International Journal Publishing
INFLUENCE: International Journal of Science Review
Volume 6, No. 1, 2024
https://internationaljournal.net/index.php/influence/index

Research Article

Synthesis of hydroxamic fatty acids (FHA) from coconut oil using lipase as a catalyst

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Academic Editor: Nguyen Ngoc Anh

ABSTRACT. Social communication uses verbal and non-verbal language. We examined the degree of confidence and brain activity when verbal and facial expressions are incongruous. Fourteen healthy volunteers viewed photographs of 8 people with pleasant (smile) or unpleasant (disgust) expressions alone or combined with a verbal expression [positive/negative]. As an index of trust, subjects were asked to offer a donation when they were told that the person in the photo was in financial difficulty. Positive emotions and confidence were assessed using the visual analogue scale (VAS). Event-related potentials (ERPs) were obtained at 170–240 ms after viewing the photographs. Brain activity in incongruent conditions was localized using standardized low-resolution brain electromagnetic tomography (sLORETA). The VAS scores for the × positive smile condition were significantly higher than those for the other conditions (< 0.05). Gift giving was significantly lower for incongruence between verbal and facial expressions, particularly for the × negative smile condition. The EEG showed more activity in the parietal lobe with incongruent conditions than with congruent conditions. The incongruence [negative × smile] elicited the least positive emotion, confidence level, and supply quantity. Our results indicate that incongruent sensory information increases activity in the parietal lobe, which may be a basis for mentalizing.

Keyword: *Coconut Oil, Enzymatic Synthesis, Fatty Hydroxamic Acids, Lipase*

A. INTRODUCTION

Hydroxamic acids, hydroxamic acids, are chelating agents derived from hydroxylamines and carboxylic acids, therefore hydroxamic acids are also called N-hydroxy carboxylic amide with the general formula R-CO-NHOH. Recently, hy droxamic acid and its derivatives have received serious attention from many researchers due to their biological activities, such as growth promoters, antibiotics, antifungals (Kurzak et al. 1992), inhibitors enzymes (Anandan et al. 2007), anti-tumors (Holmes et al. 2001), prevention of iron corrosion (Deng et al. 2008) and antioxidants (Liu et al. 2008). The complex between hydroxamic acid and various metal ions is widely used for chemical analysis purposes, including as a reagent for gravimetric and spectrometric determination of metals (Pacco 2008), as a chemical sensor in the determination of metals tiny (Isha et al. 2007), as a collector of rare earth elements (Agrawal & Kaur 1999) and to extract metal ions from solution (Suhendra et al. 2005a & 2005b).

The very diverse uses of hydroxamic acids and the need for chelating agents for research and teaching purposes in the field of analytical chemistry are high, not accompanied by sufficient availability of material. Indeed, the literature review carried out shows that there have been numerous studies to synthesize hydroxamic acids from various basic ingredients, and even many that are already available in the form of commercial products. However, so far has not found a commercial product with acids, long-chain hidroksamik.

For the purposes of teaching analytical chemistry, particularly in the separation or refinement of metal ions, many chelating agents are necessary. However, in recent times the price of chemicals, especially chelating agents, is very high, so one should think about looking for chelating agents that are cheap and can be prepared from the available basic ingredients. There are two ways to separate metals or extract metals from solutions using a chelating agent, namely solvent extraction and immobilization of the chelating agent in a non-polar polymeric support. For this purpose, a chelating agent is required that has both hydrophobic and hydrophilic properties. Viewed from its functional group, hydroxamic acid is polar (hydrophilic), so medium to long chain alkyl groups must also be hydrophobic.

The main component of vegetable oil is triacylglycerol, which is a fatty acid ester with glycerol. The synthesis of hydroxamic acids using vegetable oil as a precursor was carried out from soybean oil (Servat et al. 1990), and palm olein oil (Suhendra et al. 2005a). The main component of soybean oil is linoleic acid (54%), while the main component of palm oil olein is palmitic acid which is 37% (Zamora 2005). Judging from the length of the fatty acid chain, the main components of both oils are long-chain fatty acids. Indeed, for the purpose of extracting metals in solution, these two types of oils already meet the requirements as a basic ingredient in the synthesis of hydroxamic acids, but lately the prices of these two raw materials are quite Dear. Therefore, in this study, coconut oil was used as the basis for the synthesis of the chelating agent hydroxamic acid. With consideration; a relatively cheaper feedstock, abundant availability, and its main fatty acid content is lauric acid which is 47% (Zamora 2005), which is a medium chain fatty acid.

The synthesis of hydroxamic acids can be carried out by two methods, namely chemical and enzymatic. The first way is done in an alkaline atmosphere and at a sufficiently high temperature. For the synthesis of hydroxamic acids from vegetable oils, this method is not suitable because there are constituent fatty acids in the oil that have double bonds. Under alkaline conditions and high temperatures, double bonds can be oxidized. The second path is the path that seems most appropriate. This is due to the operation of the enzymes in a neutral atmosphere and at low temperatures, in addition to this it is environmentally friendly and the enzymes used can be reused. This research focuses on the synthesis of fatty hydroxamic acids (FHA) from coconut oil which is catalyzed by the enzyme lipase.

B. METHOD

Hydroxyaminolis

The hydroxylaminolis procedure used refers to the procedure used by Suhendra et al. (2005a), with slight modification. The amount of coconut oil dissolved in hexane is reacted with hydroxylamine hydrochloride which has been neutralized with 6 N NaOH and a number of lipase enzymes in a 100 ml Erlenmeyer lid. The reaction mixture is then incubated in a water batch shaker with a shock speed of 100 rpm. The hydroxamic fatty acids (FHA) formed at the water-hexane interface are then separated from the water and the lipase by filtration. To obtain solid FHAs, the hexane fraction is cooled in the refrigerator (<-5oC) for five hours then filtered and rinsed with hexane several times to remove the remaining oil. The FHAs formed are dried in a vacuum desiccator over phosphorus pentoxide for 24 hours.

Product characterization

Qualitative analysis of hydroxamic acid groups in FHAS was carried out using FTIR spectroscopy (Perkin Elmer FTIR-Spectrum BX, USA). The composition of fatty acids in FHA was determined using high-performance liquid chromatography (HPLC) following a procedure developed by Gutnikov and Streng (1991), which was modified. The HPLC used is a product of Waters HPLC-USA which is equipped with various devices, namely the Waters Delta-600 pump, the Waters 600 controller, the Waters-2487 Dual l absorbance detector and the Shimadzu CTO column oven. 6A.

C. RESULT AND DISCUSSION

Effect of reaction time

Reaction time is an indicator of enzymatic performance. The effect of this reaction time is used as a reference to obtain the shortest reaction time with the best results. Determining the optimal reaction time also aims to minimize excessive expansion of the process (Yee et al. 1997). Figure 1 shows that the reaction time increases rapidly until the

first 30 hours. Over 30 hours the reaction does not show a significant increase, this is probably due to the formation of solids in the reaction so that there is an inhibition of mass transfer (mass transfer limitations). Another possibility is carrying out an equilibrium reaction, that is, the reaction in the direction of product formation is the same as the reaction of product decomposition so that the concentration of the product does not not change.

Effect of reaction temperature

Changes in reaction temperature can affect the activity and stability of enzymes and of course also affect the reaction rate (Mc Gilvery & Goldstein 1983). Figure 2 shows the increase in yield when increasing the temperature from 30oC to 40oC. However, when the temperature is raised to 70oC, the decrease decreases significantly. This is probably caused by the degradation (denaturation) of lipase at temperatures above 40oC.

Although the optimal reaction temperature in this study was 40 °C, but at scale, all reactions took place at room temperature 30–34 °C. The choice of room temperature is due not only to avoid the degradation of the double bonds of the product at high temperatures as well as the increase in yield between temperatures of 30 and 40oC is not great, therefore the implementation of the reaction at room temperature can save electrical energy.

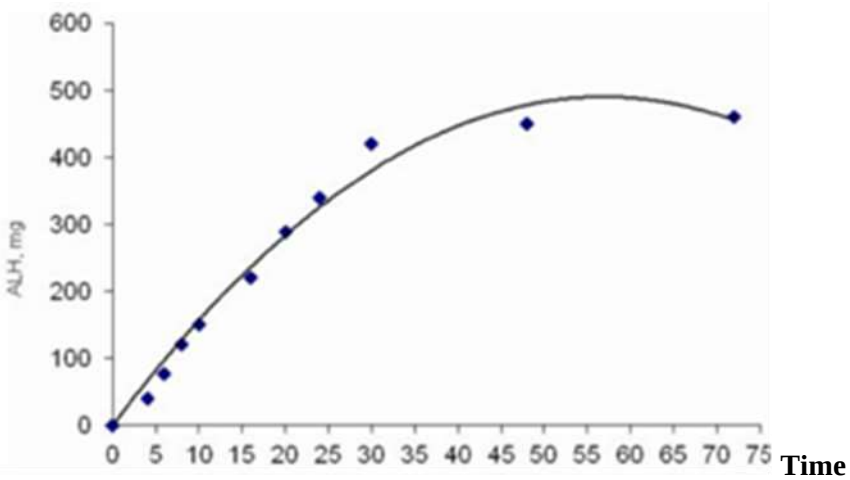
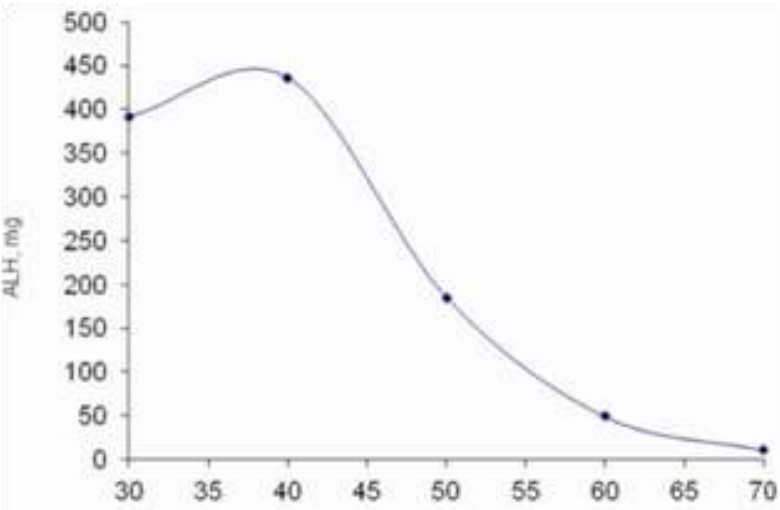


Figure 1 Effect of reaction time



Temperature

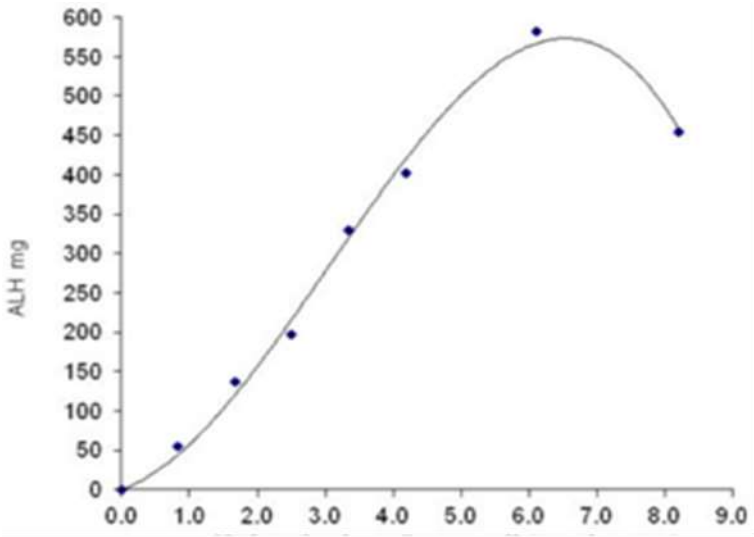
Figure 2 Effect of reaction temperature

Effect of hydroxylamine concentration

The optimal comparison of the substrate used is an important implementation in the industry (Arcos et al. 1998). Figure 3 shows an increase in the FHAS ratio with an increase in hydroxylamine concentration. These results are consistent with the results obtained by Suhendra et al. 2005, on the synthesis of FHA from palm oil. Figure 3 also shows that the higher the hydroxylamine concentration, the lower the yield. This phenomenon is consistent with the results shown by Vaysse et al. (1997), which shows the formation of inhibitors if the hydroxylamine concentration is high enough.

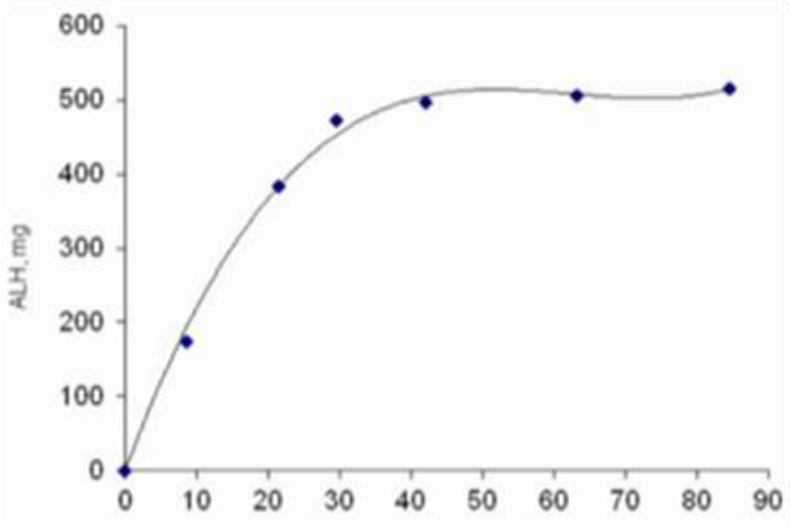
Effect of enzyme quantity

For industrial purposes, the amount of enzymes used in the reaction should be as low as possible to achieve as many results as possible. Therefore, the quantity of enzymes is very crucial to reduce production costs. The results of this study (Figure 4) show that high amounts of enzymes (enzyme:substrate ratio greater than 30) did not contribute to FHA yield.



Hydroxylamine: Coconut oil (mmol:mgram)

Figure 3 Effect of hydroxylamine concentration



[Lipozyme (mg): substrate (gram)] x 1000
Figure 4 Effect of enzyme quantity.

Characterization of FHAs. FTIR spectra

The FTIR spectrum of substrate (coconut oil) and product (FHA) is shown in Figure 5. In the spectrum of coconut oil, the strong peaks at 2848 cm-1 and 2916 cm-1 show the presence of CH stretching alkyl stretching groups. groups. This peak is supported by a peak at 1452 cm-1 which is the peak of the CH group of bending vibrations (Skoog et al. 1998). Another peak is at 1740 cm-1 which is the typical peak of C=O stretching group. The presence of OH and NH groups in the product indicates the formation of FHAS. The FHAS spectrum resembles the substrate spectrum, but the difference is clearly visible, namely the presence of OH groups marked by peaks at 3426 cm-1 which are typical areas for OH stretching and the presence of NH groups marked by peaks at 3264 cm -1 which are typical NH stretching peaks. These peaks are supported by peaks at 1662 cm-1 which are typical peaks for C=O of secondary amides.

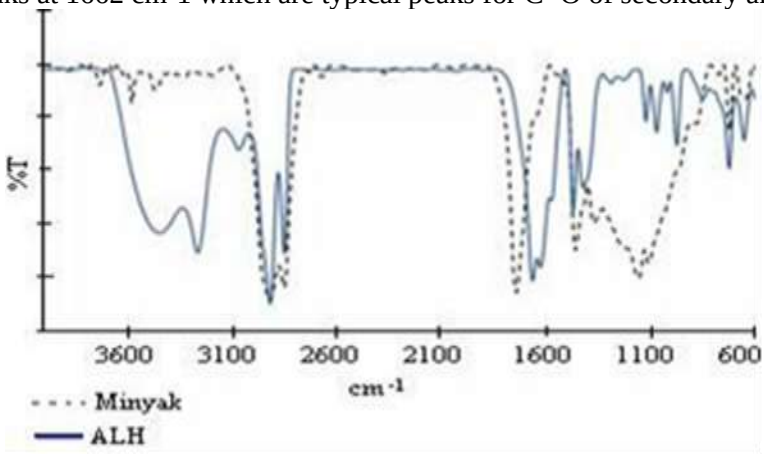


Figure 5 FTIR spectrum of coconut oil and FHAS

Figure 6 HPLC chromatogram of FHAS products

HPLC

HPLC is a currently widely used separation method. The extensive usage due to this method can be used for qualitative and quantitative analysis at the same time. Each compound in the mixture, under the desired conditions, has its own retention time (qualitative analysis) and has a signal area related to the quantity of the substance (quantitative analysis) (Meyer 1994).

The retention time (tR) of a compound is typical for that compound (Snyder & Kirkland 1979). Therefore, if the tR value of an unknown compound is known according to the tR value of a standard compound measured under the same conditions, then it is certain that the compound is the same. The composition of hydroxamic fatty acids of coconut oil is shown in Figure 6. In the photo, the composition of FHAS was compared to standard chromatograms, namely laurylic hydroxamic acid (67.5%). , hydroxyl myristic acid (20.9%), palmityl hydroxamic acid. (9.3%) and stearyl hydroxamic acid (2.3%).
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D. CONCLUSION

This research successfully synthesized FHAs from coconut oil with a commercial lipase (lipozyme) catalyst. The optimal conditions for the synthesis of FHA from coconut oil are reaction time of 30 hours, reaction temperature = room temperature (30oC), Lipozim ratio (mg): Substrate (g) = 29.5, and the Hydroclaminate (mmol): Substrate (g) ratio is 6.

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