

ADDING VALUE TO THE BY-PRODUCTS OF CEREAL PROCESSING BY FUNGAL PRODUCTION OF HIGHLY UNSATURATED FATTY ACIDS

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INTRODUCTION

The processing of cereals as agricultural crops or biofuels generates millions of tons of by-products annually. The South African beer brewing industry alone produces approximately 290 000 tons of brewers' spent grain per year. These residues not only lead to economic losses for the industry, but constitute an environmental hazard.

Currently cereal by-products are mainly utilised as animal and fish feeds. This application is limited by the high fibre and low protein levels generally present in these by-products. The presence of antinutrients (phytic acid, chlorogenic acid and other phenolic compounds) and the low levels of highly unsaturated fatty acids (HUFA) in most of these by-products also limit its use, especially as feeds for monogastric animals and fish.

HUFA such as eicosapentaenoic acid (EPA) are essential to the regulation of the cardiovascular, immune, digestive and neurological systems in mammals and fish. Arachidonic acid (ARA) is of nutritional importance in fish egg and larvae development (Ogata et al., 2004). These long chain fatty acids have to be included in the diets of mammals and fish (Dyal and Narine, 2005). Currently the main dietary source of HUFA is marine fish oil. As demand for crude fish oil for the aquaculture industry increases and the market for dietary omega-3 supplements expands by 24% annually, there are concerns over the sustainability of marine and fish sources of HUFA (Jang et al., 2000).

Recent research has focused on HUFA production by micro-organisms as a sustainable and safe alternative to fish oil (Ward and Singh, 2005). Fungi of the genus *Mortierella* are used for industrial production of some of these valuable HUFA and could be grown directly on the cereal by-products.

The aim of this study was to enhance the quality of the by-products and to provide an alternative application for cereal by-products as a source of HUFA. Cereal by-products enriched with HUFA could find applications as food, feed, or pharmaceutical or veterinary products.

MATERIALS AND METHODS:

Substrate preparation

Dried solid brewers' spent grains (BSG) and sunflower press cake (SPC) was treated by one of two methods before inoculation. In both treatments 20g aliquots of cereal substrate were distributed in conical flasks and a 70% moisture level obtained by adding water. Ten percent (w/w) linseed oil (LSO) was then added to half of the substrate treatments. The rest of the treatments were not supplemented with LSO. All treatments were sterilised by autoclaving (121°C, 20 min).

Fungal isolates and inoculum preparation

A liquid inoculum was prepared from each of 8 fungal strains representing the genus *Mortierella* (Table 1). These indigenous fungal strains were originally isolated from soil and maintained in the culture collection of the University of Stellenbosch, South Africa.

Cultivation conditions

Triplicate cultures, representing each isolate, were each inoculated with 2 ml of the homogenised inoculum for both LSO treatments. The inoculated cultures were incubated at 22°C for 3 days to obtain optimal fungal growth. The cultivation temperature was subsequently lowered to 16°C to enhance HUFA production and the cultures were incubated for a further 8 days (Figure 1).



Figure 1. *Mortierella spp* growing on agar medium and brewers' spent grain.

Analyses

The cultures were harvested, dried and analyses were performed on the total dried fermented bioproduct containing the fungal biomass (Jacobs et al., 2009). The total lipids, EPA and ARA levels of the dried fermented biomass were determined by gas chromatography with flame ionisation detection.

RESULTS AND DISCUSSION

It is generally accepted that the target concentration of omega-3 HUFA required in marine fish diets is ~1% m/m, depending on the fish species. In fresh water fish species, less omega-3 but relatively higher levels of dietary omega-6 are required (Ogata et al., 2004).

Neither of the cereal substrates tested had significant initial levels of EPA or ARA. Solid-state fermentation with *Mortierella spp* improved the composition of the total lipids by increasing the levels of omega-3 EPA and omega-6 ARA in both the fermented BSG and SPC. The highest EPA yield of 0.6% (m/m) was achieved with *Mortierella alpina* Mo 46 in the sunflower substrate supplemented with LSO (Table 1).

The omega-6 ARA production by the *Mortierella* isolates was improved by the addition of linseed oil to the substrate (Figure 2). In the sunflower by-product the highest ARA yield of 2.4% (m/m) was achieved with *Mortierella alpina* Mo 46.

Table 1. Lipid and EPA production during fermentation of LSO supplemented cereal by-products with *Mortierella spp*.

Fungal strain number	Species	Total lipids ^a		Eicosapentaenoic acid ^b	
		BSG ⁺	SPC ⁺	BSG ⁺	SPC ⁺
	Substrate control	14.4	22.6	0.2	0.2
Mo 46	<i>Mortierella alpina</i>	9.2	13.4	1.7	6.4
Mo 47	<i>M. selenospora</i>	11.0	12.8	1.1	2.4
Mo 50	<i>M. alpina</i>	9.2	13.8	1.2	4.8
Mo 88	<i>M. basiparvispora</i>	7.2	13.5	1.5	5.8
Mo 101	<i>M. epicladia</i>	10.7	13.6	2.5	4.2
Mo 102	<i>Mortierella spp</i>	10.9	16.3	2.0	0.5
Mo 114	<i>Mortierella spp</i>	9.9	16.3	1.5	0.6
Mo 130	<i>Mortierella spp</i>	12.0	15.6	0.2	1.7
Mean		10.0	14.4	1.5	3.3
Std Dev		1.5	1.4	0.7	2.3

a: gram lipid per 100g oven dried bioproduct

b: mg EPA per g oven dried bioproduct

+: 10% (w/w) LSO was added to substrate before fermentation

EPA: eicosapentaenoic acid; BSG: brewers' spent grain; LSO: linseed oil;

SPC: sunflower press cake

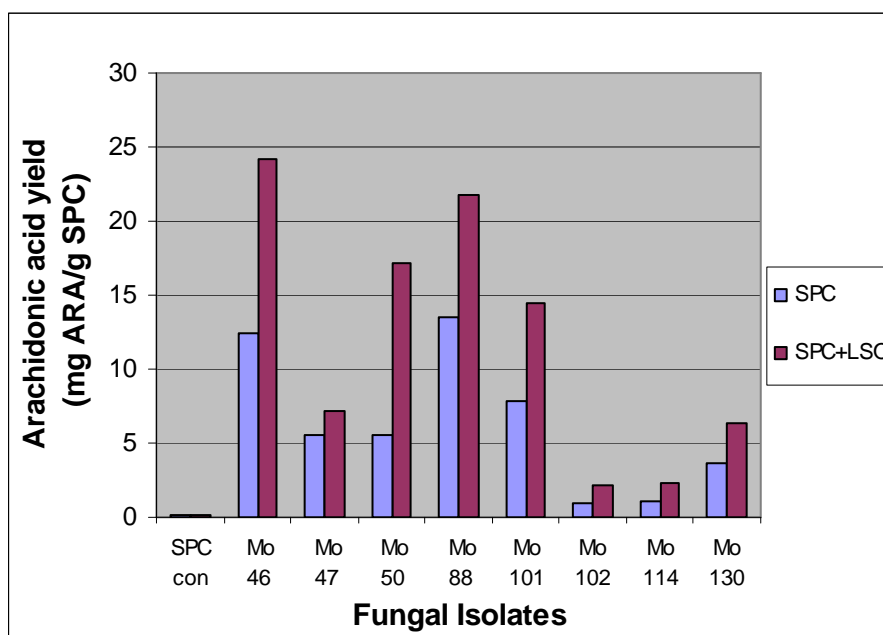


Figure 2. Effect of the addition of LSO on the levels of arachidonic acid produced during fermentation of SPC with fungi of the genus *Mortierella*.
 ARA: arachidonic acid; LSO: linseed oil; SPC: sunflower press cake

CONCLUSION

Fermentation with oleaginous fungi of the genus *Mortierella* produced HUFA-enriched cereal bioproducts. *M. alpina* Mo 46 produced the highest levels of EPA and ARA and could be investigated for larger scale fermentation and value-addition to cereal by-products. HUFA-enriched bioproducts have potential applications as animal or fish feed or could be a source of EPA and ARA for the growing HUFA market in the infant formula, nutraceutical and therapeutics industry.

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