Screening carrot varieties for biocatalytic reduction of acetophenone to 1-phenylethanol

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Summary

Many aroma-active compounds can be produced by means of plant-mediated reactions. Biotransformation using whole plant cells is a field of green chemistry, whose purpose is to minimize the impact on the environment in combination with the advantages of high chemo-, regio- and stereoselectivity. One such example is the asymmetric reduction of corresponding prochiral ketones to chiral alcohols. In this work, twenty-one different carrot varieties were screened as a whole-cell biocatalyst for bioreduction of acetophenone to 1-phenylethanol, an important aroma compound. The reaction catalysed by grated carrot roots was carried out in an orbital shaker, under controlled conditions (30 °C, 2.83 Hz, 24 h). The substrate and product concentrations were analysed during the reaction by gas chromatography. All tested carrot varieties showed interesting biocatalytic activity, with substrate conversion degree in the range of 55.1–85.0 %. When using Tendersweet variety, which showed the highest biocatalytic activity, the enantiomeric excess of (*S*)-1-phenylethanol reached 86.4 %. The influence of sugar content in carrot roots on enzyme activity was not confirmed. The results provide useful information on the application of carrot roots as a promising source for the biocatalytic conversion of acetophenone to 1-phenylethanol.

Keywords

1-phenylethanol; acetophenone; carrot root; chiral alcohol; sugar; whole-cell biocatalysis

During the last years, a significant effort has been made to develop green, eco-friendly technologies allowing to convert agricultural raw materials into high value-added products. There is a growing interest in natural compounds widely used across various industries, for example to produce health-promoting foods and beverages containing natural flavours. Consumer demands are also reflected in legislative approaches to sustainable agriculture and natural resource management [1]. In terms of industrial practice, this means that flavour compounds must either be isolated from natural sources or produced by means of controlled biotechnological processes.

A large number of flavours and fragrances can be produced by means of enzyme-catalysed reactions [2]. Enzymes catalyse a wide range of regio- and stereoselective reactions [3], e.g. the enantioselective reduction of prochiral ketones to chiral alcohols [4, 5]. During the past years, there has been an extensive interest in cost-effective ways to obtain enantiopure alcohols, since they are the key building blocks required for the synthesis of a variety of pharmaceuticals, agrochemicals, aroma compounds and other fine chemicals [6].

Biocatalysis can be performed using isolated enzymes or whole cells. Both types of catalysts have their benefits, but also shortcomings limiting their use in the synthesis of chiral alcohols [4]. The use of isolated enzymes is often preferred because of a higher volumetric productivity and the absence of side reactions. However, there are some significant drawbacks in the use of isolated enzymes, the most important of which is their

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cost [7]. Recently, Bozan et al. [8] reported production of enantiomerically pure 1-phenylethanol by lipase kinetic resolution of (*R,S*)-1-phenylethanol performed by a transesterification reaction.

Alcohol dehydrogenases can be used to reduce aldehydes and ketones. However, these enzymes vary in terms of structure and substrate specificity. Therefore, a suitable alcohol dehydrogenase must be found for each application [9]. Baker's yeast is by far the most widely used source of alcohol dehydrogenase for the reduction of prochiral ketones yielding the corresponding optically active alcohols. Unfortunately, recovery of the desired product might not be straightforward and often reduction of carbonyl compounds is carried out by enzymes requiring costly cofactors (NADH, NADPH) [10]. These complications can be overcome by using whole plant cells as a source of enzymes for biotransformation of selected substrates to specific products. Such approach can play an important role in the development of green chemistry as a versatile tool for the preparation of natural compounds with the aim of minimizing impacts on the environment, coupled with the characteristics of high selectivity, chemo-, regio- and stereoselective reactions [11, 12]. Enzymes that are used in whole-cell biotransformations are often more stable due to the presence of their natural environment inside the cell [13]. Bioreductions mediated by intact cells from cut plants, vegetables or fruits are attractive alternatives and could facilitate the transition towards a more bio-based economy [14]. The disadvantage of application of plant whole-cell systems is that they are influenced by seasonality, as well as fluctuations in enzyme activity depending on climate, soil conditions and phenophase of the plant. Nevertheless, this cost-effective method offers an alternative way to biosynthesize natural value-added compounds. The most important advantage of using whole-cell biotransformation is that the entire reaction system for cofactor regeneration is present within the cells themselves [15].

In the recent past, the biocatalytic activities of various plants were studied. Chang et al. [16] reported on asymmetric reduction of prochiral aromatic ketone to produce the corresponding chiral alcohol using apple, carrot, cucumber, onion, potato, radish or sweet potato as the biocatalyst. Majewska and Kozłowska [17] investigated the bioreduction of *trans*-4-phenylbut-3-en-2-one to (*S*)-*trans*-4-phenylbut-3-en-2-ol using the comminuted roots of carrot, celeriac or beetroot in isooctane. Bennamane et al. [18] evaluated the potential of stereoselective reduction of prochiral ketones by means of ginger root, strawberry tree or mandarin. Utsukihara and Horiuchi [19] studied the biotransformation of acetophenone and 1-arylethanol derivatives using various vegetables (carrot, sweet potato, bell pepper, onion, Japanese radish, cucumber) as catalysts. Atak et al. [20] studied asymmetric reduction of acetophenone to (*S*)-1-phenylethanol, resulting in > 99 % enantiomeric excess and 40 % conversion degree, using Ispir bean (*Phaseolus vulgaris*) with acetone powder methodology.

Based on articles published previously [14, 21, 22], carrot roots seem to be reliable, affordable and easy-to-handle biocatalysts for the enantioselective reduction of prochiral ketones, a widely

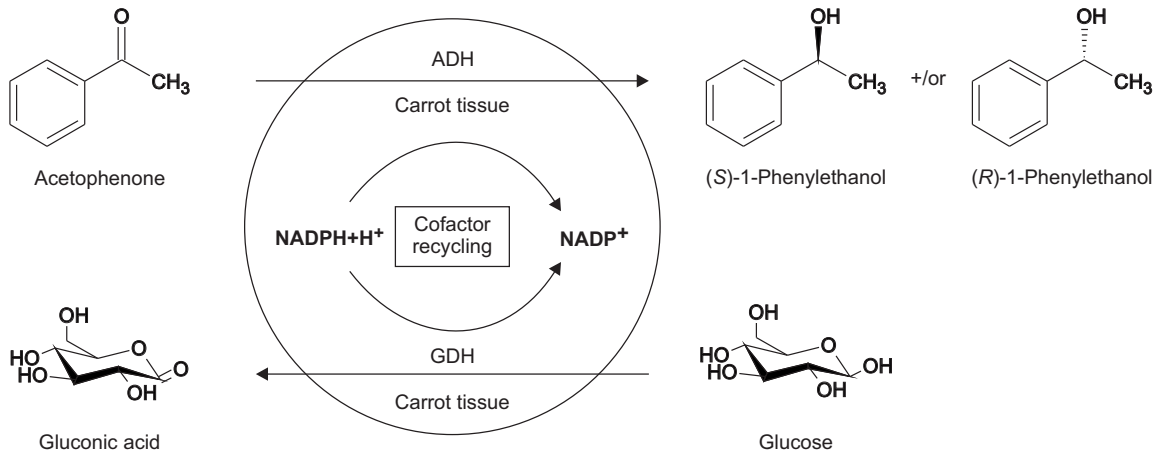

Fig. 1. Asymmetric bioreduction of acetophenone catalysed by carrot root (*Daucus carota*).

Modified scheme of Atak et al. [20].
ADH – alcohol dehydrogenase, GDH – glucose dehydrogenase, NADP – nicotinamide adenine dinucleotide phosphate.

used method for the preparation of chiral alcohols. The reaction is very simple to set up. No aseptic procedure is needed and the reaction is carried out in aqueous environment under mild conditions. In addition, downstream processing is much easier compared to other biocatalytic systems using simple filtration followed by liquid-liquid extraction [22, 23]. KAZICI et al. [15] studied asymmetric reduction of a group of aldehydes or ketones using the freeze-dried carrot as an eco-friendly whole-cell biocatalyst. OMORI et al. [23] investigated biocatalytic activities of orange, yellow and purple carrots for the enantioselective reduction of acetophenone. Comparatively, the purple carrot was a better biocatalyst than the common orange variety. LIU et al. [5] reported that the whole-cell enzyme system of carrot roots exhibited enantioselectivity, leading predominantly to the formation of (*S*)-alcohols, such as (*S*)-1-phenylethanol with 98% abundance.

1-Phenylethanol is one of the most commonly available chiral alcohols. This alcohol, also known as styrallyl alcohol, belongs to the aromatic compounds containing one monocyclic ring system consisting of benzene. In nature, it occurs in various plants, either in free or bound form. It is present in cocoa (*Theobroma cacao*) beans [24] and also, as a glycoside together with its hydrolase β-primeverosidase, in tea (*Camellia sinensis*) flowers [25, 26]. It is also reportedly present in cranberries, grapes, chives, Scottish spearmint oil, cheeses, cognac, rum, white wine, black tea, filbert, cloudberries, beans, mushrooms and endives [27].

1-Phenylethanol is formed by the catalytic action of alcohol dehydrogenase from acetophenone (Fig. 1). The mechanism of catalysis was previously described in various publications [4, 15, 20]. Fig. 1 shows that 1-phenylethanol is available in racemic or optically active forms. It is worth noting that enantiomers (*R*) and (*S*) exhibit different flavour properties. While (*R*)-1-phenylethanol has a floral, earthy-green and honeysuckle aroma, (*S*)-1-phenylethanol is characterized by a mild hyacinth and gardenia smell with the addition of strawberries [28]. (*R*)-1-phenylethanol is an important substance especially used as a fragrance, solvatochromic dye, ophthalmic preservative and as an inhibitor of cholesterol intestinal adsorption in pharmaceutical, cosmetic and chemical industries [29]. (*S*)-1-phenylethanol is used as a safe flavour in food and beverages such as bakery products, chewing gum, frozen dairy products, gelatines, puddings, non-alcoholic beverages or soft candies [27]. (*R*)- and (*S*)-phenylethanols are useful building blocks for the synthesis of complex molecules and are attractive compounds for a wide range of potential applications in the pharmaceutical industry [20]. Their esterified derivatives have many applications in perfumeries, soaps, detergents, cosmetics, room sprays, deodorants and flavours to imitate butter, apple, apricot, caramel, honey, vanilla or beer [30].

The main objective of this work was to demonstrate, that roots of carrot (*Daucus carota*) are not only an important highly nutritious, functional and health-promoting food [31], but are also a promising source of alcohol dehydrogenase for biotransformation of acetophenone to 1-phenylethanol. The screening of 21 carrot cultivars to evaluate the ability of biocatalytic production of this aromatically active compound was carried out in two basic steps. In the first step, Erlenmeyer flasks containing a mixture of grated carrot roots, substrate and water were shaken in an orbital shaker and after the reaction completion, the product was separated by centrifugation. The second step was the extraction of the product using ethyl acetate. Moreover, the possible influence of the content of sugar in the carrot roots on the relevant enzyme activity was investigated.

MATERIALS AND METHODS

Chemicals

(*R*)-1-phenylethanol and (*S*)-1-phenylethanol were obtained from Acros Organics (Geel, Belgium). Acetophenone, (*R*,*S*)-1-phenylethanol, ethanol 96% and ethyl acetate (ACS reagent, ≥ 99.5 %) were obtained from Merck (Darmstadt, Germany). Hydrogen (SIAD Slovakia, Bratislava, Slovakia) was used as a carrier gas.

Source of plant enzymes

As a source of the enzyme system for the biotransformation of acetophenone to 1-phenylethanol, twenty-one different carrot varieties were obtained from the Research Institute of Plant Production (National Agricultural and Food Centre, Piešťany, Slovakia). The plant material was sown on the field experimental plots of this institute. The latitude and longitude of the experimental field were 48° 35’ 6.4” N, 17° 48’ 46” E, altitude was 163 m. The location of Piešťany belongs to the maize production area. The climate is typically lowland, slightly dry and slightly windy. The average annual temperature is 9.2 °C and the long-term average annual precipitation is 595 mm.

The samples of carrots were collected at a stage of full maturity and immediately used for experiments. Based on the supplier information,

a more detailed description of the tested varieties is as follows:

- Marion F1 is an early Nantes type, root length 15–17 cm, cylindrical shape having blunt tip, rich orange colour with no green shoulders, vegetation period 90–95 days.
- Cascade F1 is a mid-early hybrid of Chantenay type, root length is up to 22 cm, conical shape, orange colour, vegetation period 90–130 days. It is one of the tastiest carrots, juicy and sweet, very balanced in shape and size.
- Naomi is an early Nantes-type variety, suitable for early spring and field cultivation, root length is 16–18 cm, cylindrical shape with a fine, smooth surface, orange colour, vegetation period 90 days.
- Aron F1 is a very early carrot hybrid variety similar to Nantes type, having an unusual half-long stumpy shape, cylindrical with a blunt tip, orange colour, root length up to 10 cm, vegetation period 75–85 days.
- Katrin is a mid-early carrot variety of Chantenay type, root length 10–13 cm, conical with a blunt tip, orange colour, vegetation period 125–130 days.
- Karotela is an early variety of the Nantes type, root length up to 14 cm, conical shape, intense orange colour, vegetation period 100–110 days.
- Nantes 3 is a mid-early Nantes-type variety, root length 16–20 cm, cylindrical shape, intense orange colour, vegetation period 115–125 days. It is a traditional variety of very high quality intended for direct consumption from summer to autumn and for industrial processing.
- Chamare is a mid-early variety of Chantenay type, root length approximately 10 cm, robust, short and wide, broadly conical in shape and bluntly pointed, intense orange skin and darker medium red pulp, vegetation period starts on average from 70 days after sowing. It belongs to a less traditional variety, suitable for juicing and fresh market.
- Calibra F1 is a very early carrot hybrid of Nantes type, root length 18–23 cm, of slightly conical shape, orange colour, vegetation period 90 days.
- Lenka is an early variety of the Nantes type, root length 15–18 cm, cylindrical, longer and thinner with blunt tip, good orange colour intensity, vegetation period 90–125 days.
- Kráska is a mid-early hybrid variety of Berlicum type, root length 18–20 cm, long, thin cylindrical shape with blunt tip, good orange colour, vegetation period 134 days.
- Jitka F1 is an early, high-yielding hybrid carrot of Berlicum type, root length 18–22 cm, cylindrical, smooth blunt roots of orange-red colour, vegetation period 135 days. It is recommended for fresh consumption.
- Vanda is an early variety of Nantes type, root length 16–20 cm, cylindrical, blunt, of bright orange colour, vegetation period 115–120 days.
- Maxima F1 is a hybrid of Danvers type, very large, late, storable carrot for industrial processing. The conical root is 25–30 cm long, vegetation period 150–180 days.
- Nectar F1 is an early hybrid of Nantes type, root length 18–20 cm, cylindrical, blunt-ended, with orange colour, vegetation period 105–120 days.
- Olympus is a late, high-yielding variety of Flakkeer type, root length 23–25 cm, long, conical, orange-red colour, vegetation period 160–170 days.
- Bolero F1 is a mid-early hybrid of Berlicum and Nantes varieties, root length 18–20 cm, cylindrical, bright orange colour, vegetation period 110–120 days.
- Maestro F1 is a medium-late to late variety of Nantes type, root length 18–20 cm, cylindrical shape with a blunt tip, bright orange colour, vegetation period is 120–130 days.
- Olympia is a very late variety of Flakkeer type, root length 16 cm, almost cylindrical, narrowed at the bottom, orange-red colour, harvest maturity is after 180–185 days.
- Sugarsnax 54 F1 is an extra sweet variety of Imperator type, root length 25 cm, long, tapered, cylindrical, contains high levels of beta-carotene giving it a rich orange colour, harvest maturity after 63 days.
- Tendersweet is an early Imperator type variety, root length 22–25 cm, straight, tapering, deep orange colour, the period from germination to technical ripeness is 75 days.

Determination of reducing sugars

The content of glucose, fructose and saccharose in carrots was determined using high-performance liquid chromatography with refractometric detection (HPLC-RID). Carrot roots were homogenized in a high-speed blender Grindomix GM 200 (Retsch, Haan, Germany) at 130 Hz for 20 s. Then, 5.0–7.0 g of the homogenous sample was weighed into a plastic centrifuge tube with a cap and 15 ml of deionized water was added, followed by agitation on an orbital mixer OS-20 (Biosan, Riga, Latvia) at 4 Hz for 10 min and then in an ultrasonic bath UC 005 AJ1 (Tesla, Prague, Czech Republic) for another 10 min. The sample was finally centrifuged at 17 000 ×*g* using a centrifuge 2-16KC (Sigma Aldrich, St. Louis, Missouri,

USA) for 10 min. An aliquot volume of the supernatant was filtered through a syringe microfilter (regenerated cellulose/polypropylene, pore size of 0.45 μm, 13 mm diameter) and analysed by HPLC model PU 4003 (Pye Unicam, Cambridge, United Kingdom) equipped with a refractometric detector RID-10A (Shimadzu, Kyoto, Japan). Chromatographic separation of sugars took place in a Kromasil 100-5-NH2 column (250 mm × 4.6 mm; particle size 5 μm; EKA Chemicals, Bohus, Sweden) at a column temperature of 25 °C using a mobile phase acetonitrile-water 80 : 20 (v/v) at a flow rate of 1.35 ml·min^{-1}. Sample injection volume was 20 μl and detector cuvette temperature was 40 °C. Each sample was analysed in this way at 2 different weights. The results were evaluated by the software CSW version 1.7 (DataApex, Prague, Czech Republic) according to the internal calibration procedure and were expressed as an arithmetic mean with the corresponding standard deviation in grams per kilogram of fresh product.

Preparation of glass and grater

Before each experiment, Erlenmeyer flasks with lids were sterilized in an autoclave for 20 min (120 °C, 2.5 kPa). The grater was sanitized by rinsing with 96% ethanol and allowed to dry in air.

Biotransformation of acetophenone to 1-phenylethanol by carrot roots

To prevent microbial contamination (mainly with *Galactomyces candidus*), the fresh carrot roots were sanitized before processing in a bath by a commercial disinfectant SAVO (Unilever, London, United Kingdom) containing 47 g·kg^{-1} sodium hypochlorite diluted 1 : 1 (v/v) with tap water, for approximately 2 min. Then, they were rinsed thoroughly with tap water. The washed carrot roots were carefully shredded using a hand grater on largest holes into thin slices approximately 2 cm long.

To carry out the biotransformation of acetophenone to 1-phenylethanol, 60 g of grated carrot roots were transferred to 500 ml Erlenmeyer baffled flasks (Carl Roth, Karlsruhe, Germany) and 300 ml of an aqueous acetophenone solution (concentration 1.5 g·l^{-1}) was added to start reaction. Subsequently, the flasks were incubated for 24 h at 30 °C and 2.8 Hz in a thermostated orbital shaker Innova 40 (New Brunswick Scientific, Edison, New Jersey, USA). The reaction kinetics were determined by analysing substrate consumption and product formation by sampling of the reaction mixture after 2 h, 19 h and 24 h. The progress of the reaction was monitored by gas chromatography (GC).

Conversion degree was derived from the ratio of the product peak area to the sum of the substrate and product peak areas on GC chromatogram [32] according to the formula given in Eq. 1.

$$C = \frac{A_{PE}}{(A_{PE} + A_{ACP})} \times 100 \quad (1)$$

where C is conversion degree (in percent), A_{PE} is peak area of 1-phenylethanol, A_{ACP} is peak area of acetophenone.

Sample preparation for analysis

A volume of 4 ml of the liquid phase of the reaction mixture was pipetted out from the shaking flask into a 15 ml centrifuge tube containing 1 g of NaCl and 2 ml of organic extraction solvent (ethyl acetate). The solution was vortex-mixed (LQ1; Heidolph, Schwabach, Germany) for 2 min and centrifuged using model Universal 320 R centrifuge (Hettich, Kirchlengern, Germany) at 3 000 ×g and 18 °C for 5 min. The organic phase (supernatant) was transferred by pipette into a collection tube. An additional volume of 2 ml of ethyl acetate was added to the lower phase in the centrifuge tube and the extraction, shaking, centrifugation and withdrawal process was repeated once more. The organic phases were combined producing a total volume of 4 ml. From this, 1 ml sample was taken for gas chromatography with flame ionization detection (GC-FID) analysis of acetophenone and 1-phenylethanol concentration.

Analysis of acetophenone and 1-phenylethanol

The substrate and product in reaction samples were analysed using a gas chromatograph Agilent 7890A (Agilent Technologies, Palo Alto, California, USA) coupled to FID detector, equipped with an Optima FFAP capillary column (30 m × 250 μm × 0.25μm; Macherey-Nagel, Düren, Germany). Hydrogen was used as a carrier gas at a constant flow rate of 1.5 ml·min^{-1}. One microliter of the sample was injected using a split mode in the ratio of 20 : 1, the injection port temperature was 280 °C. The initial column temperature was 60 °C. This temperature was held for 1 min, then increased at a rate of 15 °C·min^{-1} to 170 °C and subsequently increased again at 20 °C·min^{-1} to 220 °C.

Determination of enantioselectivity

The enantioselectivity of the bioreduction reaction mediated by the carrot root material was determined using a gas chromatograph Agilent 7890A (Agilent Technologies) coupled to FID detector, equipped with a Cyclosil-B capillary column (30 m × 320 μm × 0.25μm; Agilent Tech-

nologies). Hydrogen was used as a carrier gas at a constant flow rate of 3.0 ml·min^{-1}. Various volumes of sample ranging from 0.05 μl to 0.1 μl (based on predicted concentration of analytes) were injected using a split mode in the ratio of 120 : 1, the injection port temperature was 260 °C. The initial column temperature of 40 °C was held for 1 min, then increased at a rate of 10 °C·min^{-1} to 190 °C. Standards of (*R*)-1-phenylethanol and (*S*)-1-phenylethanol were used to determine the retention time of analytes.

The (*S*)-phenylethanol enantiomeric excess (*EE*) was calculated using Eq. 2.

$$EE = \frac{(S - R)}{(S + R)} \quad (2)$$

where *S* and *R* are the respective peak areas of the two enantiomers.

Statistical analysis

Each sample was analysed minimally in duplicate and results are reported as mean concentration ± standard deviation. Excel XP Software (Microsoft, Redmond, Washington, USA) was used for construction of calibration curves as well as for determination of differences between means by analysis of variance (ANOVA). In this analysis, the difference was taken as significant at $p < 0.05$ (95% confidence level).

Standard deviation of the contents of individual sugars (fructose, glucose and saccharose) was calculated using the formula given in Eq. 3.

$$\sigma_x = \sqrt{\frac{\sum_{i=1}^{n}(\bar{x} - x_i)^2}{n - 1}} \quad (3)$$

where σ_x is standard deviation, n is the number of data points in the data set, x_i is value of the ith point in the data set and $\bar{x}$ is the mean value of the data set.

Standard deviation of the total sugar content (expressed as the sum of fructose, glucose and saccharose contents) was calculated using the formula given in Eq. 4.

$$\sigma_T = \sqrt{\sigma_F^2 + \sigma_G^2 + \sigma_S^2} \quad (4)$$

where σ_T is standard deviation of the total sugar content and σ_F, σ_G, σ_S are standard deviations of individual sugars (fructose, glucose and saccharose).

To construct the calibration curve, the following stock solutions of standards were used: fructose 10.112 g·l^{-1}, glucose 10.052 g·l^{-1}, saccharose 10.054 g·l^{-1}.

RESULTS AND DISCUSSION

Limited comprehensive information is available in the literature on the chemical composition and biocatalytic activity of the carrot varieties studied in this work. Only general data or information about other varieties are available [5, 15, 22, 23, 31]. Although the previously reported reaction times for biotransformation of acetophenone to 1-phenylethanol ranged from 28 h to 100 h [5, 10, 22, 23, 33], we decided to focus on the reaction time of 24 h, because, in our opinion, it should be sufficient to assess the biocatalytic activity of the screened twenty-one different carrot varieties. Furthermore, if the reaction time was extended, there would be an increased risk of microbial contamination due to non-aseptic conditions.

As shown in Tab. 1, all tested carrot varieties showed varied enzyme activities, with substrate conversion degree ranging from 55.1 % to 85.0 %. The best conversion degree after 24 h was shown by variety Tendersweet (85.0 %), followed by Maxima F1 (84.3 %) and Olympia (84.0 %.). On the opposite side, the lowest conversion degree was achieved using varieties Aron F1 (55.1 %) and Maestro F1 (58.8 %).

In the case of the most enzymatically active variety Tendersweet, which has been proposed for the continuation of experiments, we decided to determine the enantiomeric composition as well. The reaction mixture was found to contain 93.2 % of (*S*)-enantiomer and 6.8 % of (*R*)-enantiomer. The enantiomeric excess of the (*S*)-enantiomer was thus 86.4 %.

Due to various conditions and reaction times, it is not easy to compare the achieved results with the works of other authors. Costa and Omori [22] reported 67 % conversion degree of acetophenone to 1-phenylethanol in the presence of 1.5 % of Tween 20 using carrot root slices as a biocatalyst after 24 h of reaction. Omori et al. [23] described the conversion degree of acetophenone to 1-phenyletanol of 95 %, 96 % and 97 %, using orange, purple and yellow carrot, respectively, after 48 h of reaction. Utsukihara and Horiuchi [19] described reduction of acetophenone using carrot, with 58 % yield and very high enantiomeric excess of (*S*)-1-phenylethanol (99 %) after 5 days reaction. Liu et al. [5] described the enantioselective reduction of acetophenone to 1-phenyletanol catalysed by carrot with the conversion degree of 97 %, and enantiomeric excess of 96 % after 28 h of reaction. Yang et al. [33] obtained the yield of 1-phenylethanol of 78.4 % and enantiomeric excess of 95 % in the form of (*S*)-enantiomer,

Tab. 1. Time course of acetophenone conversion to 1-phenylethanol by 21 carrot varieties.

No.	Variety	Earliness	Type	Conversion degree [%]		
				Time 2 h	Time 19 h	Time 24 h
1	Marion F1	Early	Nantes	4.3	69.3	76.5
2	Cascade F1	Mid-early	Chantenay	3.7	67.8	75.2
3	Naomi	Early	Nantes	3.3	68.7	75.4
4	Aron F1	Very early	Nantes	2.7	50.1	55.1
5	Katrin	Mid-early	Chantenay	3.1	65.2	71.9
6	Karotela	Early	Nantes	3.8	74.3	81.7
7	Nantes 3	Early	Nantes	4.3	77.1	83.4
8	Chamare	Mid-early	Chantenay	3.5	57.8	66.3
9	Calibra F1	Very early	Nantes	4.0	69.1	76.5
10	Lenka	Early	Nantes	4.2	75.0	82.7
11	Kráska	Mid-early	Berlicum	3.9	65.9	75.5
12	Jitka F1	Early	Berlicum	4.9	74.0	81.6
13	Vanda	Early	Nantes	4.6	70.9	77.9
14	Maxima F1	Late	Danvers	4.8	74.7	84.3
15	Nectar F1	Early	Nantes	4.9	63.4	70.7
16	Olympus	Late	Flakkeer	4.2	61.0	70.7
17	Bolero F1	Early	Berlicum/Nantes	2.9	62.4	69.2
18	Maestro F1	Mid-late	Nantes	2.8	52.5	58.8
19	Olympia	Late	Flakkeer	4.4	76.4	84.0
20	Sugarsnax 54 F1	Mid-late	Nantes x Imperator	2.6	67.6	74.5
21	Tendersweet	Early	Imperator	2.6	65.0	85.0

Values represent the average of 2 measurements.

Tab. 2. Sugar content in carrot varieties.

No.	Variety	Sugar content [g·kg^{-1}]			
		Fructose	Glucose	Saccharose	Total sugars
1	Marion F1	2.90 ± 0.29	4.92 ± 0.16	39.24 ± 1.08	47.06 ± 1.13
2	Cascade F1	6.92 ± 0.34	9.87 ± 0.60	27.04 ± 1.02	43.83 ± 1.23
3	Naomi	8.13 ± 0.56	13.78 ± 0.76	19.80 ± 1.17	41.71 ± 1.50
4	Aron F1	3.14 ± 0.35	4.57 ± 0.33	22.52 ± 2.26	30.23 ± 2.31
5	Katrin	6.23 ± 0.32	8.32 ± 0.04	30.21 ± 1.80	44.76 ± 1.83
6	Karotela	4.32 ± 0.18	7.91 ± 0.17	28.52 ± 0.72	40.75 ± 0.76
7	Nantes	4.74 ± 0.13	6.00 ± 0.07	32.05 ± 1.39	42.79 ± 1.40
8	Chamare	3.62 ± 0.21	5.39 ± 0.37	34.76 ± 1.97	43.77 ± 2.02
9	Calibra	6.04 ± 0.13	8.90 ± 0.67	19.31 ± 1.53	34.25 ± 1.68
10	Lenka	3.85 ± 0.44	6.53 ± 0.68	29.23 ± 2.53	39.61 ± 2.66
11	Kráska	12.86 ± 0.59	13.56 ± 0.71	26.21 ± 1.39	52.63 ± 1.67
12	Jitka F1	4.67 ± 0.33	5.89 ± 0.99	32.31 ± 2.69	42.87 ± 2.89
13	Vanda	5.16 ± 0.52	8.28 ± 0.49	25.87 ± 1.43	39.31 ± 1.60
14	Maxima F1	1.65 ± 0.20	3.32 ± 0.58	36.77 ± 1.51	41.74 ± 1.63
15	Nectar F1	10.34 ± 0.92	12.42 ± 1.45	33.85 ± 1.42	56.61 ± 2.23
16	Olympus	7.39 ± 0.55	12.01 ± 0.97	21.02 ± 0.89	40.42 ± 1.43
17	Bolero F1	4.24 ± 0.16	6.23 ± 0.57	30.41 ± 2.80	40.98 ± 2.86
18	Maestro F1	6.76 ± 0.25	7.87 ± 0.96	26.13 ± 2.14	40.76 ± 2.36
19	Olympia	11.11 ± 0.23	12.56 ± 0.19	22.04 ± 0.98	45.71 ± 1.02
20	Sugarsnax 54 F1	6.39 ± 0.19	9.28 ± 0.72	22.07 ± 0.95	37.74 ± 1.21
21	Tendersweet	12.15 ± 0.65	15.74 ± 0.28	11.24 ± 0.90	39.13 ± 1.14

Values represent mean ± standard deviation (n = 2). Total sugars are expressed as a sum of fructose, glucose and saccharose.

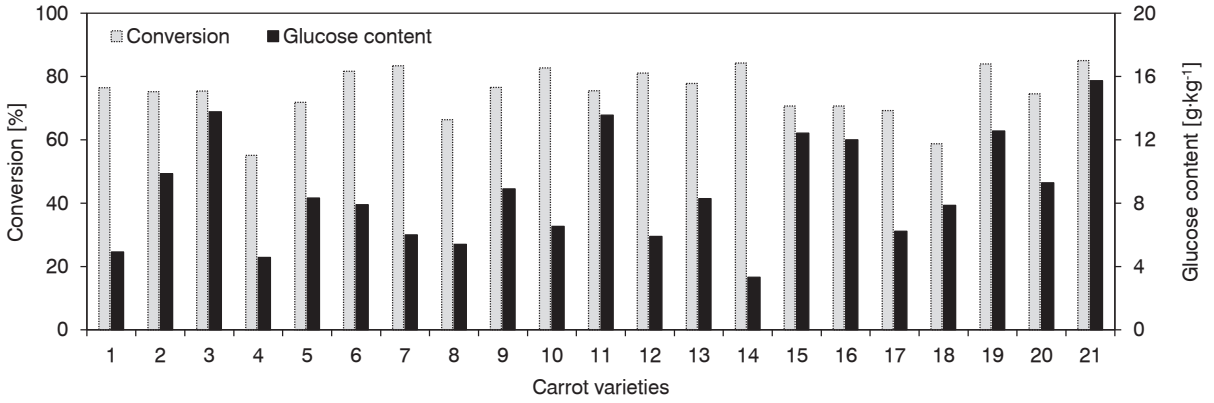

Fig. 2. Glucose content in carrot varieties and conversion degree of acetophenone to 1-phenylethanol after 24 h of reaction.

Designation of carrot varieties is given in Tab. 1.

after 50 h of biocatalytic reduction of acetophenone using carrot.

An accompanying goal of the study was to evaluate the possible influence of the sugar content in carrot roots on the conversion degree of the substrate. We supposed that the biocatalytic activity could be influenced by the content of some endogenous sugars and glucose needed for cofactor regeneration. The results of the experiments presented in Tab. 2 showed that the dominant sugar in carrot roots was saccharose. The content of individual sugars (expressed per kilogram of fresh carrot) ranged from 1.65 g·kg^{-1} (Maxima F1) to 12.86 g·kg^{-1} (Kráska) for fructose; from 3.32 g·kg^{-1} (Maxima F1) to 15.74 g·kg^{-1} (Tendersweet) for glucose; and from 11.24 g·kg^{-1} (Tendersweet) to 39.24 g·kg^{-1} (Marion F1) for saccharose. The total content of reducing sugars, expressed as the sum of fructose, glucose and saccharose contents, ranged from 30.23 g·kg^{-1} (Aron F1) to 56.61 g·kg^{-1} (Nectar F1). Augspole et al. [34] reported similar values (expressed per kilogram of fresh product): 11.1–14.0 g·kg^{-1} for fructose, 11.2–18.7 g·kg^{-1} for glucose and 18.1–48.2 g·kg^{-1} for saccharose. Sharma and Sharma [31] reported the content of reducing sugars in the range of approximately 20 g·kg^{-1} for orange carrot and 21 g·kg^{-1} for purple carrot.

As can be seen from Fig. 2, the glucose content in carrot roots had no provable effect on the studied biocatalytic activity. For example, low values of both, conversion degree (55.1 %) and glucose content (4.57 g·kg^{-1}) were measured for the Aron F1 variety, and the highest value of conversion degree (85.0 %) was measured for the Tendersweet variety, with relatively high content of glucose (15.74 g·kg^{-1}). Inconsistently, the variety Maxima F1 with the lowest glucose content (3.32 g·kg^{-1}) showed a high substrate conversion degree of 84.3 %. Similar conclusions could be drawn when comparing the content of fructose, saccharose and total sugars in the tested carrot varieties with the achieved values of substrate conversion degree. These results showed that the substrate conversion degree was not related to the content of glucose or other sugars in carrot roots. However, these assumptions may need to be verified in further experiments in which the influence of the addition of glucose during the reaction on the biocatalytic activity will be investigated.

CONCLUSIONS

The aim of this research was to perform an extensive screening for an ecofriendly asymmetric reduction system employing carrot roots for bioreductive catalysis of acetophenone to 1-phenylethanol. The main advantages of using carrot roots as a whole-cell biocatalyst are the good availability and low price of carrots, simple isolation of the product and elimination of the need to add expensive cofactors. On the other hand, the biocatalytic activity of carrot roots can vary due to several external factors that include soil composition, climatic conditions and phenophase. In summary, all 21 carrot varieties tested showed good to excellent conversion degree ranging from 55.1 % to 85.0 % after 24 h of reaction. The assumption that conversion degree of acetophenone to 1-phenylethanol depends on the glucose or sugar content in carrot roots was not confirmed. The most enzymatically active Tendersweet variety showed also high enantioselectivity, with the enan-

tiomeric excess of 86.4 % for (*S*)-enantiomer. The experimental results of this study expanded the knowledge about the use of carrot roots as a promising source of enzymes for the asymmetric bioreduction of acetophenone to 1-phenylethanol with a high degree of enantioselectivity.

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