 °C.

2.4. Experimental design of the proof of concept study

To get first indications about the fermentation performance and the microbial potential to degrade phenolic acids in sunflower substrates, this study was designed as a proof of concept trial. Therefore, three replicates of the same sunflower substrate lot were used in one assay and inoculated with four different bacterial strains from the same inoculum, respectively. For analyses of cell counts, organic acids, phenolic acids and oligosaccharides, samples of the triplicates were taken during fermentation. Sample preparation and chemical analyses were carried out with media, standards and eluents that were prepared the same day in this single assay with threefold repetition.

2.5. Experimental design of fermentation

Pasteurized (85 °C, 10 min) sunflower flour suspension and sunflower protein concentrate suspension (both 10% w/v) were inoculated with the four lactic acid bacteria in order to evaluate the suitability of the substrates for lactic fermentation. To exclude an inhibitory growth effect of the lower carbohydrate content of the protein concentrate, the sugar content of the sunflower protein suspension was adjusted to that of sunflower flour (0.57% galactose, 4.07% sucrose, 2.75% raffinose, 0.22% stachyose). Each sunflower suspension (50 ml) was inoculated with 10^8 colony forming units per milliliter (cfu/ml) of 24–48 h-old precultures: after cell enumeration, a calculated aliquot of the culture was centrifuged (9,055*g*, 10 min), resuspended and used for fermentation. In order to verify the competitiveness of the inoculated bacteria, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) measurements were conducted using