ISSN 1330–9862
(FTB-1473)

original scientific paper

Enhancement of Nutritional Value of Cereals with γ-Linolenic Acid by Fungal Solid-State Fermentations

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Received: January 21, 2005
Revised version: July 6, 2005
Accepted: July 8, 2005

Summary

The capability of lower filamentous fungi to utilize and to enrich cereals with γ-linolenic acid (GLA) by solid state fermentation was investigated. *Thamnidium elegans* was selected as a production strain and a variety of cereal materials were tested with the emphasis on achieving cereal-based bioproducts with high GLA content. It was found that dry substrate/water mass ratio of 1:1.5 was optimal for GLA formation. Also, the addition of spent malt grains (SMG) as an internal support to substrates at a mass ratio of 1:3 increased lipid accumulation in bioproducts up to 1.5-fold and GLA yield in bioproduct up to 4-fold. The study revealed that linoleic acid in substrates mixed with SMG was more efficiently transformed to GLA than without SMG. Similarly, an increase in the concentration of linoleic acid in fatty acids of substrates mixed with SMG was accompanied by direct GLA accumulation in bioproduct. Fungal growth on the mixture of wheat bran and SMG (3:1) and on the mixture of spelt wheat flakes and SMG (3:1) resulted in maximal level of GLA in fatty acids (13–14 %). However, the highest yield of GLA (7.2 g/kg bioproduct) was reached when the mixture of spelt wheat flakes and SMG at a ratio of 3:1 was used as substrate.

Key words: cereals, fungi, γ-linolenic acid, solid state fermentation

Introduction

Cereal grains are associated with virtually every day of the history of civilization. Eight cereal grains (wheat, maize, rice, barley, sorghum, oat, rye, and millet) provide 56 % of the food energy and 50 % of the protein consumed on the Earth (*1*). Because humanity has become dependent on cereal grains for the majority of its food supply, it is necessary to understand the nutritional implications of cereal grain consumption on human health.

From the point of view of lipids, cereal grains are quite low in fats averaging 3.6 % of fat in their total caloric content. While linoleic acid is the major fatty acid of n-6 family found in grains, α-linolenic acid (n-3 family) is detected only in small quantities in cereals (*1*). Therefore, cereal-based diets tend to have a high n-6/n-3 ratio and, moreover, are deficient in other essential polyunsaturated fatty acids (PUFAs) of both n-6 (γ-linolenic acid – GLA, dihomo-γ-linolenic acid, arachidonic acid) and n-3 families (eicosapentaenoic acid and docosahexaenoic acid). The importance of these PUFAs is that they serve as precursors for the synthesis of eicosanoids (prostaglandins, prostacyclins, thromboxanes and leukotrienes), potent hormone-like substances having a variety of effects including regulation of platelet aggregation, thrombosis and inflammation (*2*). Insufficient dietary consumption of PUFAs leads to increased occurrence of

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Abbreviations: GLA γ-linolenic acid, PUFA polyunsaturated fatty acid, SMG spent malt grains, SSF solid-state fermentation

atopic eczema, abnormalities in cardiovascular, endocrine, nervous, inflammatory, immune, respiratory and reproductive systems, *etc*. It has been argued that a diet based primarily on cereal grains probably encourages deficiencies of PUFAs and may result in an increased incidence of atherosclerosis (*3*). These facts also underscore the importance of a proper dietary balance between n-6 and n-3 fatty acids (*4*).

One possible approach how to enhance the content of PUFAs in cereal diet is to add these essential compounds to the diet. However, there is an inadequacy of conventional agricultural and animal oils rich in PUFAs. Therefore, attention has been focused on the development of suitable bioprocesses for their production. Success in the microbial PUFA production has led to a flourishing interest in developing fungal fermentation processes and enabled several processes to attain commercial production levels (*5,6*). One of these biotechnological methods is based on the application of fungal solid-state fermentation (SSF) that simulates the fermentation reactions occurring in the nature (*7*). SSF is characterized as a process in which microorganisms grow on a moist solid substrate in the absence of free water (*8,9*). SSFs allow microbial utilization of cheap raw agro-materials (*e.g.* cereals) or byproducts of the agro-food industries and convert them to useful metabolites (*10*).

The production of fermented food is one of the oldest food processing technologies. Many of these foods have been manufactured because their unique flavour, aroma and texture attributes are much appreciated by the consumer (*11*). Moreover, because filamentous fungi simultaneously decrease anti-nutrient compounds and partially hydrolyze substrate biopolymers, prefermented bioproduct may be used as an inexpensive food and feed supplement that may support marketing claims (*12*). Some genera of oleaginous lower filamentous fungi, mainly *Mortierella*, *Cunninghamella*, *Mucor*, *Thamnidium*, *Pythium* and *Thraustochytrium* have been reported as good PUFA producers (*13–15*). This paper deals with effectivity of several lower filamentous fungi to synthesize γ-linolenic acid during their growth on cereals. Selected strain of *Thamnidium elegans* was further used with the aim to transform various cereals into cereal-bioproducts enriched with γ-linolenic acid by SSF process.

Material and Methods

Microorganisms

Fungal strains listed in Table 1 were obtained from the Culture Collection of Fungi (CCF), Department of Botany, Charles University, Prague, Czech Republic. The cultures were maintained on modified Czapek-Dox agar slants with yeast extract (2.5 g/L) at 4 °C. The spore suspension for inoculation was prepared by washing the mycelium with sterilized distilled water to reach the final concentration of $1–2 \cdot 10^6$ spores per mL.

Substrates and cultivation conditions

Autoclavable microporous polypropylene bags (157 × 265 mm^2) were filled with 10 g of dry substrates (see Table 2), moistened by the addition of 15 mL of distilled

Table 1. Lipid content in the bioproduct (BP) and γ-linolenic acid (GLA) content in fatty acids (FA) and bioproduct after solid-state fermentation of peeled barley and oat flakes by several fungal strains at 24 °C for 4 days. Values are presented as the means of triplicates that varied between 4–8 %

Substrate	Microorganisms	*w*(lipid)	*w*(GLA)	
		(% in BP)	(% in FA)	(g/kg BP)
Peeled barley	*Thamnidium elegans* CCF-1465	2.4	6.8	1.11
	Cunninghamella echinulata CCF-103	1.9	6.5	0.82
	Cunninghamella elegans CCF-1318	2.5	5.5	0.94
	Mucor mucedo CCF-1384	2.4	7.0	1.15
	Mucor circinelloides CCF-127	1.5	5.7	0.55
	Mortierella isabellina CCF-14	2.8	3.3	0.64
	Mortierella isabellina CCF-1088	2.4	3.0	0.49
	Rhizopus oligosporus CCF-792	1.5	2.3	0.22
	Rhizopus microsporus CCF-1362	1.6	1.9	0.20
	Rhizopus arrhizus CCF-465	2.1	2.5	0.35
Oat flakes	*Thamnidium elegans* CCF-1465	9.3	6.6	4.82
	Cunninghamella echinulata CCF-103	7.5	6.2	3.58
	Cunninghamella elegans CCF-1318	8.8	4.7	3.23
	Mucor mucedo CCF-1384	9.5	6.1	4.56
	Mucor circinelloides CCF-127	6.7	5.5	2.80
	Mortierella isabellina CCF-14	10.4	2.3	1.90
	Mortierella isabellina CCF-1088	9.1	2.5	1.78
	Rhizopus oligosporus CCF-792	5.6	1.9	0.79
	Rhizopus microsporus CCF-1362	5.9	2.2	0.97
	Rhizopus arrhizus CCF-465	7.1	3.1	1.68

Table 2. Lipid content and fatty acid composition in substrates (S) of various cereals with and without the addition of spent malt grains (SMG). Values are presented as the means of triplicates that varied between 4–8 %

Substrate	*w*(lipid)	*w*(fatty acids)/%						18:2/18:3
	(% in S)	16:0	18:0	18:1	18:2	γ18:3	α18:3	
Rice	1.9	20.7	2.3	40.4	32.8	0.0	1.2	27.3
Barley	2.7	18.1	2.3	12.5	54.8	0.0	4.3	12.7
Peeled barley	1.6	20.7	2.3	14.9	55.1	0.0	4.5	12.2
Wheat	2.1	16.7	1.7	15.7	59.3	0.0	4.4	13.5
Wheat bran	1.4	17.4	1.5	17.3	58.8	0.0	3.9	15.1
Spelt wheat	1.7	15.2	1.1	19.8	58.1	0.0	2.7	21.5
Spelt wheat flakes	1.9	16.1	0.6	22.3	58.5	0.0	2.2	26.3
Oat	4.1	15.0	1.3	37.0	38.9	0.0	2.1	18.5
Oat flakes	4.8	16.7	1.0	40.0	38.5	0.0	1.0	38.5
Rye	2.4	17.2	1.4	37.0	40.9	0.0	2.3	17.8
Rye bran	2.1	16.1	1.8	37.4	42.3	0.0	1.6	26.4
Buckwheat	1.8	14.8	1.5	32.4	45.6	0.0	4.1	11.1
Millet	3.3	7.3	1.5	20.8	64.4	0.0	1.1	58.5
Amaranth	4.2	23.7	4.3	24.8	46.5	0.0	0.5	96.2
Rice/SMG (3:1)	2.5	26.7	1.8	41.3	28.4	0.0	0.5	56.8
Barley/SMG (3:1)	2.9	20.4	2.0	19.3	50.7	0.0	3.8	13.3
Peeled barley/SMG (3:1)	1.9	24.2	2.2	18.5	48.7	0.0	4.1	11.9
Wheat/SMG (3:1)	3.2	30.4	2.6	13.5	48.2	0.0	3.0	15.9
Wheat bran/SMG (3:1)	2.1	19.0	1.8	17.6	55.0	0.0	4.3	12.7
Spelt wheat/SMG (3:1)	2.5	23.2	1.4	20.1	51.5	0.0	3.4	15.0
Spelt wheat flakes/SMG (3:1)	2.6	25.7	1.5	19.3	48.9	0.0	3.5	14.2
Oat/SMG (3:1)	4.5	17.9	1.8	41.8	35.2	0.0	1.6	22.0
Oat flakes/SMG (3:1)	4.9	16.9	1.2	42.1	37.4	0.0	1.1	34.0
Rye/SMG (3:1)	2.9	19.7	1.6	39.1	37.1	0.0	1.8	20.6
Rye bran/SMG (3:1)	2.6	18.6	2.0	34.1	40.6	0.0	3.9	10.4
Buckwheat/SMG (3:1)	2.6	20.6	1.7	32.8	39.5	0.0	4.5	8.8
Millet/SMG (3:1)	4.1	13.2	1.1	28.7	54.1	0.0	0.7	77.3
Amaranth/SMG (3:1)	5.1	24.9	4.4	27.7	41.1	0.0	0.6	64.4

water, soaked for 2 h at laboratory temperature and sterilized in autoclave (120 kPa, 120 °C, 20 min). The bags filled with oat flakes and spelt wheat flakes were sterilized and then moistened with 15 mL of sterile distilled water. In some experiments, spent malt grains (SMG) were added to substrates before sterilization as an internal support (SMG was washed twice before the addition to substrates in order to remove undesirable compounds). Substrates were inoculated with 2 mL of the spore suspension. Each bag was closed with sterile cotton plug and inoculated substrates were arranged to obtain substrate layer of about 1 cm thickness in the bags. Cultivation was carried out statically at 24 °C for 4 days. In order to assure homogenous growth of the fungi, prefermented material was gently mixed once a day during the first two days. To assess reproducibility, triplicate SSF experiments for each substrate were prepared and analyzed individually (values are presented as the means of triplicates, the reproducibility varied in the range of 92--96 %).

Lipid isolation

Prefermented cereal materials (bioproduct) were gently dried at 65 °C for 10 h and weighed. Lipids from both substrates and homogenized bioproducts were isolated with chloroform/methanol (volume ratio of 2:1) and purified according to Certik *et al*. (*16*), while total lipids were determined gravimetrically.

Fatty acid analysis

Fatty acids of total lipids were analyzed as their methyl esters (*17*) by gas chromatography (GC-6890 N, Agilent Technologies) using a capillary column DB-23 (60 m × 0.25 mm, film thickness 0.25 μm, Agilent Technologies) and a FID detector (constant flow, hydrogen 35 mL/min, air 350 mL/min, 250 °C) under a temperature gradient (130 °C for 1 min; 130–170 °C at program rate 6.5 °C/min; 170–215 °C at program rate 2.7 °C/min; 215 °C for 7 min; 220–240 °C at program rate 2 °C/min) with hydrogen as carrier gas (flow 2.1 mL/min, velocity

49 cm/s, pressure 174 kPa) and a split ratio of 1/50 (Inlets: heater 230 °C, total hydrogen flow 114 mL/min, pressure 174 kPa). The fatty acid methylester peaks were identified by authentic standards of C_4-C_{24} fatty acid methylesters mixture (Supelco, USA) and quantified by an internal standard of heptadecanoic acid (C17:0, Supelco, USA).

Results and Discussion

Selection of fungal strain

Lower filamentous fungi belonging to Mucorales were tested for their effectivity to produce GLA during their growth on both peeled barley and oat flakes. These two substrates were chosen with the aim to compare fungal growth and GLA formation on materials with their different lipid content (peeled barley 1.6 %, oat flakes 4.8 %) and different level of linoleic acid (precursor of GLA) in total fatty acids (peeled barley 55.1 %, oat flakes 38.5 %). Fungi listed in Table 1 were selected according to their capacity to synthesize GLA during their growth on submerged media with glucose (*18*) and sunflower oil (*19*). It should be noted that the surfaces of substrates were covered by the fungal mycelium within 2 days of cultivation. Moreover, microscopic observations revealed that fungal hyphae also penetrated into the substrate particles. It is interesting that while lipid content in the peeled barley bioproducts was almost unchanged (1.5–2.5 %), lipid accumulation in oat flakes-based bioproducts increased about twice in comparison with the substrate and varied from 5.6 to 10.4 % (Table 1). GLA levels in isolated lipids depended mainly on microorganisms used in this process. Strains of *Thamnidium*, *Cunninghamella* and *Mucor* showed higher capacity to synthesize GLA (4.7–6.8 % of fatty acids) than strains of *Mortierella* and *Rhizopus* (1.9–3.3 % of fatty acids). Fungal GLA was accumulated in the bioproducts and its yield was affected not only by fungal strain but also by both lipid content in the bioproduct and GLA concentration in fatty acids. Although high yields of GLA in the bioproduct were identified for strains of *Thamnidium elegans* and *Mucor mucedo* grown on both substrates, oat flakes were a more preferable substrate than peeled barley. Cultivation of these two fungi on oat flakes finally resulted in a maximum 4.8 g GLA/kg of bioproduct by *T. elegans* and 4.6 g GLA/kg of bioproduct by *M. mucedo*, respectively. High yield of GLA was also reported for *T. elegans* during utilization of apple pomace (*20*) and pearled barley supplemented with additional nutrients (*21*). Subsequent experiments confirmed that *T. elegans* converted substrates to GLA more effectively than *M. mucedo* (data not shown). Therefore, this fungus was selected as a GLA-producing strain for further investigation.

Fatty acid composition of various cereals

In order to investigate the utilization of various cereals by *T. elegans*, it was necessary to know their lipid content and fatty acid profile. Information available from literature dealing with lipid composition of cereals was difficult to use in this study because the data published so far have been taken from diverse varieties of cereal grains and from different locations. Lipid characteristics of cereals that were employed as substrates for SSF were therefore analyzed. Table 2 also displays fatty acid profile of cereals with the addition of spent malt grains (SMG) that were used as a support. Lipid quantity in the listed cereals was generally between 1.4–4.8 %, where more than 4 % of total lipid was detected in amaranth, oat and oat flakes (Table 2). Results further illustrate that linoleic acid was dominant fatty acid in most cereal substrates and its amount ranged from 32.7 % (rice) to 64.4 % (millet). On the other hand, only small levels of α-linolenic acid were determined in cereals (0.5–4.5 %). Calculated ratio of n-6 (18:2)/n-3 (18:3) fatty acids varied from 11–12 (buckwheat, barley, wheat) to 96 (amaranth). It should be emphasized that α-linolenic acid is a precursor of essential fatty acids of n-3 family. Its balanced intake is important for normal development of brain and reduces risk of coronary heart diseases as well (*22*). In addition, biologically active GLA was not detected in any substrates. Endogenous formation of GLA, the rate limiting Δ^6-desaturase metabolite of linoleic acid, is low or impaired in elder age and variety of diseases (*2,23*). From this viewpoint the consumption of cereal-based diet deficient in this fatty acid should be therefore limited for people suffering from atopic eczema, diabetes, premenstrual syndrome and inflammatory problems.

Cultivation of T. elegans on various cereals

In order to optimize the potential of *T. elegans* to transform substrates into desired bioproducts, variety of cereal materials were tested with the emphasis on achieving bioproducts with high GLA content. Cereals are well-balanced sources of assimilable carbon with adequate levels of organic nitrogen and other nutrients necessary for fungal proliferation. However, suitable moisture of the substrate is also required for satisfied fungal growth during SSF (*8,24*). Preliminary trials with peeled barley, wheat bran and mixture of wheat bran and SMG at 3:1 suggested that dry substrate/water mass ratio of 1:1.5 was favourable for both lipid and GLA production (data not shown). Similar moisture level between 60–75 % of water in the substrate was reported as optimal for growth and GLA formation by Mucorales fungi (*20,21*). These authors pointed out that such ratio might be associated with better fungal assimilation of starch from cereals because starch gelatinization occurs in well-moistened substrates. Moreover, substrate moisture used in our experiments was not advantageous for bacteria and thus prevented bacterial contamination. Table 3 illustrates that *T. elegans* utilized substrates with various effectivity and enriched them with oil containing GLA. Lipid content in bioproducts was generally 1.5–2 times higher than in corresponding substrates and ranged from 2.4 % in peeled barley to more than 9 % in all oat flakes substrates. GLA concentration in isolated fatty acids was permanently low in bioproducts prepared from rice and rye bran (less than 5 %). On the other hand, the fungal growth on wheat bran/SMG and spelt wheat flakes/SMG constantly resulted in maximal quantities of GLA in fatty acids (13–14 %). However, the highest yield of GLA (7.2 g/kg bioproduct) was reached when mixture of spelt wheat bran/SMG (3:1) served as a substrate for the SSF process. Other promising substrates for biopro-

Table 3. Lipid content, fatty acid composition and γ-linolenic acid (GLA) content in cereal-based bioproducts (BP) after solid-state fermentation of cereals by *T. elegans* at 24 °C for 4 days. (SMG – spent malt grains). Values are presented as the means of triplicates that varied between 4–8 %

Bioproduct	*w*(lipid)	*w*(fatty acids)/%						*w*(GLA)
	(% in BP)	16:0	18:0	18:1	18:2	γ18:3	α18:3	(g/kg BP)
Rice	4.1	19.6	3.2	48.6	22.5	4.6	0.0	1.36
Barley	4.2	18.5	3.8	28.7	38.7	6.4	2.3	1.95
Peeled barley	2.4	19.4	4.8	31.6	33.6	6.8	1.2	1.11
Wheat	3.6	14.6	5.1	32.1	37.2	6.1	2.1	1.58
Wheat bran	3.3	15.1	3.6	32.6	40.1	5.3	1.6	1.24
Spelt wheat	2.7	17.1	2.8	33.5	38.1	6.2	1.1	1.16
Spelt wheat flakes	5.8	18.4	4.4	35.1	32.7	8.3	0.9	3.61
Oat	6.5	16.7	2.5	43.9	27.6	5.4	1.1	2.66
Oat flakes	9.3	15.5	1.9	45.1	30.0	6.6	0.2	4.82
Rye	3.2	16.7	1.2	38.3	35.4	5.8	1.4	1.31
Rye bran	3.6	16.3	1.8	40.8	33.9	4.8	0.9	1.23
Buckwheat	4.8	16.2	3.5	46.4	22.9	7.6	1.8	2.67
Millet	5.6	12.4	1.7	24.4	49.6	7.8	0.5	3.26
Amaranth	7.5	20.3	3.6	33.2	35.9	5.4	0.6	3.09
Rice/SMG (3:1)	4.2	19.8	1.1	47.8	24.5	5.1	0.4	1.55
Barley/SMG (3:1)	4.5	17.5	1.5	28.7	39.2	10.4	2.1	3.42
Peeled barley/SMG (3:1)	3.4	16.1	1.2	31.4	38.4	9.6	2.2	2.31
Wheat/SMG (3:1)	3.7	22.3	2.7	20.3	40.9	10.0	2.9	2.65
Wheat bran/SMG (3:1)	4.7	16.1	2.8	30.0	34.1	14.3	1.3	4.96
Spelt wheat/SMG (3:1)	3.6	16.9	2.6	26.6	42.5	9.6	1.7	2.46
Spelt wheat flakes/SMG (3:1)	7.2	16.5	2.6	34.5	28.3	13.1	2.7	7.23
Oat/SMG (3:1)	7.1	15.4	1.8	39.4	32.1	6.9	2.3	3.75
Oat flakes/SMG (3:1)	9.5	14.3	1.6	37.4	35.6	7.9	0.9	5.91
Rye/SMG (3:1)	4.2	17.6	1.3	39.3	32.7	6.7	1.2	2.04
Rye bran/SMG (3:1)	5.4	18.4	3.9	33.9	30.7	10.5	0.9	4.22
Buckwheat/SMG (3:1)	6.2	18.1	3.4	39.3	26.0	10.1	1.4	4.73
Millet/SMG (3:1)	6.7	10.1	2.2	31.8	42.1	12.7	0.4	6.47
Amaranth/SMG (3:1)	7.8	18.9	4.3	32.9	33.6	7.8	0.5	4.67

ducts enriched with GLA were found to be oat flakes/SMG and millet/SMG with yields of GLA 5.9 and 6.5 g/kg bioproduct, respectively. Thus, biotransformation of cereal substrates by *T. elegans* led to cereal-based bioproducts nutritionally enriched with GLA.

Implications of the addition of spent malt grain to cereal substrates

According to our preliminary experiments (data not shown), the substrate/SMG mass ratio of 3:1 was found to be optimal for the fungal biotransformation of cereals to GLA-bioproducts. Cereals without SMG in most cases led to agglomeration of substrate particles and created more compact mass, which in turn interfered with microbial respiration and affected substrate utilization negatively. On the other hand, substrate/SMG ratio higher than 3:1 provided probably limited surface for microbial attack and thus poorer availability of assimilable compounds from substrates (*24*). Fig. 1 clearly indicates that the addition of SMG to the substrate (3:1) increased lipid accumulation in bioproducts up to 1.5 times (rye bran). GLA was also synthesized more effectively by *T. elegans* under SMG presence in substrates. Its content in fatty acids was maximally elevated in bioproducts based on rye bran and wheat bran (2.2 and 2.7 times, respectively) when SMG was added to these substrates. The ratios of GLA yields in SMG-bioproduct to GLA yields in non-SMG-bioproduct depended on substrate type and varied between 1.1 (rice) and 4.0 (wheat bran). However, it is noteworthy that SMG connection with bran-based substrates supported GLA formation more efficiently than with other cereals. Positive effect of SMG on GLA production is also demonstrated in Fig. 2 where *T. elegans* grown in the SMG presence converted linoleic acid from substrates to GLA more effectively. Fig. 2 undoubtedly proves that increase of linoleic acid concentration in fatty acids of substrates mixed with SMG is accompanied by GLA accumulation in bioproduct more rapidly than without SMG. It should be emphasized that the relationship between the level of linoleic acid (as a GLA precursor) in the substrate and GLA yield in the bioproduct has not been investigated yet. This is the first paper that deals with such studies. In order to discuss this phenomenon, it is necessary to know that both

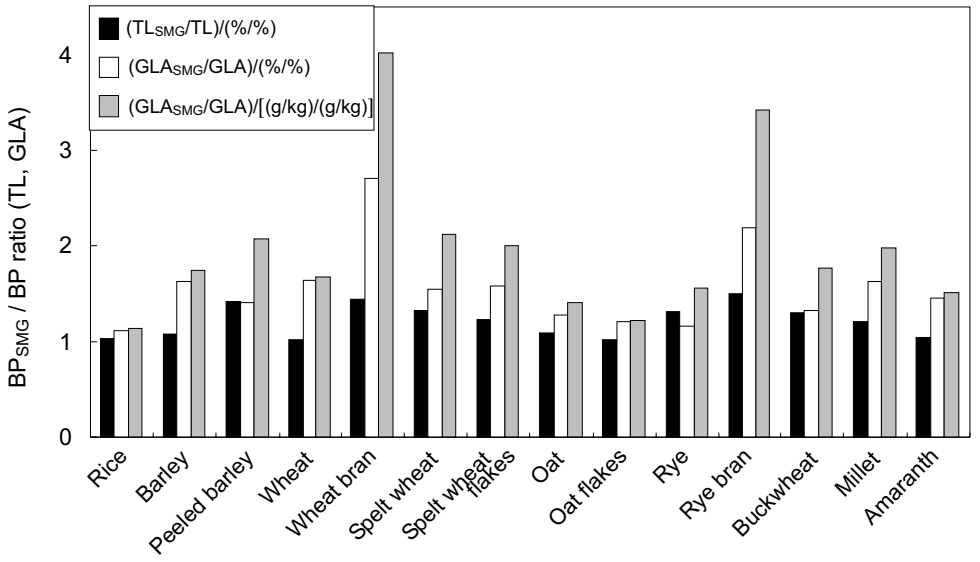

Fig. 1. Ratios of the accumulation of total lipids in (TL_{SMG}/TL)/(%/%), percentage composition of GLA in fatty acids in (GLA_{SMG}/GLA)/(%/%) and GLA yields in bioproducts in (GLA_{SMG}/GLA)/[(g/kg)/(g/kg)] calculated from the bioproduct with spent malt grains (BP_{SMG}) and without spent malt grains (BP) after solid-state fermentation of cereals by *T. elegans* at 24 °C for 4 days

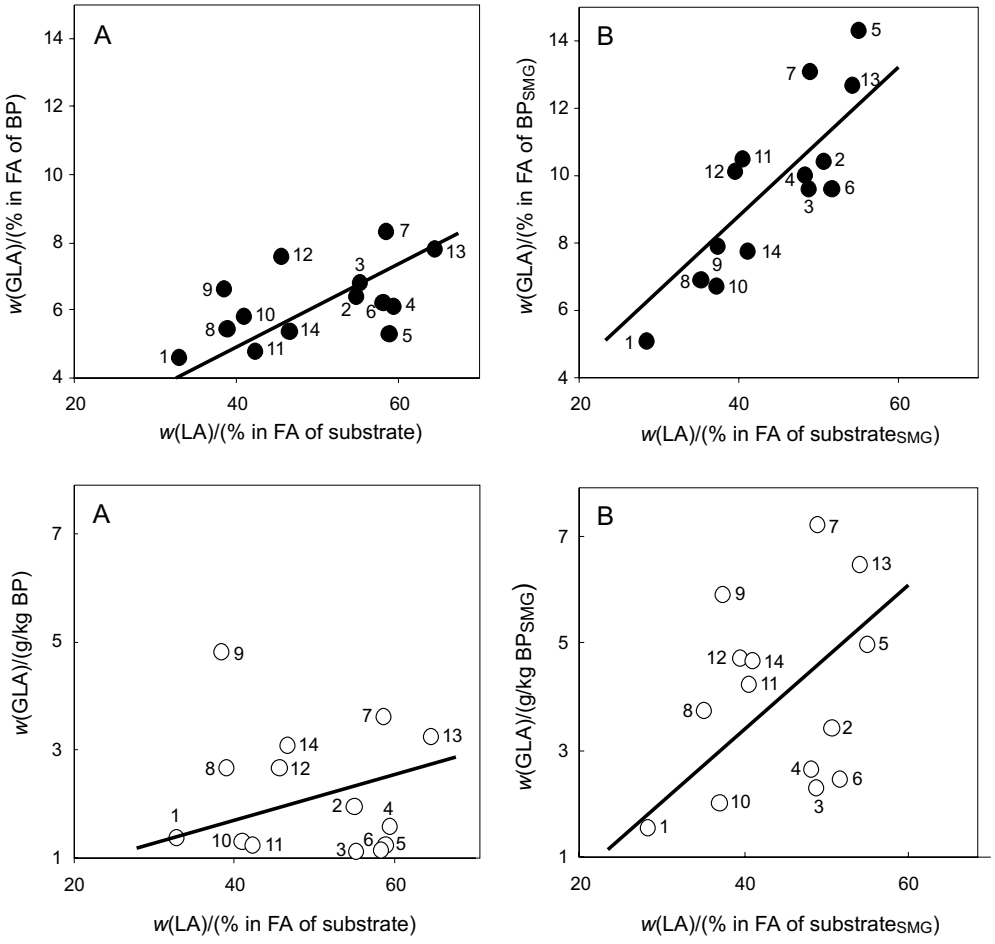

Fig. 2. Effect of the addition of spent malt grains (SMG) on bioconversion of linoleic acid (LA) from substrates to GLA in bioproducts after solid-state fermentation of used cereals by *T. elegans* at 24 °C for 4 days. Used cereals: 1 – rice, 2 – barley, 3 – peeled barley, 4 – wheat, 5 – wheat bran, 6 – spelt wheat, 7 – spelt wheat flakes, 8 – oat, 9 – oat flakes, 10 – rye, 11 – rye bran, 12 – buckwheat, 13 – millet, 14 – amaranth. (**A** – cultivation without SMG, **B** – cultivation with SMG, filled symbols – relationship between LA content in fatty acids of substrate and GLA content in fatty acids of the bioproduct, open symbols – relationship between LA content in fatty acids of the substrate and GLA yield in the bioproduct)

fungal growth and linoleic acid transformation to GLA by Δ^6-desaturase require high oxygen availability. Porous SMG-substrates provided better respiration and aeration efficiency due to an increased inter-particle space (*24*). This resulted in improved bioconversion of linoleic acid from substrates to GLA. Moreover, SMG may also help to remove the heat generated during fermentation and to promote GLA accumulation in bioproduct.

Conclusion

The association of lower filamentous fungi with cereals can be applied for preparation of new cereal-based bioproducts enriched with GLA. These value added bioproducts may be used as an inexpensive food and feed supplement and may create new perspectives for nutritional requirement of PUFA-enhanced cereal-based diet. Nevertheless, further work needs to be focused on improving GLA yield in bioproducts and on industrial scale-up of this bioprocess.

Acknowledgement

This work was supported by the grant VEGA No. 1/2390/05 from the Slovak Grant Agency of Ministry of Education, Slovak Republic and by National Research Project No. 2003 SP 27/028 0E 02/028 0E 02 from the Ministry of Agriculture, Slovak Republic.

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Povećanje prehrambene vrijednosti žitarica γ-linolenskom kiselinom fermentacijom na čvrstoj podlozi s fungima

Sažetak

Ispitana je sposobnost nižih filamentoznih funga da fermentacijom na čvrstoj podlozi obogaćuju žitarice γ-linolenskom kiselinom. Kao proizvodni soj odabran je *Thamnidium elegans* te ispitan niz žitarica kako bi se proizveli bioprodukti s velikim udjelom γ-linolenske kiseline. Za njezino nastajanje optimalni je omjer mase supstrata i vode 1:1,5. Prethodno

korištena zrnca slada, koja se dodaju supstratima u masenom omjeru 1:3, povećala su akumulaciju lipida u bioproduktima za 1,5 puta, a količina γ-linolenske kiseline povećala se 4 puta. Utvrđeno je da se linolna kiselina u supstratima, pomiješana s prethodno korištenim zrncima slada, uspješnije transformira u γ-linolensku kiselinu nego bez tog dodatka. Slično je i povećanje koncentracije linolne kiseline u masnim kiselinama supstrata, pomiješane s prethodno korištenim zrncima slada bilo popraćeno izravnom akumulacijom γ-linolenske kiseline u bioproduktu. Fungalni rast na smjesi pšeničnih mekinja i prethodno korištenih zrnaca slada (3:1) te na smjesi prethodno korištenih pšeničnih pahuljica i prethodno korištenih zrnaca slada (3:1) davao je maksimalnu količinu γ-linolenske kiseline u masnim kiselinama (13–14 %). Najveće iskorištenje γ-linolenske kiseline (7,2 g/kg bioprodukta) postignuto je uporabom smjese prethodno korištenih pšeničnih pahuljica i prethodno korištenih zrnaca slada u omjeru 3:1, kao supstrata.