Lynette Whelan
Editor

Oleic Acid

Production, Uses and Potential Health Effects

NOVA

Biochemistry Research Trends

BIOCHEMISTRY RESEARCH TRENDS

OLEIC ACID

PRODUCTION, USES AND POTENTIAL HEALTH EFFECTS

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PREFACE

Oleic acid is a monounsaturated fatty acid and natural constituent of a number of foods, particularly vegetable oils. On the basis of proven beneficial health effects it is also a possible ingredient in processed functional foods. However, due to its high energy content it is not recommended to increase the consumption of any particular fat, but to substitute other lipids with oleic acid. While there is a well-established consensus that replacing saturated fats in the diet with oleic acid or other unsaturated fats contributes to the maintenance of normal blood cholesterol levels, a series of other effects has also been studied, including the modulation of inflammatory markers, blood pressure, insulin sensitivity, gastrointestinal functions and even various cancers. This book discusses oleic acid's health effects, as well as its production, and how it is used.

Chapter 1 – In the current study the influence of aeration rate, inoculum size and fermentation medium volume on the sophorolipids production from the yeast *Candida bombicola* have been studied. Using the data obtained from a two-level Placket-Burman experimental design, linear and cubic models were obtained to understand the interaction amongst the ingredients. The cubic model was used to find the optimal aeration rate, inoculum size and the fermentation medium volume. The maximum production of SLs is predicted to be obtained when the medium volume is 10 mL (in 125 mL Erlenmeyer flask), is inoculated with 5% of the inoculum and incubated at 350 rpm.

Chapter 2 – Various studies in the literature suggested a link between the consumption of olive oil and different food products enriched with oleic acid (OA) and various positive health effects. The central focus of this research field is on learning and predicting how OA intake induces these health benefits. In recent years, there is a growing interest in understanding the biological role of this monounsaturated *cis* fatty acid in regulating cell membranes and its effect on biological processes. In this context, it is interesting to explore the effect of its incorporation on the model membrane characteristics and properties. These studies are considered as first steps towards a deeper understanding of the molecular mechanisms underlying OA beneficial health effects and their association with the biological membrane properties.

This chapter summarizes recent studies conducted on the influence of OA and its counterparts (saturated and *trans* fatty acids) on model lipid membranes. In particular, the main focus is to present recent investigations on the structural characterization and also the potential applications of lipidic non-lamellar self-assembled nanostructures loaded with OA. These lyotropic liquid crystalline (LLC) phases and microemulsions are attractive as drug delivery systems. The most investigated LLC phases are the inverted-type hexagonal (H_2) and the inverted-type bicontinuous cubic (V_2) nanostructures. These unique inverted type self-assembled systems are compatible, digestible, and bioadhesive matrices that are able to co-exist under equilibrium conditions with excess water. They display nanostructures closely related to those observed in biological membranes and posess interesting characteristics such as the high interfacial area (specific interfacial area up to ~400 m^2/g), the high solubilization capacities of drugs with different physicochemical properties (hydrophilic, amphiphilic, and hydrophobic molecules), and the potential of controlling drug release. In particular, there is an enormous interest in testing the possibility of utilizing these LLC phases for enhancing the solubilization of poorly water-soluble drugs, obtaining sustained drug release, and improving the *in vivo* performance of various drug substances.

The scope of this chapter also covers recent studies that have attempted to shed light on the possible fragmentation of these inverted type self-assembled nanostructures for forming nanoparticlulate formulations attractive for food and pharmaceutical applications. These nanostructured aqueous dispersions (mainly cubosomes, hexosomes, and micellar cubosomes) in which the submicron-sized dispersed particles envelope distinctive well-defined self-assembled nanostructures can be utilized in different applications owing to their low viscosity as compared to the corresponding non-dispersed bulk liquid crystalline phases and their biological relevance.

Chapter 3 – Oleic acid is a monounsaturated fatty acid and natural constituent of a number of foods, particularly vegetable oils. On the basis of proven beneficial health effects it is also a possible ingredient in processed functional foods. However, due to its high energy content it is not recommended to increase the consumption of any particular fat, but to substitute other lipids with oleic acid. While there is a well-established consensus that replacing saturated fats in the diet with oleic acid or other unsaturated fats contributes to the maintenance of normal blood cholesterol levels, a series of other effects has also been studied, including the modulation of inflammatory markers, blood pressure, insulin sensitivity, gastrointestinal functions and even various cancers. Commercial communication of such effects is only ethical where such effects are relevant to human health and proven using the highest possible standards, preferably with well-performed, double-blind, randomised, placebo-controlled human intervention trials. Most intervention studies investigating the health effects of oleic acid are performed using vegetable oils which also contain other fatty acids and minor constituents. This represents a possible confounding factor and makes interpretations difficult. In this chapter, the health effects of oleic acid are discussed together with the possibilities of using oleic-acid-related health claims on foods in commercial communications in the European Union.

Chapter 4 – Oleic acid is a monounsaturated fatty acid found in high concentrations in vegetable oils, presenting a broad number of applications in many industrial areas, such as food, pharmaceutical, cosmetic, oleochemical and biodiesel industries. Due to the lipophilicity, unsaturation and acidic characteristics that this compound presents, oleic acid can be effectively used in esterification and acidolysis, among other reactions. Recent studies have used oleic acid as an efficient substrate for synthesis of trimethylolpropane esters by esterification using lipase from *Candida Antarctica*, since this polyol ester is widely applied in hydraulic fluids with several applications. Other studies used *C. antarctica* lipase for improving the lipophilicity of bioactive molecules, such as ferulic acid and L-ascorbic acid by esterification with oleic acid, which is very interesting, taking into account that it increases the solubility of these molecules in hydrophobic environments, resulting in higher biological activities. On the other hand, some studies showed that lipases can be used to convert oleic acid into epoxies, which are useful intermediates in organic synthesis due to the high reactivity they present. They are used to produce plasticizers that increase flexibility, workability or distensibility of plastics, hence rendering them suitable for several applications. One study reported biodiesel production by esterification of oleic acid with aliphatic alcohols using immobilized *Candida antarctica* lipase, showing high yields of biodiesel (above 90%) in less than 24 h with ethanol, n-propanol and n-butanol; whereas with methanol, the enzyme was inactive after ten cycles of reaction. In addition to the various reactions involving oleic acid as a promising substrate for various reactions, oleic acid can also be used to induce microbial lipase production, as seen in a study using the fungal strain *Rhizopus arrhizus*. Therefore, different high-added-value compounds can be obtained using oleic acid as a cheap and efficient substrate for microbial lipases, which can be considered as environmentally friendly alternatives for chemical catalysts. Within this context, this chapter reviews some studies and trends on the use of oleic acid as an efficient substrate for microbial lipases.

Chapter 5 – Recently, due to inevitable exhaustion of the fossil petroleum reserves, and the environmental impact generated by the green-house effect gas emission, to develop efficient processes for the production of fuels and chemicals from the renewable feedstock has been pursued researchers in worldwide. In this sense, since the oleic acid is a common component of vegetal oils and animal fatty, it raise as a highly attractive raw material, due to its high availability and affordability. In general, the oleic acid is present in different feedstock as a free fatty acid or as glyceryl ester. Several chemicals of interest for plentiful industries can be obtained via different catalytic reactions starting from the oleic acid as source, such as alkyl esters or ethers and epoxide-derivatives. Particularly, alkyl oleate esters are useful as lubricant, surfactant, emulsifying agent, emollient, fuels additive and biodiesel. Actually, the main component of biodiesel is in general the methyl or ethyl oleate, which is manufactured by the alkaline transesterification of edible or non-edible vegetable oils via a well-established industrial process. However, the conventional alkaline homogeneous process results in large generation of effluents and residues of neutralization, in addition the laborious steps to remove the non-reusable catalyst, being because of these reasons a non-friendly environment process. In this work, the authors wish the recent advances achieved in the development of catalytic processes for the production of alkyl esters of oleic acid via acid catalysis, however, using recyclable catalysts. They will pay special attention to development of homogeneous catalysts that can be recovery and reusable without loss of activity in the oleic acid esterification reactions. These catalysts are solid when pure and soluble in the reaction being thus recovered after solvent distillation and extraction of products. Numerous industries in all parts of world have crescent demand by developing of environmentally friendly technologies for the production of biodiesel and chemicals, which are especially attractive when are based on reusable catalysts. Herein, the authors focus the use of two different sorts of catalysts: the former, Lewis acid such as tin compounds, and the second one, Brønsted acid catalysts, which are based on Keggin-type heteropolyacids. The catalysts performance it was assessed in the esterification reactions with short chain alkyl alcohols (i.e., methyl, ethyl, propyl, isopropyl and butyl alcohols).

A comparison with the traditional catalysts used in these reactions also was performed. The development of new, efficient, and environmentally benign catalytic processes that may lead to high value added products, starting of renewable raw material such as oleic acid, is still an challenge to be overcome. The authors hope that this work can significantly contribute to improvement of this important research field.

Chapter 6 – Most vegetable oils are obtained from beans or seeds, which furnish valuable and high quality oil commodities in the world oil market. Seed oil quality is related to oil percentage and fatty acid composition and defines the oil's value for industry. With emerging new markets and increased concerns about the health risks of foods, changes in the oil quality of various crops have been demanded. Plant breeders have been successful in developing novel oil types in sunflower, soybean, peanut and others with increased percentages of oleic acid. Genotype is the most important factor that defines the oil fatty acid composition, but environmental factors, particularly during the grain-filling period, can widely affect both oil content and oleic acid percentage. Various environmental factors including temperature (heat and cold, day/night differences), solar radiation, humidity, day length and moisture availability (rainfall distribution and intensity, drought or flooding) affect seed oil percentage and composition. When environmental factors deviate from the optimal quantity or intensity for the crop plant, stress is caused. Changes in both oil percentage and fatty acid composition caused by environmental stress could have a dynamic effect on the quantity and quality of oil that is extractable by seed processors. Temperature is a major environmental factor that determines the rate of oil accumulation. Generally warm temperatures during the entire growing season or a period of heat stress during grain-filling favors the production of oleic acid, while cooler temperatures favor the production of linoleic acid in traditional oil crops. However, not all genotypes are similarly affected by temperature and show strong genotype by environment interaction. Generally the novel sunflower genotypes with increased oleic acid contents display more stable oleic to linoleic acid ratios across different environments than standard types with high linoleic acid percentages. In novel soybean varieties, the high oleic acid content fluctuates with temperature differences. In order to improve oil quality in traditional oil crops, it is necessary to understand the temperature effects on oleic acid content. In addition, since agricultural and management practices can alter temperature and other important environmental factors that plants are exposed to during grain-filling, altered production practices could contribute to modified oleic acid contents in vegetable oil crops.

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Chapter 1

OPTIMIZATION OF THE MEDIA VOLUME, AERATION RATE AND INOCULUM SIZE FOR SOPHOROLIPID PRODUCTION FROM *CANDIDA BOMBICOLA* ATCC 22214

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ABSTRACT

In the current study the influence of aeration rate, inoculum size and fermentation medium volume on the sophorolipids production from the yeast *Candida bombicola* have been studied. Using the data obtained from a two-level Placket-Burman experimental design, linear and cubic models were obtained to understand the interaction amongst the ingredients. The cubic model was used to find the optimal aeration rate, inoculum size and the fermentation medium volume. The maximum production of SLs is predicted to be obtained when the medium volume is 10 mL (in 125 mL Erlenmeyer flask), is inoculated with 5% of the inoculum and incubated at 350 rpm.

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Biosurfactants have become increasingly popular in the recent times owing to their environmental friendly properties. One of the biosurfactants that is gaining attraction for its biological properties are Sophorolipids (SLs). SLs are low-molecular weight biosurfactants produced by yeasts such as *Candida bombicola, Yarrowia lipolytica, Candida apicola, and Candida bogoriensis* when grown on carbohydrates and lipophilic substrates. [1] The biological properties of the compounds include anticancer [2], antibacterial [3], antifungal [4], antiviral [5] and spermicidal activity. [6] In addition, SLs have also shown to be an effective septic shock antagonist [7,8] and have been proposed to have applications in food thickening, herbicide and pesticide formulations, consumer product manufacturing (e.g. detergents and cosmetics), and lubricant formulations. [9]

Not many studies have been published to optimize the fermentation conditions for obtaining maximum SL yields. In our recent study, we optimized the fermentation medium for the maximum production of SLs using the yeast *Candida bombicola* ATCC 22214. [8] Sixteen different media ingredients were screened and the fermentation medium composed of sucrose, malt extract, oleic acid, K_2HPO_4 and $CaCl_2$ was shown to provide the highest yield of the glycolipids. However, no physical parameters were optimized in the earlier study. Using a two-level Placket-Burman design, three physical process parameters are optimized in the current study to obtain high yields of SLs under batch fermentation. The process parameters are aeration rate, medium volume and the age of the inoculum. Aeration rate and medium volume are critical in determining the amount of oxygen transferred into the fermentation medium. Oxygen supply is important in the SL fermentation because the yeast is very sensitive to the oxygen limitation during their exponential growth phase Guilmanov et al. have carried out a detailed investigation on the influence of oxygenation on the SL production under fed-batch conditions using shake-flask method [9]. They reported that higher levels of oxygenation resulted in increased SL formation and that the oxygen transfer rate has to be between 50 and 80 m*M* O_2/L h^{-1} for obtaining high yields. The study however was carried out using an un-optimized media of glucose, yeast extract and urea, and also included a step of centrifuging the cells from the inoculum media before introducing them into the fermentation media. In our preliminary study, we found that centrifugation of cells before introducing them to the fermentation media decreases the yield of SL (data not shown). Thus, the process parameters of media volume and agitation rate were selected in the current study. As the culture flasks will be of identical size, cultures of higher medium volumes represent lower oxygenation rate and those with smaller volumes represent higher oxygenation. Higher aeration rate results in higher oxygenation rate, and smaller aeration rates results in lower oxygenation rate. Inoculum volume was selected as the third parameter because it is known that the production of SL begins only when the nitrogen in the fermentation media is depleted. [10] The inoculum size would determine how many yeast cells are introduced in the fermentation medium and hence the rate at which the nutrients are utilized.

Candida bombicola ATCC 22214 was used for SL production. The protocol described in Rispoli et al. [8] was used for Sophorolipid production. The fermentation was carried out in 125 mL Erlenmeyer flasks and the fermentation media was composed of sucrose, 125 g/L; oleic acid, 166.67 g/L; CaCl2, 2.5 g/L; K2HPO4, 1.5 g/L and malt extract 25 g/L. The amount of fermentation medium in the flask and the volume of inoculum added to the media were varied as per the experimental design described in Table 1. The flasks were incubated for 8 days at 30 ± 1.5 °C in a rotary shaker. The extraction and estimation of SLs was carried out following the protocol described earlier [8] A Plackett-Burman two-level experimental design was obtained with one block for three independent variables. Fusion Pro version 7.3.20 (S-Matrix Corp., USA) software was used to obtain the design. The obtained design is shown in Table 1. The statistical analysis of data was carried out using Statistica release 8 (StatSoft Inc., USA).

Table 1. Experimental design matrix and the obtained yields of Sophorolipids under each condition

Experiment Number	Aeration (rpm)	Media volume[a] (mL)	Inoculum (%)	SL Yield (g/L)
1	50	10	5	26.14
2	50	10	15	23.33
3	50	40	5	9.67
4	50	40	15	7.85
5	200	25	10	15.49
6	350	10	5	87.84
7	350	10	15	74.2
8	350	40	5	15.29
9	350	40	15	15.2

[a] The media volume is the final volume in the flask after addition of the inoculum.

As can be seen in Table 1, the media volume in the flask was varied from 1/10 (10 mL) of the total flask volume to 1/3 (40 mL). Similarly, the aeration was varied from 50 rpm to 350 rpm. Thus, experiment number 6 and 7 which have a volume of 10 mL and were incubated at 350 rpm receive highest oxygenation. Whereas experiment number 3 and 4 have the lowest oxygenation. SL yield indicates that the highest yield was obtained when the yeast received high amount of oxygen. When one compares the SL yield obtained in experiment 1 and 3, 6 and 8 it can be concluded that increasing the media volume decreases the production of SLs. These comparisons were carried out because between the experiments, the other two variables have same value. Comparison between experiments 1 and 6, 2 and 7, indicates that increasing aeration has a positive influence on the yield.

Table 2. Linear and cubic model obtained by analyzing the data described in Table 1

Variable	Linear model	Cubic model
	$R^2 = 0.71$	$R^2 = 0.94$
x_1	50.72	86.30
x_2	-21.53	8.13
x_3	14.74	21.79
$x_1.x_2$	-	-80.68
$x_1.x_3$	-	-35.43
$x_2.x_3$	-	-23.61
$x_1.x_2.x_3$	-	37.16

Both a linear and a cubic model were obtained using regression analysis (Table 2). The primary effect of each of the variables can be evaluated based on a liner regression model. Based on the coefficients, aeration has the highest positive influence on the yield, whereas media volume has a strong negative influence. The amount of inoculum added also has a positive influence on the production of SLs. The low fit of the linear model with the experimental data is an indication that apart from the primary effect for each independent variable, there is a high degree of interaction that is undetected by the linear model. The quadratic model result has R^2 value of 0.94. The improvement of the R^2 value from 0.70 to 0.94 is due to the two-way and three-way interaction terms incorporated into the cubic model. Interestingly, the cubic model shows that the primary effects of all the variables (including media volume) are positive and the observed overall effect for each variable is due to the interactions with other variables. The model shows that all the two-way interactions are negative. Confirmation of the interaction can be obtained from