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Esterification of conjugated linoleic acid by yeasts for increasing the value of plant materials

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ABSTRACT

Yeast-catalyzed esterification of free *cis*-9,*trans*-11-conjugated linoleic acid (*c*9,*t*11-CLA) was studied to develop a bioprocess for incorporation of *c*9,*t*11-CLA produced in plant materials into triacylglycerol (TAG). Two *Yarrowia lipolytica* strains and one *Saccharomyces cerevisiae* strain, each as growing and stationary phase cultures, were used to define their capacity to accumulate and esterify exogenous *c*9,*t*11-CLA in TAG. In both culture types all three strains incorporated *c*9,*t*11-CLA into neutral lipids and the highest *c*9,*t*11-CLA content in TAG, 58 mg/g dry matter and 0.26 mg/mL was reached in growing cultures of *S. cerevisiae* B-72021. Instead, *Y. lipolytica* ATCC 20373 was more efficient in non-growing mode for esterification, the highest values in TAG being 31 mg/g d.m. and 0.16 mg/mL. *S. cerevisiae* B-72021 was chosen to esterify *c*9,*t*11-CLA formed in soy okara by *Propionibacterium freudenreichii*. In spite of the presence of okara-based free fatty acids, *c*9,*t*11-CLA concentration of 23 mg/g total d.m. in TAG was obtained corresponding to 0.23 mg/mL of okara-yeast suspension. Then, 81% of the *c*9,*t*11-CLA taken into cells ended in TAG and the proportion of *c*9,*t*11-CLA in total TAG was 14%. Thus, combining the yeast-based esterification to natural processing of low value plant side streams can add their health beneficial properties.

1. Introduction

Research on conjugated fatty acids, especially on conjugated linoleic acid (CLA), has brought evidence that they may have several isomer-specific positive effects on health and wellbeing [1–3]. In food materials, the concentration of CLA is normally low and this has raised interest to search new solutions by which CLA intake could be increased *via* human diet. Milk has been enriched with CLA through manipulation of the diet of dairy cattle [4], dairy products have been fortified with synthetic CLA [5,6], and linoleic acid isomerizing starter cultures have been used for dairy products [4,7,8]. The latter approach has been studied during manufacture of fermented milks and cheese with or without addition of linoleic acid-rich plant oils [9–13].

Recently, the stereospecific microbial isomerization catalysis has been broadened to the synthesis of CLA isomers also into non-animal based food materials or their side products. In this purpose, a natural microbial process has been developed converting linoleic acid efficiently into *c*9,*t*11-CLA in preparations of soy okara, oat or camelina meal [14,15]. In this process, endogenous linoleic acid was first liberated from neutral lipids by supplementing the plant materials with lipase-active oat flour and then isomerizing the free acid by fermentation with a propionic acid bacterium [15,16].

The microbial processes studied so far for isomerization of 1,4-pentadiene fatty acids can carry out stereospecific CLA synthesis but are complicated by the fact that the isomerization occurs at the level of free fatty acids [17–20]. Several observations suggest that the addition of free unsaturated fatty acids to food products may change the acidity, and cause possible off-flavours and poor taste [19,21,22]. Decrease in palatability has been demonstrated, *e.g.* in a yoghurt-type preparation supplemented with free CLA but when CLA was added bound to triacylglycerol (TAG) the off-flavours were no more detectable [23]. Esterification of CLA into neutral lipids by current biotechnological methods is based on the use of microbial lipase preparations [19,24,25]. However, enzymatic esterification in plant slurries is hampered by the fact that they often contain water and require an extra dewatering step.

On the other hand, yeasts are known to incorporate free fatty acids even from complex matrices and esterify them in cellular TAG [26–28]. Isomers of CLA are also incorporated in yeast [29,30] and if they are esterified with sufficient efficiency, a yeast-based esterification of free CLA could be included, as a final step, in the natural CLA enrichment process previously described [15].

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This reasoning formed the basis of the present study where the capabilities of three yeasts to accumulate and esterify free *c*9,*t*11-CLA into neutral lipids were elucidated using both actively growing and early stationary phase cultures. Based on the obtained data, applicability of the method was proven using soy okara as a complex starting material.

2. Materials and methods

2.1. Microorganisms and preparation of cultures

Three yeast strains, *Yarrowia lipolytica* ATCC 20373, *Y. lipolytica* DSM 70561, and *Saccharomyces cerevisiae* B-72021 (VTT Technical Research Centre of Finland), were used for esterification studies. The inocula were cultivated in Difco YM broth at 28 °C for 24 h with 250 rpm shaking. For microbial production of *c*9,*t*11-CLA, *Propionibacterium freudenreichii* ssp. *shermanii* DSM 20270 was used. The strain was cultivated at 30 °C for 48 h in lactate medium containing per litre 10 g of tryptone, 5 g of yeast extract, and 20 g of 50% sodium lactate solution. To get a concentrated cell culture for isomerization, the bacterial strain was cultivated in 1.7 L of lactate medium, the cells were harvested by centrifugation at 5900 × *g* for 15 min and resuspended in 20 mL of saline containing per litre 8.5 g of NaCl and 1 g of bacteriological peptone.

2.2. Yeast-based esterification of synthetic c9,t11-CLA in cultivation medium

Cultivation medium contained per litre 10 g of glucose, 2 g of tryptone, 1 g of ammonium sulfate, 1.7 g of Difco yeast nitrogen base (YNB, product number 233520). YNB in distilled water was separately sterile filtered and added to the medium. Cultivations were performed in 250 mL Erlenmeyer flasks with 100 mL of medium. Synthetic *c*9,*t*11-CLA (>98%, Matreya LLC) was added in 0.5 mL of ethanol to reach a concentration of 0.5 mg/mL of cultivation medium. Same amount of ethanol was added to control cultivations without CLA supplementation.

Yeast-catalyzed esterification of *c*9,*t*11-CLA was studied using actively growing and stationary phase cultures. In cultivations for growth-associated esterification, cultivation medium with or without *c*9,*t*11-CLA was inoculated with 1% (vol/vol) of yeast culture and incubated at 28 °C for 66 h with 250 rpm shaking. When stationary phase cultures were used for esterification, glucose concentration of the cultivation medium was 20 g/L. Yeast strains were first cultivated in medium without *c*9,*t*11-CLA for 42 h into early stationary phase, *c*9,*t*11-CLA in ethanol was added to reach a concentration of 0.5 mg/mL and incubation was continued for 24 h in conditions described above. The samples taken during cultivations were centrifuged at 4300 × *g* for 15 min and the supernatants were freeze-dried prior to fatty acid analysis.

2.3. Esterification of microbially produced c9,t11-CLA in okara slurries

The process scheme used for the production of *c*9,*t*11-CLA in okara slurries followed by its esterification in yeast TAG is presented in Fig. 1.

2.3.1. Plant materials and partial hydrolysis of okara neutral lipids

Soy okara was obtained from Raisio Ltd. (Finland) and dehulled, non-inactivated oat groats, cultivar Fiia, from Korpela Mill, Ltd. (Finland). The hydrolysis of neutral lipids in freeze-dried and ground okara was performed with 10% (w/w) of non-activated oat flour at water activity of 0.70 and the progress of hydrolysis was monitored as previously described [15]. Hydrolysis period at room temperature was 17 days.

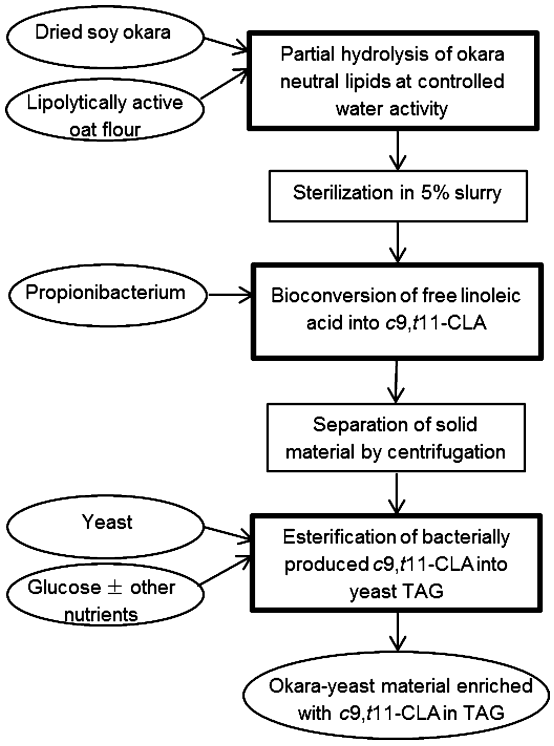

Fig. 1. Procedure used for enrichment of soy okara with microbially produced and esterified *c*9,*t*11-CLA.

2.3.2. Formation of microbially produced free c9,t11-CLA in okara slurries

Prior to isomerization of free linoleic acid in okara, the okara-oat mixture was suspended in water to yield slurries containing 5% (w/vol) dry material. The slurries were homogenized and sterilized, and the isomerization reaction was performed by non-growing cells of *P. freudenreichii* ssp. *shermanii* DSM 20270 in a 1 L fermentor as previously described [15]. Viable count of propionibacteria was 1×10^{10} CFU/mL of slurry. At the end of the 21 h fermentation, pH of the okara slurry was adjusted to 9.0 with 1 M NaOH, the slurry was heated to 50 °C in water bath, and aseptically centrifuged at 5900 × *g* for 15 min. The supernatant was collected, stored at 7 °C, and heated to 50 °C before use.

2.3.3. Yeast-based c9,t11-CLA esterification

The okara supernatant containing bacterially produced *c*9,*t*11-CLA was used for esterification experiments. Esterification medium contained per litre 750 mL of okara-CLA supernatant, 10 g of glucose, 2 g of tryptone, 1 of ammonium sulfate, and 1.7 g of Difco yeast nitrogen base. Also, an esterification medium without tryptone, ammonium sulfate and yeast nitrogen base was used. The experiments were performed in 250 mL Erlenmeyer flasks with 90–100 mL of medium. The media were inoculated with 1% yeast culture and incubated at 28 °C for 66 h with 250 rpm shaking. Samples for fatty acid and dry matter analyses, and yeast viable counts were taken at appropriate intervals. The yeast cell mass during cultivations was calculated from the increase in total dry matter.

2.4. Analytical methods

The fatty acids in freeze-dried samples were methylated and analyzed as methyl esters as previously described [15]. For the analysis of the amount and fatty acid composition of major lipid classes,