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Production of glucolipids and specialty fatty acids from sophorolipids by *Penicillium decumbens* naringinase: optimization and kinetics

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ABSTRACT

In view of global environmental concerns and the awakening to the exhaustibility of our natural resources, an increasing importance of biologically derived surfactants can be expected in the near future. Enzymatic modification of these biosurfactants allows to improve their characteristics and so extend their application field. In view of this, glucolipids are interesting substrates e.g. for the synthesis of new glycolipids with increased biological activity. Here we describe the optimization of glucolipid production from *Candida bombicola* sophorolipids by *Penicillium decumbens* naringinase and show that the enzyme might be useful for production of specialty fatty acids as well. Optimum conditions for production of glucolipids were found to be pH 7.0 and 50°C with a yield of 80% (w/w) glucolipids after 3h of incubation. The K_m for sophorolipids was 1.67 mM, while V_{max} was 0.035 mM sophorolipids/min. At pH 3.0, glucolipids were immediately further hydrolyzed and completely converted to fatty acids after 24h of incubation, offering a biological route to the synthesis of unique specialty fatty acids. The K_m for glucolipids was 11 mM while V_{max} was 0.21 mM glucolipids/min. Glucose inhibited the enzyme in a competitive way with K_I around 10-15 mM glucose. Surfactant properties of the produced glucolipids were comparable to those of the acidic sophorolipids.

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1 INTRODUCTION

Chemically derived surfactants are used in nearly every industry but due to their (eco-)toxicity, low biodegradability and petroleum-based origin the biologically derived counterparts gain more and more attention. Such biosurfactants are produced by a variety of micro-organisms when grown on hydrophobic substrates such as alkanes, oils or fats. Glycolipids are the most important class of biosurfactants since they offer a wide range of complex structures with highly specific properties and a broad range of possible applications [1-3]. However, to date only rhamnolipids from *Pseudomonas aeruginosa* and sophorolipids from *Candida bombicola* are produced by fermentation on economically competitive scales. In order to broaden the application field or to enhance specific properties of these high-yield biosurfactants, one can introduce enzymatic modifications which for example lead to the incorporation of new functional groups giving the biosurfactant better performances [4]. The use of enzymes in surfactant production is not new [5-11] since the high regioselectivity and stereospecificity of these biocatalysts can remarkably reduce protection and purification steps of conventional chemical syntheses and minimize the use of environmentally unfriendly and toxic solvents. In the search to new glycolipid analogs, glucolipids which are produced from natural glycolipids can serve as unique building blocks for further (chemo)-enzymatic syntheses. For example these

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glucolipids can be used as a substrate for different glycosyltransferases, leading to the enzymatic synthesis of a broad variety new-to-nature glycolipids with unique carbohydrate heads. As the nature of the carbohydrate head is shown to have an influence on the antibacterial activity of the glycolipid [12], interesting properties of these new glycolipids can be expected such as better biological activity and/or reduced cytotoxicity.

The enzymatic production of glucolipids from sophorolipids by glycosidases has first been described in 1999 [13]. Deacylated acidic sophorolipids, obtained after alkaline hydrolysis of the originally *C. bombicola* sophorolipid mixture, were used as a substrate and different activities in function of pH were reported in a qualitative way for 8 glycosidases. Either *Penicillium decumbens* naringinase or *Aspergillus niger* hesperidinase were considered useful for production of glucolipids. The production of glucolipids from sophorolipids by *P. decumbens* naringinase occurred in phosphate buffer at pH 9 and 40°C and led to a weight-based production yield of 80% [14]. However, to obtain this yield, a 30h incubation period was necessary. These authors also stated that the enzyme did not attack the second glucose moiety of the sophorose unit. In this paper we describe the optimization of the reaction conditions for production of glucolipids and we reached the same production yield (80% w/w) after only 3 hours of incubation. Moreover, we show that the enzyme is useful for production of specialty fatty acids as well.

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2 MATERIALS AND METHODS

2.1 Enzyme assays

Penicillium decumbens naringinase and β-nitrophenylglucoside (β-NPG) were obtained from Sigma. Deacylated acidic sophorolipids were obtained after fed batch cultivation of *Candida bombicola* ATCC 22214 and subsequent alkaline hydrolysis as described in [13]. The solution was brought to pH 4.0 and acidic sophorolipids were extracted twice with one volume of technical ethanol/ethylacetate (1/1). After vacuum evaporation of the solvent, the sophorolipid crystals were resuspended in water and freeze-dried. They consisted of > 97% deacylated acidic sophorolipid with a 17-OH-octadecenoic fatty acid tail, as revealed by LC/MS (MW 622 g). Glucolipids were produced as described in this paper. The reaction mixture was brought to pH 2 and glucolipids were extracted twice with one volume of t-butylmethylether. After vacuum evaporation of the solvent, the glucolipid crystals were resuspended in water and freeze-dried.

For activity tests on β-NPG, 200 µl naringinase solution (1.20 β-glucosidase units/ml in 0.1M phosphate buffer, pH 3.0 freshly prepared) was added to 1.8 ml preheated substrate solution (2mM β-NPG in 0.1M phosphate buffer at pH 3.0) and incubated for 5 min at 60°C. At regular time intervals, 100 µl samples were taken and added to 100 µl of Na_2CO_3 solution (0.2M in water) for spectrophotometric determination of nitrophenol release.

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For activity tests on sophorolipids, 200 µl naringinase solution (1.20 β-glucosidase units/ml in 0.1M phosphate buffer, pH 7.0 freshly prepared) was added to 1.8 ml preheated substrate solution (10 mM sophorolipids in phosphate buffer at pH 7.0) and incubated for 30 min at 50°C. At regular time intervals, 150 µl samples were taken and inactivated at 95°C during 5 minutes before measurement of glucose release by means of a YSI Select 2700 Biochemistry Analyzer. For determination of the pH optima, activity tests were performed at 37°C and substrates were dissolved in buffer at the appropriate pH. For determination of temperature optima, reactions were performed at pH 3.0. Initial reaction velocities were calculated from reaction progress curves. All experiments were done in triplicate.

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2.2 HPLC-ELSD

Samples were extracted with one volume of t-butylmethylether and analyzed for glucolipid production on a Varian ProStar HPLC (Varian) with a Chromolith® Performance RP-18e column [100 mm (l) x 4.6 mm (I.D.)] (Merck) connected to an Evaporative Light Scattering Detector (Alltech). Compounds were eluted by means of an acetonitrile/acetic acid (0.5% in water) gradient (5 /95 to 95 /5 in 40 min) under constant flow of 1 ml/min. Column temperature was set at 30°C.

2.3 Kinetics and glucose inhibition

The kinetics for production of both glucolipids and specialty fatty acids were determined under optimum reaction conditions for each process. For kinetics on

sophorolipids, a dilution series (0.3mM, 0.6 mM, 1 mM, 3 mM, 6 mM and 10 mM) was prepared in phosphate buffer at pH 7.0, while for glucolipids a dilution series (0.6 mM, 1 mM, 3 mM, 6 mM, 10 mM and 20 mM) was prepared in 1.5% Tween-20 in phosphate buffer at pH 3.0. All experiments were done in triplicate. Glucose inhibition was examined by using β-NPG as a substrate. Reaction mixtures (1 ml) contained 0.175 mM, 0.35 mM, 0.7 mM, 1.05 mM, 1.75 mM, 3.5 mM and 7 mM β-NPG in presence of 20 mM, 30 mM, 40 mM, 50 mM and 60 mM glucose respectively. Experiments were done in duplicate.

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3 RESULTS AND DISCUSSION

3.1 pH and temperature optimum for production of glucolipids

In 1999, Rau reported the production of glucolipids after a 30h incubation of *P. decumbens* naringinase with *C. bombicola* ATCC 22214 sophorolipids (acidic deacylated form) in phosphate buffer at pH 9 and at a temperature of 40°C [13]. At the end, glucolipids were extracted with t-butyl-methylether at pH 2.0 with a final yield of 80% (w/w). In a first attempt to optimize the reaction conditions, we determined pH and temperature optima for the glucosidase activity of naringinase on acidic sophorolipids and, by means of comparison, on β-NPG. From Figure 1 it is clear that the best conditions for hydrolysis of sophorolipids by naringinase are at pH 3.0 and 50°C. The fact that the temperature optimum is lower for sophorolipids as compared to β-NPG can be explained by the tendency of the sophorolipids to melt at temperatures above 50°C, forming an oil-water emulsion which is hampering access of the glucosidase active centre to the sophorolipids. Many authors have investigated optimum reaction conditions for naringinases, mostly from *Aspergillus sp.* because of its industrial application in debittering of fruit juices. However, in most cases these optima are related to the debittering action by stepwise breakdown of the bitter tasting compound naringin (4,5,7-trihydroxyflavanone-7-rhamnoglucoside) and so do not distinguish between rhamnosidase and glucosidase activitiy of the naringinase complex or focus on

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rhamnosidase activity only. Some studies report characteristics of *P. decumbens* naringinase [15-17] but also here few data concerning the glucosidase activity are published. In studies on NPG as a substrate for *P. decumbens* naringinase, the same pH optimum of 3.0 was reported by Gabor and Pittner [15], in contrast to Mamma who found a pH optimum of 7.0 in different buffer systems [18]. From our results, it was found that the naringinase lost 95% of its activity towards β-NPG at pH 7.0, while 75% of the activity towards sophorolipids was lost at the same pH.

It is important to note that at pH values below 6.0 and at room temperature, 10 mM sophorolipids were not completely soluble in the buffer system used, but were present as a white suspension instead. However, increasing the temperature to 37°C or higher, resulted in complete solubilization of sophorolipids at any of the pH values considered.

3.2 Balance between glucolipid formation and hydrolysis is pH dependent

In order to follow the production of glucolipids from sophorolipids by HPLC, a reaction was set up under optimum conditions for the glucosidase (pH 3.0 and 50°C). At regular time intervals, 1ml samples were taken and analyzed on HPLC for glucolipid production. Surprisingly, not only glucolipids appeared in the chromatogram, but nearly immediately also a third signal corresponding with free fatty acid formation was observed. As sophorolipid concentration decreased during incubation with naringinase, also glucolipid concentration decreased while the free fatty acid concentration increased confirming that glucolipids were further

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hydrolyzed. This was not expected since it has been stated earlier that naringinase from *P. decumbens* did not use glucolipids as a substrate for hydrolysis [13]. However, the authors performed the reaction at lower temperatures and at pH 9.0, conditions we found to result 95% loss of activity (results not shown). To verify if glucolipids were still hydrolyzed at less acidic pH values, the naringinase reaction described above was repeated at pH 4.0, 5.0, 6.0 and 7.0 and samples taken after 1h, 3h, 4h an 24h were analyzed via HPLC. Figure 2 shows the HPLC results of the samples taken after 1h and 3h of incubation at pH 7.0 as compared to pH 3.0. At pH 3.0, fatty acids are immediately produced from glucolipids, resulting in a strong fatty acid signal in the chromatogram already after 1h of incubation (Figure 2A). After 24 hours of incubation at pH 3.0, glucolipids are completely hydrolyzed by the naringinase and only fatty acids are detected (results not shown). With increasing pH values, remarkably less release of fatty acids takes place and no significant fatty acid production is found when the reaction is performed for 1h at pH 7.0 (Figure 2A). In the reaction mixtures at pH 3.0, 4.0, 5.0 and 6.0 the relative amount of fatty acids (as deduced from peak area percentages in the chromatograms) is continuously increasing with incubation time (3h, 4h and 24h) while that of glucolipids decreases. Though the rate of glucolipid hydrolysis is clearly slower with increasing pH values and still no fatty acid release is observed after a 3h incubation at pH 7.0 (Figure 2B). When the substrate is incubated with naringinase at pH 7.0 and 50°C for more then 3h, some minor glucolipid hydrolysis is also observed here. Based on these results, we conclude that production of glucolipids from sophorolipids by *P. decumbens*

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naringinase is optimally when the enzyme reaction is performed at pH 7.0 and 50°C. Under these optimum conditions, an 80% (w/w) yield of glucolipids is obtained after only 3h of incubation without formation of free fatty acids.

On the other hand, *P. decumbens* naringinase clearly shows activity towards glucolipids as well, and therefore might be very suitable for production of specialty fatty acids from sophorolipids or glucolipids. Fatty acids derived from sophorolipids are used for the chemical synthesis of derivatives that in their turn can serve as building blocks for e.g. chemical surfactant or polymer production [19-21] but undoubtedly, they are interesting substrates for different enzymatic esterification reactions as well e.g. for the synthesis of sugar fatty acid esters [6]. Nowadays these fatty acids are produced by acidic alcoholysis of natural sophorolipids, an easy process yet which requires the use of toxic solvents, increased reaction temperatures and rather long reaction times [20]. Though some further optimization is required, the enzymatic process described here, might be an interesting alternative to produce those fatty acids in a water environment in much shorter reaction times and without the need of toxic solvents. Moreover, it should be investigated if the specificity of certain glucosidases can be an interesting feature for particular fatty acid release from more complex glycolipid mixtures.

3.3 Kinetic parameters on sophorolipids and glucolipids

To further characterize the production of glucolipids from sophorolipids on the one hand and that of specialty fatty acids from glucolipids on the other hand, the

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kinetic parameters of the naringinase reaction were determined under optimized reaction conditions for both substrates. Since glucolipids are not soluble in phosphate buffer at pH 3.0, 1.5% Tween-20 was added. Though naringinase activity towards substrates in suspension was mentioned in the literature [10], the addition of Tween-20 resulted in a 166 % increase in activity towards glucolipids. Hanes-Wolff plots were drawn for the naringinase reaction on sophorolipids and glucolipids (Figure 3) and the kinetic parameters were derived. As to production of glucolipids from sophorolipids, the Michaelis constant K_m is 1.67 mM sophorolipids while V_{max} is 0.035 mM sophorolipids/min for 1 mg naringinase. As to the production of specialty fatty acids from glucolipids, K_m is 11 mM glucolipids while V_{max} is 0.21 mM glucolipids/min for 1 mg naringinase preparation. This higher K_m value for glucolipids as compared to sophorolipids, might explain why the rate of glucolipid hydrolysis is much higher at pH values near the optimum (pH 3.0) as compared to pH 7.0.

Incubating the naringinase with β-NPG in the presence of increasing glucose concentrations, reveals that the glucosidase activity is competitively inhibited by glucose with K_I 10-15 mM glucose as derived from the Dixon plot (results not shown). To maximize the efficiency of glucosidase activity from naringinase reactions on either sophorolipids or glucolipids, substrate concentrations must best be limited to 10 mM.

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3.4 Physico-chemical characterization of glucolipids as compared to sophorolipids

To better estimate the possible applications of the produced glucolipids as such, some physico-chemical characteristics were determined and compared to those of acidic sophorolipids. In general, glucolipids behave similar to acidic sophorolipids with respect to surface tension lowering capacity, solvent solubility and foaming capacity. The critical micelle concentration (CMC) was determined by means of the Wilhelmy plate method on a dilution series in ultrapure water and revealed the CMC for glucolipids to be 160 mg/L while that of the acidic sophorolipids was 80 mg/L. Hereby glucolipids reduced the surface tension of water from 72 mN/m to 40 mN/m which is comparable to the surface tension lowering capacities of acidic sophorolipids (decrease to 42 mM/m). These results are in accordance with earlier reported studies [22] and indicate that glucolipids are less efficient surfactants as compared to sophorolipids.

Since the most interesting application of glucolipids might be their use as a substrate for other (chemo)-enzymatic modifications, their solubility in different solvents is an important item. Since the magnitude of the polar carbohydrate head is only half of that of sophorolipids, it was assumed that glucolipids would show decreased solubility in polar solvents as compared to acidic sophorolipids. However, similar to acidic sophorolipids, glucolipids were soluble in ultrapure water at pH 7.0 , in watery solutions at neutral or basic pH or in highly polar solvents such as DMSO (Polarity Index = 7.2). To increase solubility in acidic environments, tween-20 can be added with the concentration to be added being

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proportional to the acidity of the solution. Solubility in ethanol (Polarity Index = 5.2) was weak but increased upon heating while no solubility could be obtained in solvents with lower polarity indices such as isopropanol (3.9) or chloroform (4.1). No proper model exists for calculating the HLB value of ionic surfactants of this type, but based on the results mentioned above, one can expect glucolipids to have a comparable apparent HLB value as sophorolipids (12-13), indicating their usefulness as detergent or solubilizer but not as emulsifier as was confirmed by their inability to emulsify hexane in water.

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4 CONCLUDING REMARKS

In this paper we describe the optimization of glucolipid production from sophorolipids by *P. decumbens* naringinase and we show that the enzyme has hydrolytic activity towards glucolipids as well. Optimum reaction conditions for glucolipid production are found to be pH 7.0 and 50°C. Under these conditions, an 80% (w/w) yield of glucolipids is obtained after only 3h of incubation while no significant fatty acid formation is observed. Lowering the pH of the reaction mixture results in an increase in glucolipid hydrolysis. At pH 3.0, which is the optimum pH for the glucosidase activity, glucolipids are immediately further hydrolyzed and are completely converted to fatty acids after 24h of incubation. Since the activity towards glucolipids is immediately detected after addition of the naringinase, it is assumed that complete hydrolysis is reached much earlier though no samples were taken between 4h and 24h of incubation time to confirm this. The activity of naringinase towards glucolipids is never described before and offers an interesting biocatalytic alternative for the conventional acidic alcoholysis of glycolipids to produce specialty fatty acids. This chemical process requires hazardous solvents and rather long reaction times at elevated temperatures. Though some further optimization might be necessary, we believe that this enzymatic process can reach complete hydrolysis of the glycolipids in a water environment in much shorter reaction times, reducing energy costs for heating and moreover avoiding the removal of the toxic alcohols afterwards.

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The naringinase shows a K_m of 1.67 mM for sophorolipids and a K_m of 11 mM for glucolipids while the maximal reaction velocities V_{max} are 0.035 mM sophorolipids/min and 0.21 mM glucolipids/min for 1 mg naringinase. Glucose is inhibiting the glucosidase activity in a competitive way with K_I around 10-15 mM glucose. Surfactant properties of glucolipids are comparable with those of the acidic sophorolipids, though glucolipids are slightly less efficient in lowering the surface active tension of water (CMC glucolipids = 160 mg/L, CMC sophorolipids = 80 mg/L). The most promising application of glucolipids however is their use as a substrate for further enzymatic modifications, leading to new-to-nature biosurfactants with better performances. For example, the activity of glycosyltransferases towards glucolipids might give rise to unique glycolipids with unusual carbohydrate head groups showing increased biological activity or decreased cytotoxicity. The good solubility of glucolipids in watery solutions therefore is a positive feature.

Since consumers attach more and more importance to the ecologically sound and overall low toxicity of the products they use, the attention to naturally derived surfactants is expected to further increase in the near future. Biological ways to modify the structure of these biosurfactants in order to increase their performances, will therefore gain more attention too. In view of this, glucolipids are interesting starting molecules that open a way to the enzymatic synthesis of interesting new-to-nature biosurfactants.

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5 REFERENCES

[1] Desai, J.D., Banat, I.M., Microbial production of surfactants and their commercial potential. *Microbiol. Mol. Biol. Rev.* 1997, *61*, 47-64.

[2] Banat, I.M., Makkar, R.S., Cameotra, S.S., Potential commercial applications of microbial surfactants. *Appl. Microbiol. Biotechnol.* 2000, *53*, 495-508.

[3] Kitamoto, D., Isoda, H., Nakahara, T., Functions and potential applications of glycolipid biosurfactants – from energy-saving materials to gene delivery carriers. *J. Biosci. Bioeng.* 2002, *94*, 187-201.

[4] Shah, V., Doncel, G.F., Seyoum, T., Eaton, K.M. *et al.,* Sophorolipids, microbial glycolipids with anti-Human Immunodeficiency Virus and sperm-immobilizing activities. *Antimicrob. Agents Chemother.* 2005, *49*, 4093-4100.

[5] Asmer, H.-J., Lang, S., Wagner, F., Wray, V., Microbial production, structure elucidation and bioconversion of sophorose lipids. *J. Am. Oil Chem. Soc.* 1988, *65*, 1460-1466.

[6] Sarney, D.B., Vulfson, E.N., Application of enzymes to the synthesis of surfactants. *Trends Biotechnol.* 1995, *13*, 164-172.

[7] Bisht, K.S., Gross, R.A., Kaplan, D.L., Enzyme-mediated regioselective acylations of sophorolipids. *J. Org. Chem.*, 1999, *64*, 780-789.

[8] Hu, Y., Ju, L.-K., Lipase-mediated deacetylation and oligomerization of lactonic sophorolipids. *Biotechnol. Progr.* 2003, *19*, 303-311.

For Peer Review

[9] Tarahomjoo, S., Alemzadeh, I., Surfactant production by an enzymatic method. *Enzyme Microb. Technol.* 2003, *33*, 33-37.

[10] Bisht, K.S., Bhatt, S., Muppalla, K., Synthesis of glycolipid analogs via highly regioselective macrolactonization catalyzed by lipase. *Tetrahedron Lett.* 2006, *47*, 8645-8649.

[11] Van Bogaert, I.N.A., Saerens, K., De Muynck, C., Develter, D. *et al.,* Microbial production and application of sophorolipids. *Appl. Microbiol. Biotechnol.* 2007, *76*, 23-34.

[12] Shah, V., Badia, D., Ratsep, P., Sophorolipids having enhanced antibacterial activity. *Antimicrob. Agents Chemother.* 2007, *51*, 397-400.

[13] Rau, U., Heckmann, R., Wray, V., Lang, S., Enzymatic conversion of a sophorolipid into a glucose lipid. *Biotechnol. Lett.* 1999, *21*, 973-977.

[14] Rau, U., Hammen, S., Heckmann, R., Wray, V. *et al.,* Sophorolipids: a source for novel compounds. *Ind. Crop. Prod.* 2001, *13*, 85-92.

[15] Gabor, F., Pittner, F., Characterization of naringinase from Penicillium sp. *Hoppe-Seyler's Z. Physiol. Chem.* 1984, *365*, 914.

[16] Norouzian, D., Hosseinzadeh, A., Nouri Inanlou D., Moazami, N., Production and partial purification of naringinase by Penicillium decumbens PTCC 5248. *World J. Microbiol. Biotechnol.* 2000, *16*, 471-473.

[17] Puri, M., Banerjee, U.C., Production, purification, and characterization of the debittering enzyme naringinase. *Biotechnol. Adv.* 2000, *18*, 207-217.

For Peer Review

[18] Mamma, D., Kalogeris, E., Hatzinikolaou, D.G., Lekanidou, A. *et al.,* Biochemical characterization of the multi-enzyme system produced by Penicillium decumbens grown on rutin. *Food Biotechnol.* 2004, *18*, 1-18.

[19] Biermann, U., Friedt, W., Lang, S., Lühs, W. *et al.*, New syntheses with oils and fats as renewable raw materials for the chemical industry. *Angew. Chem. Int. Ed.* 2000, *39*, 2206-2224.

[20] Zerkowski, J.A., Solaiman, D.K.Y., Synthesis of polyfunctional fatty amines from sophorolipid-derived 17-Hydoxy oleic acid. *J. Amer. Oil. Chem. Soc.* 2006, *83*, 621-628.

[21] Zerkowski, J.A., Solaiman, D.K.Y., Polyhydroxy fatty acids derived from sophorolipids. *J. Amer. Oil. Chem. Soc.* 2007, *84*, 463-471.

[22] Lang, S., Brakemeier, A., Heckmann, R., Spöckner, S. *et al.* Production of native and modified sophorose lipids. *Chim. Oggi* 2000*, 18*, 76-79.

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Figure 1.

pH (A) and temperature (B) optimum for glucosidase activity of *P. decumbens* naringinase on β-NPG and sophorolipids.

Figure 2.

HPLC chromatogram of t-butylmethylether extracts from *P. decumbens* naringinase reactions on sophorolipids at pH 3.0 and pH 7.0. A) after 1h of incubation B) after 3h of incubation. The signals at 20.5 min, 23.0 min and 27.4 min correspond with deacylated acidic sophorolipids (SL) , glucolipids (GL) and fatty acids (FA) respectively.

Figure 3.

Hanes-Wolff plot for *P. decumbens* naringinase activity on sophorolipids and glucolipids respectively. Reactions were performed under optimum conditions for each process.

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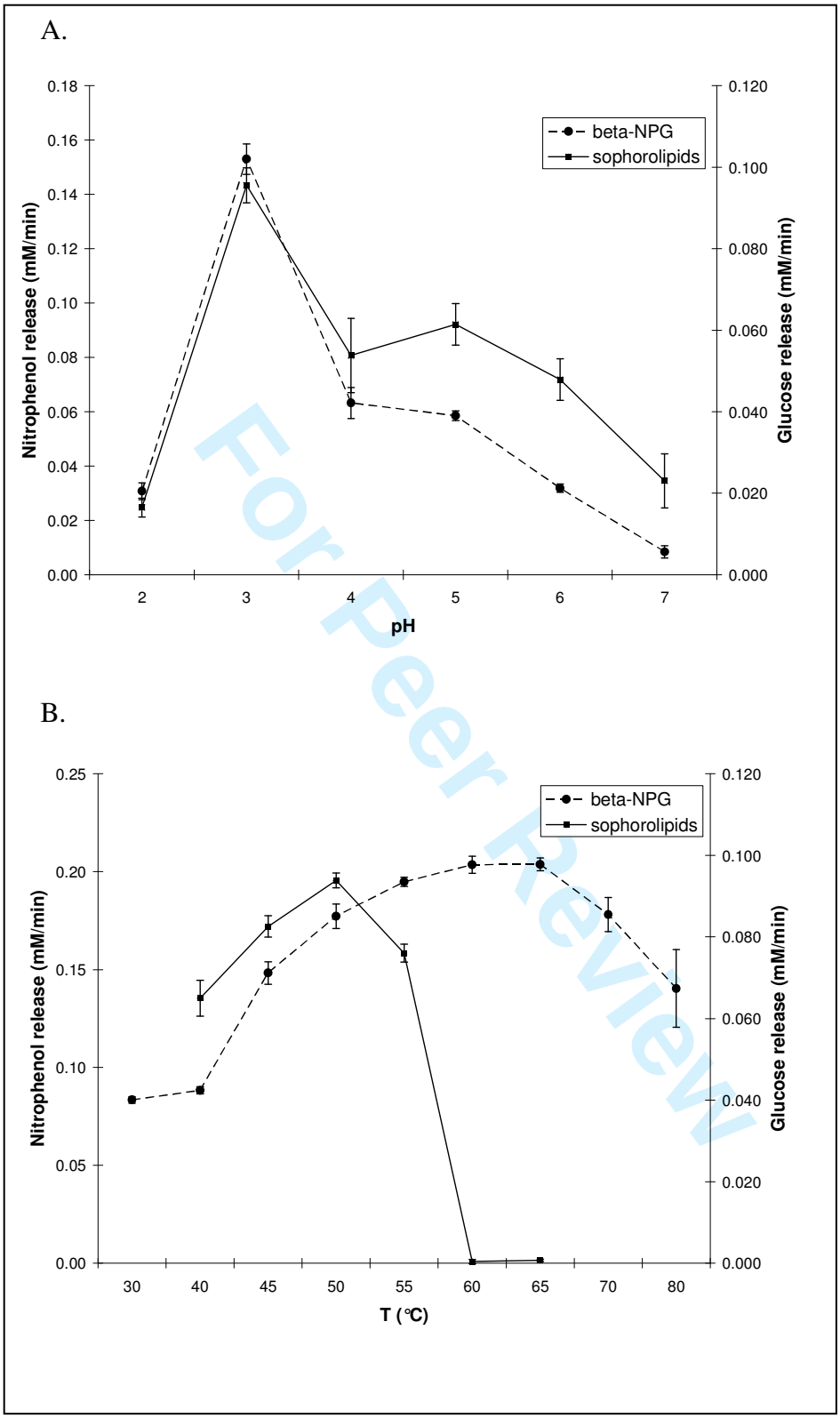
CONFLICT OF INTEREST STATEMENT

The authors have declared no conflict of interest.



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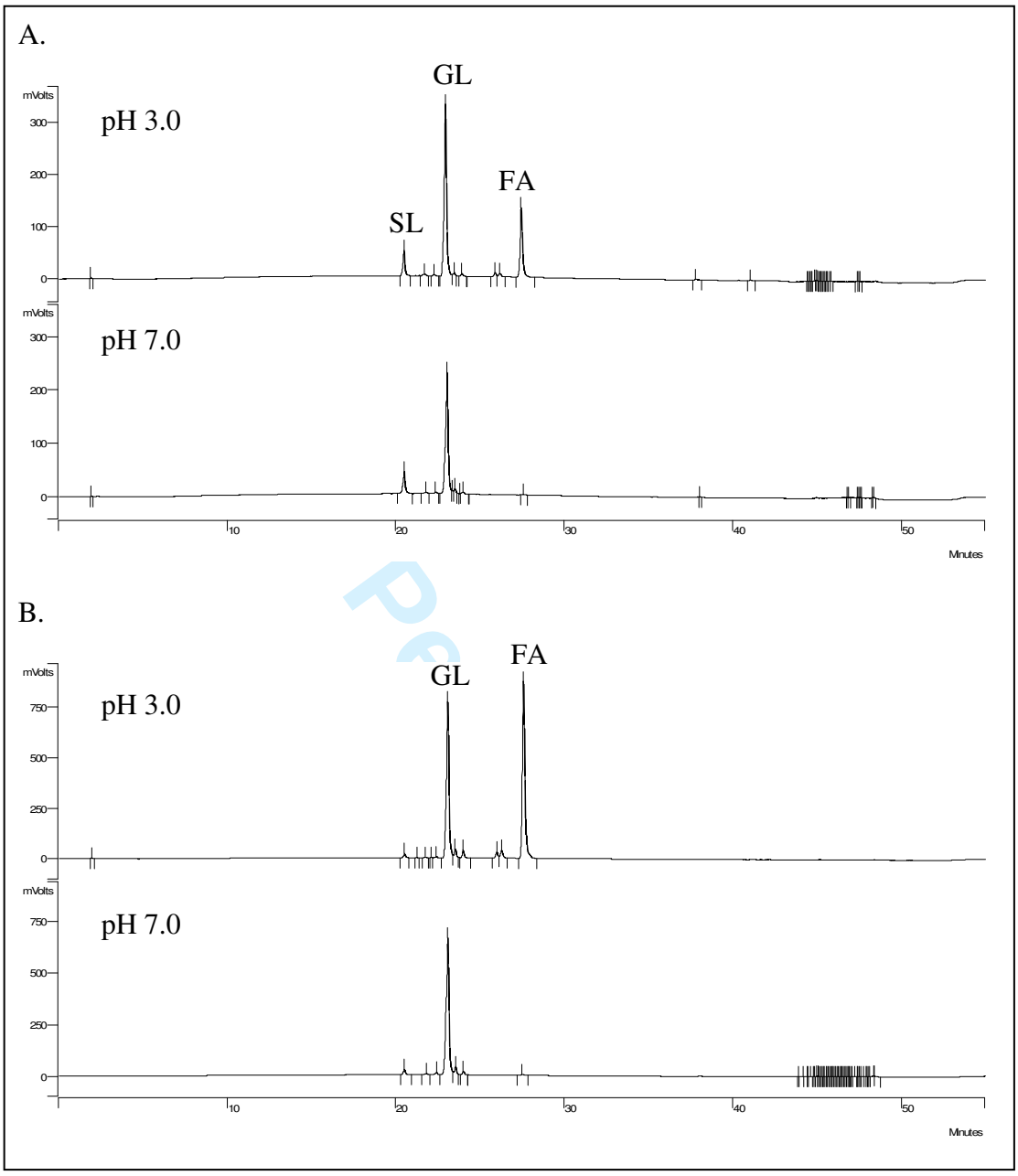
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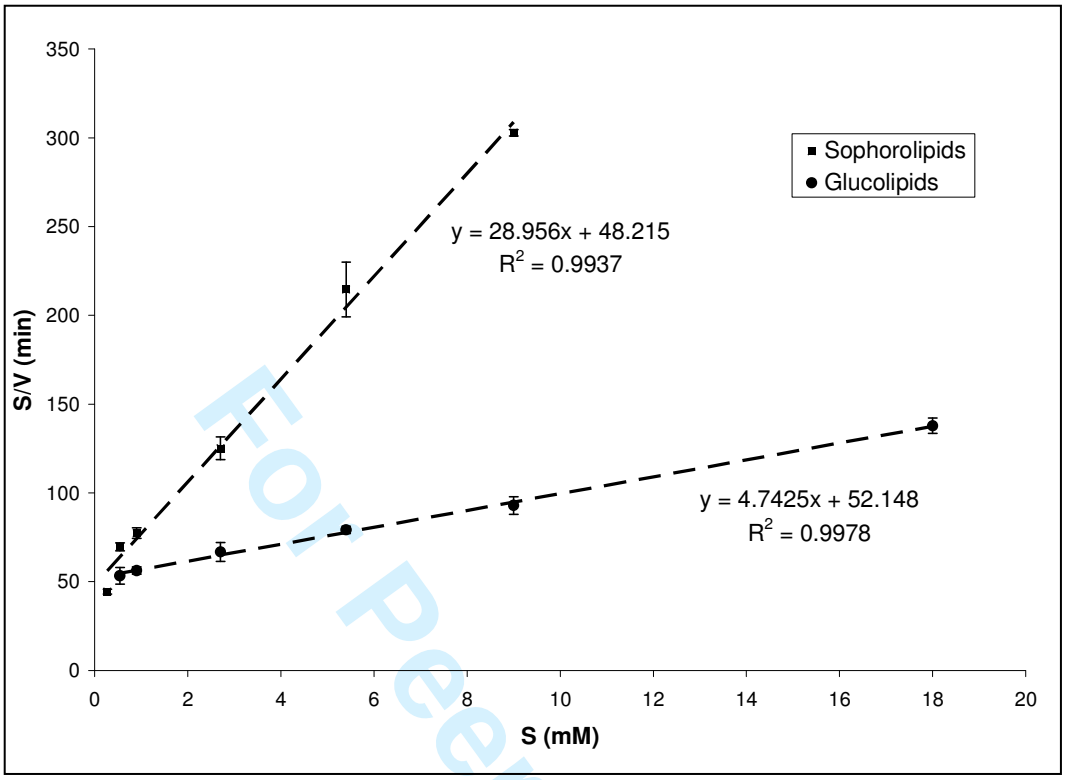

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