THE USE OF SOLID STATE FERMENTATION FOR FOOD AND FEED PLANT MATERIAL PROCESSING

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Abstract. The present study is aimed at selection of the optimal conditions for solid state fermentation of flaxseed, white lupine, defatted soy flours and Jerusalem artichoke tubers and integrated evaluation of the changes of product physico-chemical properties during the processing.

The moisture content, pH kinetic and total titratable acidity (TTA) as well as contents of D-(-) and L-(+) isomers of lactic acid, amylolytic and proteolytic activities, excreted by *Lactobacillus sakei*, *Pediococcus acidilactici* KTU05-*7* and *Pediococcus pentosaceus KTU05-8* during submerged (SMF) and solid state fermentation (SSF) of plant products were analysed.

The results showed the impact of moisture content of substrate on formation of organic acids and vitality of LAB during SSF in the analysed plant products. The fermented products of 50 % moisture were found to have the lowest values of pH and the highest TTA and content of bacteria.

The highest amylolytic activity excreted by *L. sakei* and *P. acidilactici* were measured in Jerusalem artichoke products (1280.7 and 765.7 AU g^{-1} (SMF fermentation) and 1075.0 ir 390.6 AU g^{-1} (SSF fermentation) respectively). On the contrary, plant products treated by SSF showed the lower proteolytic activity of LAB. Results indicated that SSF technology allow to produce the plant products with higher amounts of L-(+) isomers of lactic acid (from 2.8 g/100 g in fermented with *P. acidilactici* Jerusalem artichoke to 10.3 g/100 g of fermented with *L. sakei* lupine products) as well as lower formation of D-(-) isomers of lactic acid (from 0.18 g/100 g in fermented with *P. acidilactici* Jerusalem artichoke to 7.05 g/100 g in fermented with *P. pentosaceus* soy products) ($P \leq 0.05$).

Keywords: solid state fermentation, lactic acid fermentation, hydrolases, lactic acid D-(-)/L-(+) isomers.

Introduction

In recent years, solid-state fermentation (SSF) has become a reliable biotechnology used for industrial purposes. It is an alternative to submerged fermentation (SMF) in liquid state for its potential use in biologically active secondary metabolites production of food, feed, pharmacy and other industrial areas. In order to meet the rapidly changing industry and consumers'expectations and requirements, to improve production efficiency and to ensure product safety and functionality, it is necessary to have innovations in fermentation technologies in the food and feed industry. The success of the SSF technology installation industry depends on rationalization and standardization of these processes (Singhania et al., 2009). Because of the specification of the increased microorganisms'enzyme amount production, SSF receives a lot of attention (Pandey, 2003; Durand, 2003; Duchiron and Copinet, 2011). Enzymes or their complexes produced during this process help to achieve better bioavailability, solubility and the required viscosity of the product. This is why SSF is widely used in fruit and vegetable transformation (for pectinases), in bakery industry (for hemicellulases), feed industry (for hemicellulases and cellulases), bio ethanol industry (for cellulases and hemicellulases) and in beer and distilled alcoholic beverages industry (for hemicellulases). Microbial metabolism and emissive enzymes significantly influence the composition of the fermentation products as during this process macromolecular components (polysacharides, proteins, lipids) are split into the lower molecular weight compounds (dextrins, sugars, peptides, amino acids, and free fat acids) and secondary metabolism products (acids, alcohols, esters, aldehydes, ketones, vitamins, etc.) are being formed (Farnword, 2003).

The rapidity of economic changes and concern for environment induce the improvement of fermentation technologies; this is why SSF has become more relevant. Applycation of this technology would significantly increase production efficiency of processing plant raw materials, particularly those, that are rich in insoluble components (cellulose, lignin, hemicellulose, etc.).

However, inspite of all SSF virtues, few companies of the world apply advanced processing technology as it has been insufficiently validated and optimized. This is why it is very important to optimise parameters of the technological process by paying particular attention to the safety aspects of the final product.

Though fermentation (SMF and SSF) has been well known and applied in the industrial processes for a long

time, the improving research equipment and new factual material have revealed the formation of undesirable compounds during the lactic acid fermentation. One of the most dangerous compounds formed by applying these techniques are biogenic amines (BA) and D-(-) lactic acid isomers. We have identified and published BA variations in fermented plant products: lupines, soy and flaxseed (Bartkienė et al., complete for release, VetZoo).

D-(-) lactic acid isomers represent another untoward product formed during fermentation. Microbially produced lactic acid is usually a mixture of the L-(+) and D-(-) isomers of lactic acid. As the latter can not be metabolized by humans, excessive intake can result in acidosis, which is a disturbance in the acid-alkali balance in the blood. The potential toxicity of D-(-) lactic acid is of particular concern for malnourished and sick children (Motarjemi and Nout, 1996). For these reasons, it is very important to minimize the amount of this secondary metabolite in the final product during the selection of fermentation technology conditions.

Also, the specific character of fermentable substrate has a significant impact on the fermentation process. In order to remove antinutritional factors and improve the absorption of bioactive components many plant materials are fermented. Fermented soy products are related with better bioavailability; they have an especially positive impact on the digestibility (Brouns, 2002; Friedman and Brandon, 2001; Messina et al., 2002). In order to improve bioactive compounds – lignans bioconversion, it is purposeful to ferment flaxseed (Cornwell et al., 2004). Lupin seeds are known as a source of protein and have been used as animal feed for a long time (Segal, 2002; Peterson, 2000). Lupin protein has many potential antioxidant characterized materials: carotenoids, tocopherols and lecithin (Lampart – Szczapa, 2003). For a very long time, Jerusalem artichokes have been identified as an excellent substrate for lactic acid fermentation due to its exceptional carbohydrate composition (Andersen and Greaves, 1942). Subsequently, there has generated the interest in them as prebiotics – probiotic complex product development (Cummings et al., 2001).

For these reasons, the aim of this work is to choose SSF specifications for defatted flaxseed, white lupines, defatted soy flour and processing of Jerusalem artichoke tubers and to make complex evaluation of changes in the products used during this technological process.

Materials and methods

Plant products and their preparations for fermentation. Defatted flaxseed (producer *Institute of Natural Fibres, Institut Wlokien Naturalnych*); white lupines (*Lupinus albus* L.) (harvest of 2008, received from the Lithuanian Institute of Agriculture Vokė Branch); defatted soy flour (country of origin Czech Republic) and Jerusalem artichokes (*Helianthus tuberosus* L.) (harvest of 2011, received from the Lithuanian Institute of Horticulture Experimental Farm) were used in this experiment.

Lupines were milled into particles up to 3mm in diameter by laboratory disc mill (*Disc Mill RS 200*, Germany) before the fermentation. Jerusalem artichoke tubers were used for fermentation and, before the fermentation, were cut into 1–2 mm slices and dried in the vacuum oven (*Model SZG*, China) at +45°C.

Submerged (SMF) and solid state (SSF) fermentation of plant products. The lactic acid bacteria (LAB), which produce bacteriocins, were received from the Department of Food Science and Technology of Kaunas University of Technology (Cereal and cereal products group collection *(Lactobacillus sakei*; *Pediococcus acidilactici* KTU05-*7*; *Pediococcus pentosaceus* KTU05-*8)*. Before the experiment, LAB were stored at -70°C (*PRO-LAB Diagnostics*, United Kingdom). After LAB were defrosted, they were propagated in de Man Rogosa Sharpe (MRS) medium (CM 0359, Oxoid Ltd, Hampshire, United Kingdom): *Lactobacillus sakei* at 30°C temperature, *Pediococcus acidilactici* at 32°C temperature, *Pediococcus pentosaceus* at 35°C temperature by keeping for 48 hours in the thermostat. Before the use, 40 mM of fructose and 20 mM of maltose were added to the medium. Before the experiment, LAB multiplied in the agar, were attenuated by saline concentration up to 10^8 CFU/ml and were used for plant products fermentation. Fermentation was conducted by using pure LAB cultures, proportionately at 30; 32 and 35°C temperature for 48 hours in the thermostat (*Binder*, Germany). Ferments were produced from different plant products and pure LAB cultures, by applying different fermentation technologies – SSF and SMF. SSF was applied at no more than 50% of fermentable material moisture, and SMF was applied at more than 50 % of material moisture.

Method of the evaluation of LAB count. The total number of mesophilic LAB colonies forming units (LAB log CFU/g) was established according to the LST ISO15214: 2009.

Determination of the moisture required samples were dried at 105°C temperature in the thermostat till the constant mass and the percentage of the change of the mass was calculated.

pH of the fermented products was measured and recorded by applying pH-meter „*Sartorius Professional Meter PP – 15*“.

Total titratable acidity of samples (TTA) was determined by titrating solutions of samples with 0.1 M NaOH solution.

Determination of amylolytic enzymes activity in fermented products. By applying this method, amylolytic enzymes activity was evaluated at 37°C temperature for 10 min by catalysing 1 g of soluble starch hydrolase to dextrine. Consequently, 5 g of the fermented product was brewed by 50 ml distlil H_2O. The obtained content was stirred and filtered; for the further analysis the filter was used. 5 ml of the starch solution was poured into the each test-tube and kept for 10 min at 30°C in the water bath *(MEMMERT WB – 07)*. After that, 5 ml of the filtrate was poured into each test-tube and was again incubated for 10 min at 30°C in the water bath. 5 ml of distilled H_2O was poured into the secondary test-tubes. 5 ml were taken from the content of each test-tube and mixed with 50 ml

iodide solution. The abosrbance of the received solution was measured at 670 nm wave length (spectrophotometer „*GENESYS 10 UV*“).

Determination of proteolytic enzymes activity in fermented products. The essence of this method is that amino acids are formed when proteases affects casein. For the evaluation 5 g of solution was brewed by 50 ml of reagent F (0.01M sodium acetate buffer solution was mixed with 0.05M calcium acetate solution (pH=7.5)). The content was stirred and filtered (filter (I)). After that, 5 ml of 0.65 % of casein solution was poured into empty test-tubes and tubes were put into the thermostate for 10 minutes at 37°C. 1 ml of the prepared filtrate (protease solution) was poured into each secondary test-tube with casein solution and again incubated for 10 minutes at 37°C. 5 ml of reagent TCA (trichloroacetic acid) was poured into each test-tube and incubated for 30 minutes at 37°C in the thermostat. After that, the content of each test-tube was stirred and filtered (filter (II)). 2 ml of filtrate (II), 5 ml of sodium carbonate and reagent FC (F-C, prepared 10 ml of folin phenol reagent attenuated with 40 ml distil H_2O) were poured into the test-tubes. Test-tubes were heated for 10 minutes at 37°C in the thermostat. After that, each test-tube‘s absorption at 660 nm wave length was measured.

The method of lactic acid and L-(+)/D-(-) isomers quantity evaluation.

2 g of the sample, attenuated by distilled water up to 50 ml, was stirred for about 10 minutes and the essence was filtered through the paper filter. The filter was moved to the 100 ml flask and attenuated up to the mark with the distilled water. The samples prepared in this way were used for the D-(-)/L-(+) lactic acid isomers quantity analysis.

D-(-) lactic acid isomer quantity was evaluated spectrophotometrically, by evaluating changes of the colour, initiated by two functioning enzymes, and by applying enzymatic test K-DLATE 08/11 (*Megazyme International Ireland Limited*). The first reaction was catalised by D-lactate dehydrogenase (D-LDH), during which D-isomer oxidized till pyruvate, by forming nicotinamide - adenine dinucleotide (NAD+). The second reaction included conversion of pyruvate into the D-alanine and 2- oxoglutarate which occured during the ferment D-glutamate-pyruvate transaminase (D-GPT) action. NADH amount, formed during these reactions, correlated with the amount of D-(-) lactic acid isomers. NADH amount was evaluated spectrophotometrically at 340 nm wave length.

The quantity of L-(+) lactic acid isomers was evaluated by applying oxydation till pyruvate with L-lactate dehydrogenase (L-LDH), during which nicotinamide - adenine dinucleotide (NAD+) formed. Further, D-GPT was applyed and absorbation at 340 nm wave length was evaluated.

Statistical analysis

Mathematical statistical analysis of the data was conducted by applying statistical program package „*Prism 3.0*“. The arithmetic mean, total amount, and differences between average results reliability (P) were calculated. The level of the factor significance was set by the Fisher criterion, when the reliability was 95 percent.

Research results

Moisture of fermented plant products, measured after 48 hours of fermentation is presented in Fig. 1. It was determined, that by applying SSF technology, the lowest moisture products were obtained from defatted soy flour (in fermented with *L. sakei* – 38.94%; with *P. acidilactici 7* – 21.32% and with *P. pentosaceus 8* – 21.50%, respectively).

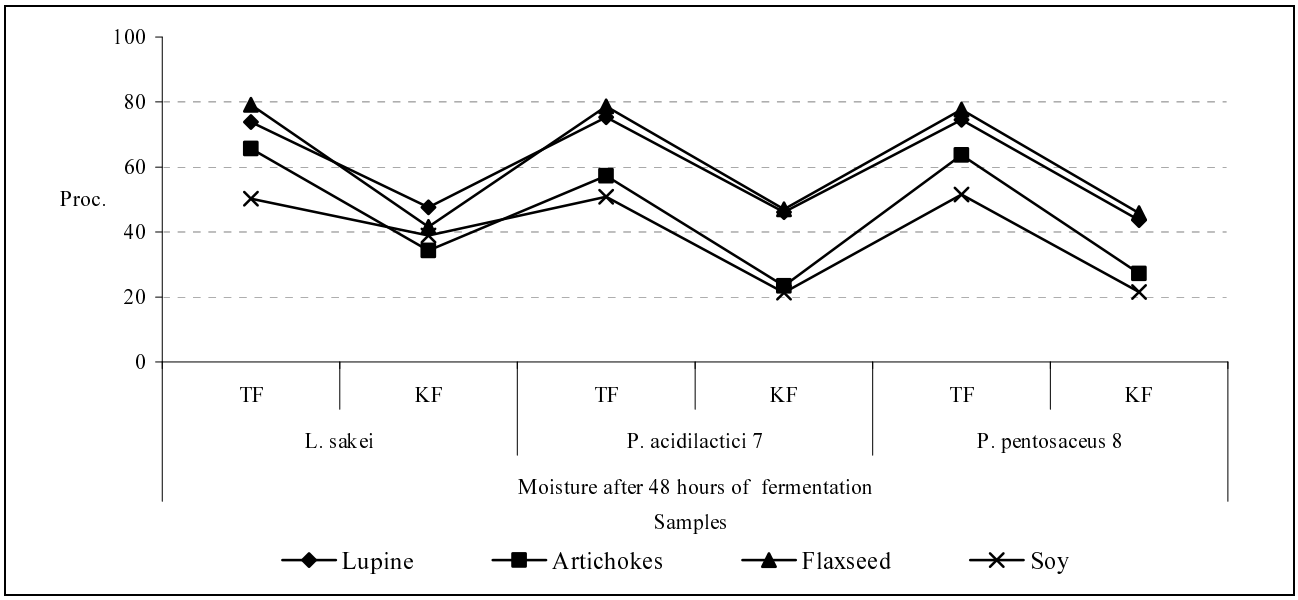

Fig. 1. **Moisture** (%) **in fermented plant products, measured after 48 hours of fermentation**

By applying SSF for lupine and flaxseed processing, it was estimated, that the final homogenous product may be received when moisture varies from 47.62% (in fermented with *L. sakei*) to 43.66% (in fermented with *P. pentosaceus 8*), flaxseed from 46.96% (in fermented with *P. acidilactici 7)* to 41.62% (in the ones fermented with *L. sakei*). Jerusalem artichokes moisture processed by applying SSF varied from 23.37% (in the ones fermented with *P. acidilactici 7*) to 34.33% (in the ones fermented with *L. sakei*). The average of moisture of processed plant products by applying SMF in the liquid phase after 48 hours varied from 78.49 (+/- 0.76) % to 50.87 (+/- 0.67) %.

pH value of the products after 24 hours of fermentation in most cases was estimated as less by fermenting SMF (Table 1). pH variation tendencies after 48 hours were the same in SMF fermented products the average pH value – 4.42 (+/- 0.04), and in SSF processed products – 5.35 (+/- 0.14), respectively. Similar tendencies were not estimated after LAB samples study. After SMF and SSF with *L. sakei*, it was estimated, that after 48 hours of fermentation, the average LAB of plant products was higher than in SMF processed products (11.0 °N) than by applying SSF (9.6 °N). By applying *P. acidilactici 7* in fermentation, the LAB in the products was different in the margin in errors; SMF was 11.6 °N, SSF 11.7 °N LAB, respectively. The reverse tendency was set only by applying *P. pentosaceus 8* during fermentation. In the latter case, higher LAB was estimated by applying SSF fermentation (13.9 °N) than SMF (9.5 °N). However, weak (R=0.6652) but reliable (P<0.0001; P reliable, when P≤0.05) correlation between fermented products and its pH was estimated. Weak but reliable correlation was estimated (R=0.4834; P=0.002; P reliable, when P≤0.05) between fermented products LAB and pH values.

After the analysis LAB CFU/g of fermented products, it was estimated, that in most cases higher LAB CFU/g was in products where SSF was applied (Table 2). By applying fermentation in the solid phase, the variation of LAB CFU/g in products was from 4.5×10^6 to 9.6×10^9 CFU/g (in *L. sakei* fermented Jerusalem artichokes and with *P. pentosaceus* fermented soy flour, respectively). The same or lower LAB CFU/g of the product in SMF fermented products was estimated, except in *P. pentosaceus* fermented flaxseed (SMF was 1.3×10^9 CFU/g, SSF was 1.8×10^8 CFU/g).

Table 1. **pH kinetics and TTA** (°N) **of SMF and SSF products**

Plant products	*L. sakei*		*P. acidilactici 7*		*P. pentosaceus 8*	
	SMF	SSF	SMF	SSF	SMF	SSF
pH, after 24h of fermentation						
Lupines	4.96±0.05	6.11±0.04	4.33±0.05	5.53±0.14	4.16±0.04	5.49±0.07
Artichokes	4.30±0.02	6.40±0.03	4.5±0.11	5.21±0.15	4.57±0.03	5.19±0.11
Flaxseed	5.98±0.05	6.00±0.01	4.85±0.05	6.04±0.07	4.61±0.03	5.83±0.12
Soy flour	6.11±0.02	6.42±0.01	6.23±0.07	6.18±0.02	6.46±0.05	6.15±0.02
avr	*5.34*	*6.23*	*4.98*	*5.74*	*4.95*	*5.67*
pH, after 48h of fermentation						
Lupines	3.72±0.04	4.36±0.07	3.68±0.03	4.25±0.04	3.8±0.09	4.04±0.03
Artichokes	4.16±0.07	6.18±0.03	4.27±0.02	6.19±0.04	4.17±0.07	6.1±0.05
Flaxseed	4.45±0.03	5.91±0.04	4.39±0.02	4.8±0.01	4.41±0.05	4.87±0.01
Soy flour	5.42±0.04	5.59±0.05	5.18±0.01	5.94±0.05	5.4±0.05	5.96±0.03
avr	*4.44*	*5.51*	*4.38*	*5.30*	*4.45*	*5.24*
Total titratable acidity, after 48h of fermentation, °N						
Lupines	14.4±0.41	19.4±0.25	16.5±0.67	20.6±0.35	13.6±0.87	24.8±0.18
Artichokes	9.4±0.72	5.0±0.12	15.4±0.78	5.4±0.42	16.0±0.91	6.0±0.54
Flaxseed	13.8±0.45	8.2±0.14	10.0±0.25	17.6±0.89	4.8±0.19	22.8±0.43
Soy flour	6.5±0.32	5.7±0.11	4.5±0.31	3.3±0.74	3.6±0.32	2.2±0.14
avr	*11.0*	*9.6*	*11.6*	*11.7*	*9.5*	*13.9*
Explanation: SMF – moisture content of fermented products > 50%, SSF – moisture content of fermented products < 50%						

By comparing separate plant groups, LAB adapted best to the soy flour substrate; LAB CFU/g in them varied from 3.2×10^9 till 9.6×10^9 CFU/g, in *P. acidilactici* SMF and in SSF fermented with *P. pentosaceus,* respectively. The lowest amount of the product LAB CFU/g was in fermented Jerusalem artichokes, it varied from 5.0×10^4 to 6.3×10^8 CFU/g in processed *P. acidilactici* SMF and in processed *P. pentosaceus* by SSF, respectively. By comparing different LAB adaptation to different substrates, the highest LAB CFU/g was by using *P. pentosaceus* for fermentation, the lowest by using *P. acidilactici*. To summarize, it is possible to state that all applied LAB in the experiment are appropriate for lupines, Jerusalem artichokes, flaxseed, and soy flour fermentation, and in order to receive higher LAB CFU/g, SSF fermentation should be applied, except with *P. pentosaceus* fermented flaxseed.

Table 2. **LAB CFU/g in SMF and SSR products**

Plant products	*L. sakei*		*P. acidilactici 7*		*P. pentosaceus 8*	
	SMF	SSF	SMF	SSF	SMF	SSF
LAB cfu/g						
Lupines	2.0×10^{7}	1.7×10^{9}	3.3×10^{6}	8.9×10^{8}	5.0×10^{8}	7.4×10^{8}
Jerusalem artichokes	2.3×10^{6}	4.5×10^{6}	5.0×10^{4}	4.6×10^{8}	3.4×10^{6}	6.3×10^{8}
Flaxseed	8.3×10^{7}	2.8×10^{9}	5.2×10^{8}	8.5×10^{8}	1.3×10^{9}	1.8×10^{8}
Soy flour	5.4×10^{9}	4.0×10^{9}	3.2×10^{9}	7.7×10^{9}	7.0×10^{9}	9.6×10^{9}
Explanation: SMF – moisture content of fermented products > 50%, SSF – moisture content of fermented products < 50%						

Amylolytic and protelytic enzymes activity in SSF and SMF processed with different LAB in plant products is given in Figs 2 and 3. During the analysis of amylolytic ferments activity in different plant products, different variations of tendencies were estimated (Fig. 2). The highest amylolytic activity was in fermented *L. sakei* and in *P. acidilactici* Jerusalem artichoke products (in SMF fermented 1280.7 and 765.7 AU g^{-1}, SFF – 1075.0 and 390.6 AU g^{-1} respectively). In other fermented products amylolytic enzymes activity varied according to the starting microorganisms, plant material particularity and substrate moisture. In fermented products, higher amylolytic activity was estimated in SMF products (with *L. sakei* fermented lupines (1.13 times), with *L. sakei* fermented soy flour (3.90 times); with *P. acidilactici* fermented lupines, flaxseed and soy flour, 1.56; 2.10 and 5.98 time respectively; with *P. pentosaceus* fermented Jerusalem artichokes and flaxseed, 1.13 and 2.57 time respectively). With *L. sakei* fermented Jerusalem artichokes and flaxseed, with *P. acidilactici* fermented Jerusalem artichokes and with *P. pentosaceus* fermented lupines and soy flour higher amylolytic activity was estimated by applying SSF 1.19; 1.54; 1.96; 4.70 and 1.62 times higher respectively.

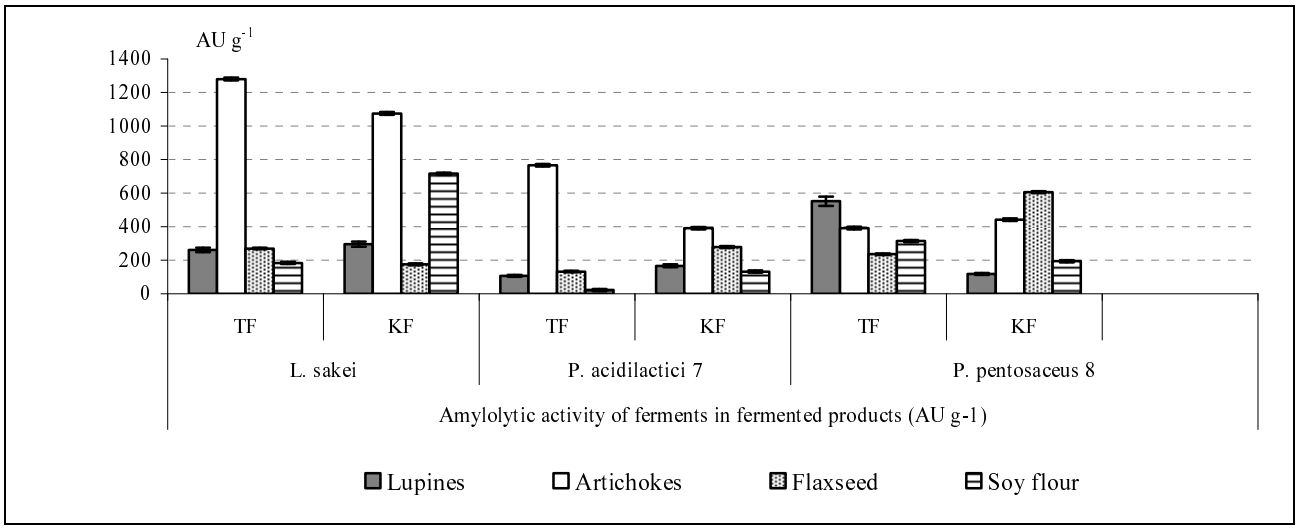

Fig. 2. **Amylolytic activity of LAB in fermented products** (AU g^{-1})

The highest activity of proteolytic enzymes was estimated in fermented Jerusalem artichokes: SMF with *L. sakei* – 1102.1, with *P. acidilactici* – 1178.2, with *P. pentosaceus* – 1094.5 AU g^{-1}, respectively and SSF 54.8; 290.9 and 225.3 AU g^{-1} less than SMF (Fig. 3). In all SSF products there was estimated lower proteolytic activity by comparing with SMF and it varied from 59.1 to 1047.3 AU g^{-1}, with *P. pentosaceus* fermented lupines and with *L. sakei* fermented Jerusalem artichokes, respectively.

L-(+) and D-(-) lactic acid isomers amount in fermented products is presented in Table 3.

In SMF processed samples L-(+), the amount of isomers varied from 0.18 g/100g (with *L. sakei* fermented Jerusalem artichokes) to 7.85 g/100g (with *P. pentosaceus* fermented soy flour). In SSF processed fermented products L-(+) the amount of isomers varied from 2.81 g/100g (with *P. acidilactici* fermented Jerusalem artichokes) to 10.3 g/100g (with *L. sakei* fermented lupines).

It was estimated that in the most samples by applying SSF during samples fermentation, the amount of L-(+) isomers was higher than in fermented samples in the liquid phase (SMF), except with *L. sakei* fermented soy flour (SMF fermented – 6.74 g/100g, SSF – 6.02 g/100g), with *P. acidilactici* fermented Jerusalem artichokes (SMF fermented – 3.79 g/100g, SSF – 2.81 g/100g), and with *P. pentosaceus* fermented flaxsed (SMF fermented – 6.34 g/100g, SSF – 3.08 g/100g). In SMF fermented products D-(-), isomer amount varied from 1.92 g/100g (with

P. acidilactici fermented Jerusalem artichokes) to 6.47 g/100g (with *L. sakei* fermented lupines). In SSF fermented samples, the amount of D-(-) isomers varied from 0.18 g/100g (in with *P. acidilactici* fermented Jerusalem artichokes) to 7.05 g/100g (in with *P. pentosaceus* fermented soy flour). According to the received results, it is possible to state that SSF in the most cases is a safer fermentation than SMF as a smaller amount of undesired D-(-) isomers is formed.

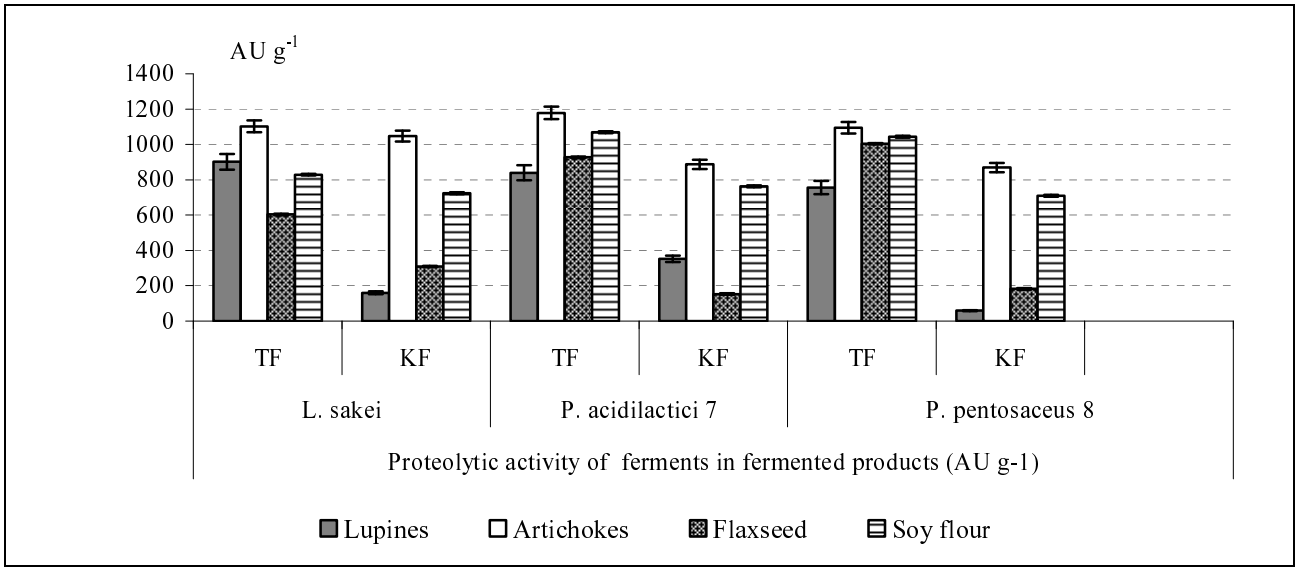

Fig. 3. **Proteolytic activity in of LAB fermented products** (AU g^{-1})

Table 3. **Amount of L-(+) and D-(-) lactic acid isomers in fermented plant products**

Plant products	*L. sakei*		*P. acidilactici 7*		*P. pentosaceus 8*	
	SMF	SSF	SMF	SSF	SMF	SSF
L-(+)lactic acid isomers amount, g/100g						
Lupines	1.96±0.02	10.3±0.11	4.55±0.14	6.69±0.11	3.08±0.02	6.02±0.11
Jerusalem artichokes	0.18±0.03	6.29±0.15	3.79±0.09	2.81±0.03	4.11±0.07	7.32±0.15
Flaxseed	2.94±0.07	5.93±0.12	6.25±0.13	6.25±0.14	6.34±0.09	3.08±0.09
Soy flour	6.74±0.12	6.02±0.09	6.74±0.21	9.37±0.23	7.85±0.16	8.17±0.24
D-(-)lactic acid isomers amount, g/100g						
Lupines	6.47±0.22	5.93±0.11	3.44±0.07	3.48±0.07	2.5±0.04	3.93±0.11
Jerusalem artichokes	2.41±0.11	3.08±0.04	1.92±0.10	0.18±0.02	2.68±0.07	4.51±0.03
Flaxseed	4.73±0.09	3.48±0.06	4.33±0.09	3.08±0.12	3.17±0.09	0.31±0.02
Soy flour	3.39±0.06	5.09±0.13	4.51±0.12	4.82±0.03	3.3±0.07	7.05±0.21
Explanation: : SMF – moisture content of fermented raw material > 50%, SSF – moisture content of fermented raw material < 50%; C – control sample						

Discussion and conclusions

SSF is the process of microorganisms' growth in the wet solid substances' environment with no free water (Moo-Young and others, 1983; Pandey, 2000). SSF systems, which during the previous two decades were termed as 'low-technology' systems, appear to be promising ones for the production of value-added 'low volume-high cost' products such as biopharmaceuticals. It has received a lot of attention by applying this technology in the industry of the secondary metabolic production (Pandey and Soccol, 2000).

During the experiment, when the appropriate SSF moisture of substrate was chosen, we estimated that products fermented by applying SSF may be processed at moisture of substrate less than 50%. Weak (R=0.6652; P<0.0001) correlation was estimated between products moisture and pH value. In the liquid phase, the average pH value of fermented products was lower than in SSF (4.42 (+/- 0.04) and 5.35 (+/- 0.14), respectively). Fermented products' LAB relied more not upon the substratum moisture but upon the starting microorganisms applied for fermentation (with *P. pentosaceus* fermented products higher LAB SSF was in the processed products (13.9 °N) than in SMF (9.5 °N)); with *L. sakei* liquid phase (11.0 °N), SSF (9.6 °N); with *P. acidilactici 7* fermented products LAB varied in bias boundaries: SMF – 11.6 °N, SSF – 11.7 °N).

LAB spores are differentiated cell types, highly specialized, and designed for the survival on adverse conditions (Sella et al, 2009). The water present in SSF

systems exists in a complexed form within the solid matrix or as a thin layer either absorbed to the surface of the particles or less tightly bound within the capillary regions of the solid (Raimbault, 1988). Environmental factors, for example, concentration of the nutrients, the amount of moisture, water activity and the amount of oxygen significantly influence growth of microorganisms and may influence their secondary metabolite production (Mudgett, 1986). Substrate moisture content is a significant process condition, especially for LAB, because these organisms are considered the most suitable for growth in higher moisture contents (Sella et al, 2009).

Though in SSF processed products the amount of secondary metabolites was lower, the tendencies of the microorganisms mass variation were reverse: in SSF processed LAB CFU/g varied from $4.5x10^6$ till $9.6x10^9$ (with *L. sakei* fermented Jerusalem artichokes and with *P. pentosaceus* fermented soy flour, respectively), and in the liquid phase in the most cases there was the same lower LAB CFU/g of the product, except with *P. pentosaceus* fermented flaxseed (SMF-1.3×10^9, SSF-1.8×10^8). The first stage of fermentation technologies scheme is the optimization of the growth conditions (Kathiresan and Manivannan, 2006). In the industry, homofermentative LAB are more commonly used as their main activity is growth of microorganisms mass and production of high amount of lactic acid. By1976, a few homofermentative LAB from the genera of *Lactobacillus*, *Streptococcus* and *Pediococcus* already had been indentified (Stanier, 1976). Different LAB have different abilities to grow on different substrates (Burgos-Rubio et al., 2000). Several factors influence LAB fermentation: starting microorganisms, cultivation, substratum, chemical consistence of carbohydrates, temperature, concentration of sugars, and amount of oxygen, pH and product concentration. All these factors may become regulators in the microorganisms' growth environment. Higher amount of LAB in the most cases was in SSF processed products. It can be explained as LAB has better adaptation to grow in the anaerobic conditions, because by applying SSF plant raw material, products were pressed to reduce the oxygen amount in the substrate, and this factor secured better starting microorganisms' reproduction.

LAB are also known as the source of proteolytic enzyme and exopolysacharide (Welman and Maddox, 2003), a lot of other secondary metabolites such as antimicrobial components (de Vuyst and Leroy, 2007), and amylolytic enzymes (Mishra ir Behera, 2008), characterized by different amylase activity units (Tonkova et al., 1993; Kathiresan and Manivannan 2006). After hydralase amount was examined, it was estimated that SSF technological schemes enable the output of products with less protelytic activity, whereas amylolytic activity depended more on the fermented substrate that on its moisture. Microorganisms with high protelytic activity may be improper for albuminous plants material fermentation (soy, lupine), as it can initiate BA formation. According to the received research results, it is possible to state that SMF is more appropriate for carbohydrates substrates fermentation and SSF for albuminous plant materials.

SSF in microorganisms growing under extreme conditions produce higher amount of lactic acid, yet LAB with amylolytic effect applied in the industry tend to produce stereospecific lactic acid isomers (Reddy et al., 2008). In order to ensure the safety of the products, not only the amount of lactic acid but also D-(-) and L-(+) lactic acid isomers is important. We determined that SSF warrants higher L-(+) isomers amount in products; in comparison with fermentation in liquid phase (SSF – L-(+)) the isomer amount varied from 2.81 g/100g with *P. acidilactici* fermented Jerusalem artichokes till 10.3 g/100g with *L. sakei* fermented lupines; with SMF processed products L-(+) isomers amount varied from 0.18 g/100g with *L. sakei* fermented Jerusalem artichokes till 7.85 g/100g with *P. pentosaceus* fermented soy flour). Reverse tendencies were estimated after the analysis of D-(-) isomers amount; their higher amount was formed in the liquid phase. In SMF processed products, their amount varied from 1.92 g/100g to 6.47 g/100g (with *P. acidilactici* fermented Jerusalem artichokes and with *L. sakei* fermented lupines respectively); and in SSF processed products from 0.18 g/100g to 7.05 g/100g (with *P. acidilactici* fermented Jerusalem artichokes and with *P. pentosaceus* fermented soy flour respectively).

Lactic acid (2-hydroxy propionic acid) is the simplest hydroxy acid with an asymmetric carbon atom and exists in two optically active configurations. L-(+) isomer is produced in humans and other mammals, where both the (D-(-) and L-(+)) enantiomers are produced in bacterial system (Garlotta, 2002). Most often lactic acid is produced in industry by fermenting starchy plant materials LAB, by optimizing technological conditions or by modifying microorganisms in desirable direction, which produce lactic acid exceptionally (Hartmann, 1998). The highest L-(+) isomer amount producing LAB are: *L. bavaricus*, *L. casei*, *L. maltaromicus* and *L. salivarius*. Other stems, for example, *L. delbrueckii*, *L. jensenii* or *L. acidophilus* produce more D-(-) isomers or D-(-) and L-(+) compound (Hartmann, 1998). This LAB can convert high amount of carbohydrates at the standard fermentation conditions, i.e. relatively low (to neutral) pH, approximately at 40°C temperature and small oxygen concentrations (Kharas et al, 1994).

We have determined that by applying different starters (LAB) and by minimalising the moisture of the substrate, it is possible to secure fermented products' safety, as higher L-(+) isomer amount and lower D-(-) isomers amount were estimated in the most samples.

Conclusions

1. Fermentation in the solid-state phase of the lupine, Jerusalem artichokes, defatted flaxseed, and defatted soy flour, is effective when moisture of the substrate is less than 50%, (products are lower pH and TTA and higher CFU/g LAB).

2. Fermentable substrate has a significant influence on amylolytic enzymes activity, and the highest amylolitic activity was obtained by fermented Jerusalem artichoke products with *L. sakei* and *P. acidilactici* (in SMF

fermented 1280.7 and 765.7 AU g^{-1}, SSF – 1075.0 and 390.6 AU g^{-1} respectively), whereas proteolitic enzymes activity by applying SSF technologic scheme, in all cases was lower.

3. SSF is a safe technological process, which insures higher L-(+) isomers obtention in products (L-(+) varied from 10.3 g/100g in with *L. sakei* fermented lupine till 2.81 g/100g in with *P. acidilactici* fermented Jerusalem artichokes) and lower D-(-) isomers formation (varied from 0.18 g/100g in with *P. acidilactici* fermented artichokes to 7.05 g/100g in with *P. pentosaceus* fermented soy flour) ($P \leq 0.05$).

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