PREPARATION OF DECADIENAL AND DECATRIENAL THROUGH BIOTRANSFORMATION

Vladimír Sitkey[1], Peter Dočolomanský[1], Natália Bokros Jeriga[1], Iveta Čičová[2]

[1]Axxence Slovakia s.r.o., Mickiewiczova 9, 811 07 Bratislava, Slovakia

[2]NPPC VÚRV, Bratislavská cesta 122, 921 68 Piešťany, Slovakia

Abstract

Many aroma active compounds can be produced by means of plant enzyme-catalysed reactions. The ability of the eggplant fruit enzymes to catalyse biotransformation of distilled sunflower and linseed oils to two important flavour compounds: (2E,4E)-decadienal, and (2E,4E,7Z)-decatrienal was investigated under various conditions. The results show that maximum yields were reached after 20 minutes of reaction for both compounds (2E,4E)-decadienal (1.24 g/kg of egg fruit) and (2E,4E,7Z)-decatrienal (1.4 g/kg of egg fruit), respectively. The extraction efficiency of ethyl acetate was higher compared to the use of isoamyl alcohol for both compounds.

Keywords: *biocatalysis, decadienal, decatrienal, eggplant, hydroperoxid lyases, lipoxygenases*

1. INTRODUCTION

During the last years, a considerable effort has been paid to the development of green, eco-friendly technologies allowing to convert agricultural raw materials into chemical products having high added value. The EU has also made a significant boost to the production of agro-chemical specialty chemicals in order to increase farmers' incomes and supply the market with organic and healthy products [1]. There is a growing interest in natural substances that have a wide range of uses, for example as components of natural aroma in the cosmetics and food industries. Current legislation is addressing increasing consumer demands for the production of healthy food and beverages containing natural flavours [2].

In industrial practice this means that the components of the aroma must either be isolated from natural sources or obtained from natural products using controlled biotechnological processes [3]. The application of biocatalysis is currently an attractive and rapidly developing area [4, 5]. Such innovative processes lead to increased safety and quality, i.e. "more natural" and "more bio" foods for consumers, which is in line with the EU Strategy for Green Growth.

Plant enzymes play an important role in the development of green chemistry as a versatile tool for the preparation of natural aroma compounds through biotransformation. They are able to catalyse regio- and stereospecific reactions and therefore can be used for the production of desired substances [6].

Many flavours and fragrances (F&F) can be produced by means of enzyme-catalysed reactions, e.g. by hydroperoxidation of free fatty acids, followed by cleavage of linoleic and linolenic hydroperoxides by the sequential action of lipoxygenase (LOX) and hydroperoxide lyase (HPL).

The key step toward successful biotechnological production of natural flavours is economically competitive biocatalytic conversion of substrates to wanted products. The basic prerequisite for this process is the access to raw materials containing suitable enzymes. In principle, it is possible to consider processes using LOX and HPL obtained from a single source [7, 8].

Among the commercially interesting natural substances complying criteria of naturalness belong currently also (2E,4E)-deca-2,4-dienal (CAS number: 25152-84-5; FEMA 3135) hereinafter DDAL, and (2E,4E,7E)-deca-2,4,7-trienal (CAS number: 51325-37-2; FEMA 4089) hereinafter DTAL.

DDAL is created by catalytic action of LOX on linoleic acid at the presence of oxygen to form 9-hydroperoxylinoleic acid that is subsequently transformed by suitable HPL to DDAL. The mechanism of enzyme action is complicated because of the complexity of chemical and physical nature of their substrates with respect to degree of branching of the metabolic pathway [9].

DDAL belongs to the class of organic compounds known as medium-chain aldehydes. They are aldehydes with a chain length containing between 6 and 12 carbon atoms. Thus, DDAL is considered to be a fatty aldehyde lipid molecule. DDAL is a very hydrophobic molecule, practically insoluble (in water), and relatively neutral. DDAL is a fat, fatty, and fried tasting compound. At lower concentration (1 ppm), it has the odour of citrus, orange or grapefruit. In concentrations of 10 ppm is used to impart a deep fat flavour in beef, lamb, chicken, potato chips and French fries [10]. The main use of DDAL is to impart a fried character to flavours that include an element of heated fat or oil within their overall character [11].

DTAL belongs also to the class of organic compounds known as medium-chain aldehydes. DTAL is used as perfuming or flavouring ingredient [12]. It is used as odour and/or flavour in citrus and green [13].

LOX is a common enzyme, present in plant cells and can be found also in many animals. Extremely rich LOX sources are soy beans [14] and potatoes [15]. In addition, LOX was isolated from sources such as tomato leaves, cucumbers, wheat flour and many others [16]. The fruit of eggplant (*Solanum melogena)* also belongs to the potential source of LOX [17, 18].

This work presents the possibility to prepare two products having high added value by means of biotransformation of distilled sunflower and linseed oils: DDAL, and DTAL, using eggplant fruit as a source of enzymes. The biocatalytic production of these aroma active compounds acids is realized in three basic steps. First, mixture of eggplant, substrate and water is blended in mixer and subsequently separated by centrifugation, followed by product extraction using organic solvents.

2. MATERIALS AND METHODS

2.1 Source of plant enzymes

Fresh eggplant from local market was used as a source of enzymes for DDAL and DTAL preparation.

2.2 Biotransformation of natural distilled oils to DDAL and DTAL

For DDAL preparation, distilled sunflower oil Nouracid HE 30 (OLEON GmbH, Germany) containing C16-C18 unsaturated fatty acids, dominantly linoleic acid (C18:2) polyunsaturated fatty acid was used as a substrate.

For DTAL preparation, distilled linseed oil Nouracid LE 80 (OLEON GmbH, Germany) containing (C16-C18 and C18) poly unsaturated fatty acids abundant for linolenic acid (C18:3) up to 50% and linoleic acid - up to 20% was used as a substrate.

Reaction conditions for DDAL preparation: 300 g of eggplant, 900 ml of tap water, 13.3 ml of Nouracid HE 30 and 0.13 ml of Tween 80 were put into a bench mixer Concept and mixed for 2 minutes at maximum speed, followed by mixing at reduced speed on the 1st stage, 18 minutes.

Reaction conditions for DDAL preparation was the same as described above, except of substrate changing for Nouracid LE 80.

The experiments themselves were performed under model conditions (temperature, pH, stirring, reaction time and eggfruit/substrate ratio). Time course of the reaction was monitored by sampling with subsequent analysis by GC and GC-MS.

2.3 Analytical methods

Sample preparation consisted in: 6 ml of the reaction mixture was subjected to repeated extraction with 2x3 ml of ethyl acetate (EA). After stirring vigorously (2 min) in a vortex, the mixture was centrifuged using Hettich Rotixa 50 RS (6000 RPM/5 minutes) and then the organic phase was separated. The procedure was repeated once more. After joining of the organic phases, the composition of the extract was determined by gas chromatography.

Analysis of DDAL and DTAL was performed using an Agilent 7890A gas chromatograph with FID detector, fitted with an Agilent OPTIMA-FFAP capillary column with the dimensions 30 m x 250 µm x 0.25 µm. Conditions of analyses: The injection port temperature was set at 250°C and the optimized oven temperature programme began at 95°C held for 6 minutes, ramped to 120°C at a rate of 10°C/minutes, and then ramped to 220°C at a rate of 20°C/minutes and held for 17 minutes. Sample injection mode was splitless with a sampling time of 2 minutes followed by a split ratio of 25:1 using hydrogen (SIAD Slovakia) as carrier gas pumped through the column at a constant flow rate of 1.5 mL/minute.

GC-MS measurements were performed on Agilent 7890A mass spectrometer with quadrupole mass detector Agilent 5975C.

3. RESULTS AND DISCUSSION

3.1 Study of kinetics of DDAL production with the addition of Nouracid HE 30

The main objective of the sets of experiments was to determine the most relevant parameters necessary for the subsequent process optimization and scale-up in the production facility. The aim of the present work was also to study the kinetics of DDAL production as a function of temperature and pH.

The result of the experiment is shown in Fig. 1, where we can observe a slight decrease of DDAL concentration in the 20th minute, which is probably caused by the higher temperature at the end of the reaction from 18 °C to 36 °C. This temperature elevation was caused by the overheating of the mixer. During reaction, we observed a minimal change of pH, from the value 5.8 at beginning to 5.7 at the end. After repeated experiments with the same results, we no longer observed a change in the pH of the reaction mixture in further experiments. The results suggest that the total yield appear to reach a maximum after 17 minutes of reaction, approximately 1.24 g DDAL/kg of egg fruit.

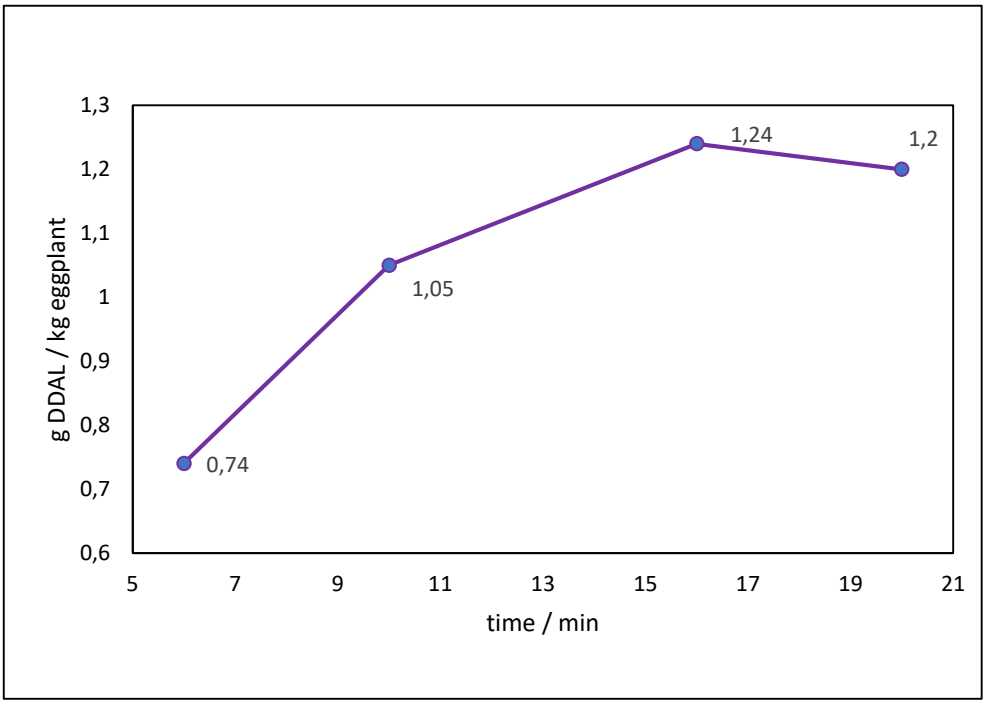


Fig. 1. Production of DDAL by eggplant enzymes

3.2 Study of kinetics of DDAL production without the addition of Nouracid HE 30

To compare the possibility of reaction without the addition of substrate (exploiting only the internal pool of fatty acids in cells), we performed the experiment under the same conditions as in the previous case, except of the addition of substrate. After GC analysis, we found that DDAL was not produced without the addition of Nouracid HE 30.

3.3 DDAL production by eggplant enzymes, with solids separation before substrate addition

Due to high content of solids in reaction mixture and foam formation during stirring, we tried to simplify process efficiency by solids removal before substrate adding.

The primary procedure was adapted as follows: 277 g of eggplant and 500 ml of water were mixed 15 seconds at maximum RPM, followed by filtration through sintered glass filter frit S1. In the next step, 550 mL of filtered eggplant juice was connected with 500 ml of water, 13.3 mL of Nouracid HE 30 and 0.13 ml of Tween 80 and mixed on the 1st stage of the mixer for 22 minutes. The reaction mixture was extracted with 2x 200 ml of EA (200 ml of EA were added directly to the reaction mixture and agitated, followed by centrifugation (4500 rpm, 10 min). Due to the creation of emulsion in the organic phase, the procedure was repeated once more adding next 200 ml of EA to the organic phase. The result of experiment is shown in Fig. 2. We can see that using the procedure with solids removal before substrate addition, we observed a slower reaction kinetics. The highest production was reached approximately 22 minutes after the reaction start time.

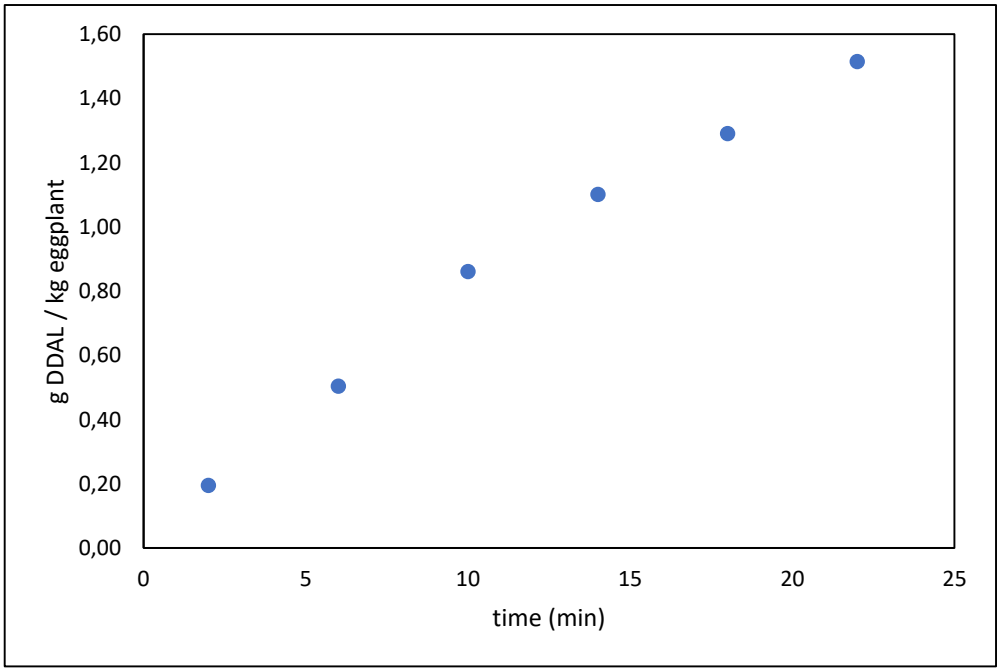


Fig. 2. DDAL production by eggplant enzymes, with solids separation before substrate addition; substrate: Nouracid HE 30

3.4 Study of DTAL production

DTAL was prepared in the same manner as DDAL, except that the substrate Nouracid HE 30 was replaced by Nouracid LE 80. After GC analysis and comparison with the standard on GC-MS we found that in our mixture there are 3 isomers DTAL: (2E, 4Z, 7Z)-decatrienal and 2 others have not been identified yet) and in addition there are also 2 isomers of DDAL: (E2, Z4)-decadienal, and (E2, E4)-decadienal, the occurrence of which is caused by the presence of linoleic acid in the used substrate. Production of DDAL and DDAL is shown in Fig. 3 (samples were extracted by ethyl acetate).

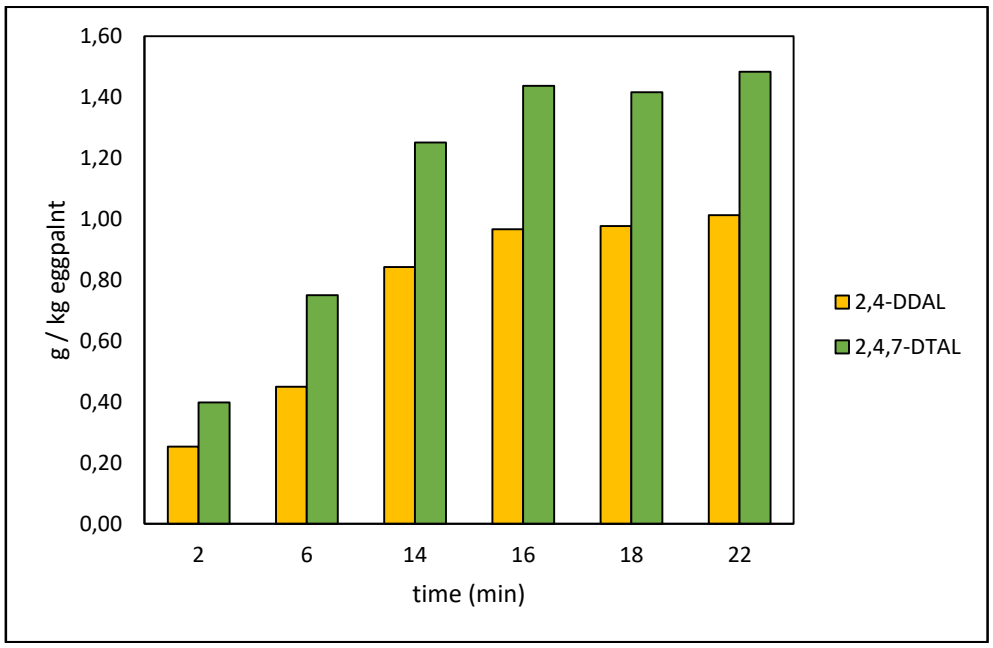


Fig. 3. Production of DDAL and DTAL using eggplant enzymes.

3.5 Comparison of the effectiveness of organic solvents

In another series of experiments, two extractants, ethyl acetate (EA) and isoamyl alcohol (IA) were compared for both substrates. The mixture was shaken in a separation funnel after the addition of the solvent. Fig. 4 illustrates extraction of reaction mixture for the substrate Nouracid HE 30. The results show that the use of EA for DDAL extraction is more efficient compared to the use of IA.

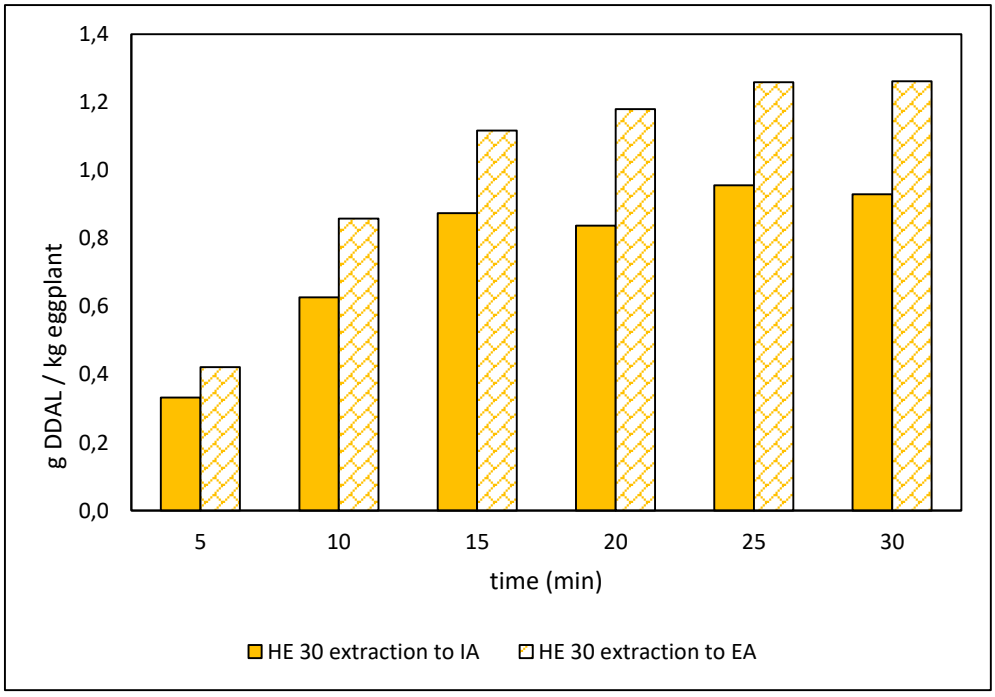


Fig. 4. Comparison of the effectiveness of organic solvents for DDAL extraction at laboratory temperature; used substrate: Nouracid HE 30

Fig. 5 illustrates extraction of reaction mixture for the substrate Nouracid LE 80. The results show that the use of EA extraction is more efficient for both, DDAL and DTAL, resp., compared to the use of IA.

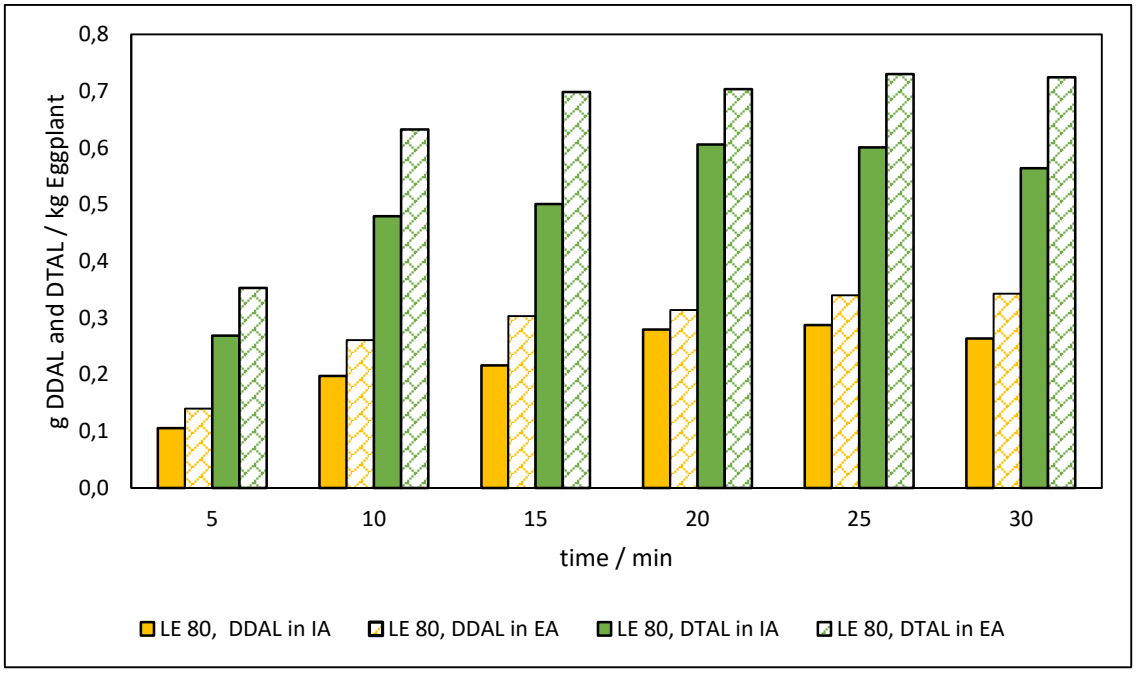


Fig. 5. Comparison of the efficiency of organic solvents in the extraction of DDAL and DTAL at room temperature; substrate used: Nouracid LE80.

CONCLUSIONS

Eggplant seems to be promising source of enzymes for (2E,4E)-deca-2,4-dienal and (2E,4E,7E)-deca-2,4,7-trienal preparation.

The biocatalytic action of the eggplant enzymes is related to several factors that affect the efficiency of biotransformation of substrates to the required products. These factors include in particular, the soil and climatic conditions, phenological growth stages of the plant, harvesting conditions (weather and temperature), mechanical damage of plants/fruits during harvesting, post-harvest processing, length/conditions during storage, etc.

In the future, it is necessary to solve the problems associated with the foam separation, which arise when stirring the reaction mixture, e.g. by adding diatomaceous earth or bentonite to the reaction mixture and subsequent filtration.

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