EFFECT OF REACTION TIME AND STRENGTH OF HYDROLYZING AGENT ON ACID HYDROLYSIS OF TRIGLYCERIDES OF NATURAL ORIGIN

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ABSTRACT: *Fatty acids are naturally distributed as components of animal and vegetable fats (triglycerides of saturated fatty acids) and oils (triglycerides of unsaturated fatty acids). Fatty acids have found their many commercial applications today thus their production on commercial scale is the aim of fatty acid producing industries. The present research work is aimed to find out the suitable route to hydrolyze the triglycerides of natural sources "tallow". For this purpose meat tallow is hydrolyzed at a constant temperature of 80 °C, achieved on hotplate equipped with continuous stirrer, with strong hydrochloric acid while varying its strength from 2M to 10M and effects of acid strength on degree of release of target fatty acid has been studied. Another important parameter that remains the focus of this work is the effect of reaction time on successful release of fatty acid under the same set of reaction conditions. Different tests i.e. degree of hydrolysis, acid value, saponification value, free fatty acid value and Ester value helped to characterize the process success. These entire characterization tests agree on the point that increasing the strength of hydrolyzing acid increase the percent release of fatty acids from their triglycerides. Increasing the time duration for hydrolysis also makes it possible to achieve high yield of fatty acids from its natural source "tallow". The factor of hydrolyzing acid strength is important and a controlling factor but high yields can only be achieved at high reaction time.*

Keywords: *Triglycerides, acid strength, degree of hydrolysis, saponification value, ester value.*

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INTRODUCTION

Fatty acids are naturally distributed as components of animal and vegetable fats and oils. They are the characteristic constituent of lipids and play vital role in functions such as fluidity, flexibility and material transfer in bio-membranes. Fat or oil, can be hydrolyzed into corresponding fatty acids and glycerine. Besides their biological importance fatty acids have also revolutionized the modern industries as they are widely used in food, pharmaceuticals, soaps, detergents, lubricants, softeners, cosmetics, coatings, shoe and metal polishes, suppositories and ointments [1].

Octadecanoic acid commonly called "Stearic acid" is a saturated fatty acid obtained from animal and vegetable fats. Being chemically benign and inexpensive stearic acid finds many applications in industries and end user products such as soaps, detergents and cosmetics i.e. shampoos, shaving creams etc. Soaps are not the direct product of stearic acid; however they are indirectly obtained by saponifying the triglycerides of stea.ic acid esters, with glycol stearate, glycol distearate and ethylene glycol that produce a pearly effect in shampoos, soaps and cosmetic products. On the other hand detergents derived from stearic acid are amides and quaternary alkylammonium stearates [2].

Infect the bifunctional character of stearic acid, with a non-polar chain that conduce its solubility in non-polar solvents and a polar head group that allow the attachment of metal cations, exploit its applications in soap and cosmetic industries [3].

An important component of almost all the greases is lithium stearate. Other stearate salts, used to soften the PVC, are zinc stearate, cadmium stearate, lead stearate and calcium stearate. In textile industries to prepare textile softeners stearic acid is used along with castor oil [4].

The present research work is aimed to find out the suitable route to extract the stearic acid from its natural source "tallow". It has been a challenge for a chemist to isolate and elucidate the fatty acid structure [5]. Recently the interest in high value fatty acids has increased for the chemical and pharmaceutical industry due their unusual structural features. Nowadays oils that can provide a single fatty acid in high concentration are the main roots of modern research [6]. The basis of pharmaceutical industry actually lies on accessible fossil oil resources, which have been estimated to be extinguished in 5-10 years. Vegetable oils, in contrast to fossil oil resources, are renewable and environment friendly since CO_2 is neutral product of their thermal decomposition [7].

MATERIALS ANDMETHODS

EXTRACTION OF LIPIDS

Oven dried the washed sample at 105 °C for 30 mins then weighed accurately 150g of dried tallow in two petri dishes separately. Transferred the both samples into two 500ml round bottom flasks separately and added 300 ml of n-Hexane to each weighed sample. Equipped one of the round bottom flasks with condenser and refluxed for 60 mins at mild flame while keeping the other under same conditions but at room temperature. Then filtered the contents of both flasks immediately using wattman filter paper and let the filtrate to evaporate first at room temperature than on hotplate. Finally oven dried to remove even traces of solvent. Weighed the extracted lipids and stored at -4 °C.

ACID HYDROLYSIS OF EXTRACTED LIPIDS

Firstly weighed three samples, 20g each, of lipid extracted by n-Hexane and transferred to 250 ml Erlenmeyer flasks separately and added 100 ml of 2M, 8M and 10M HCl respectively to each sample and refluxed at 80 °C on hotplate for 1h,

1.5h, 3h, 6h and 12h. Then allowed to cool and stored at room temperature under aluminum foil. On solidifying numerous washings were given to each sample under tape water than with distilled water until washings reach neutral point. Finally the Degree of hydrolysis, Acid value, Saponification value, Free Fatty acid value and Ester Value was calculated by following the standard procedures.

CHARACTERIZATION

DEGREE OF HYDROLYSIS

Weighed 1g of hydrolyzed sample in 250 ml conical flask.

Added 5 ml of neutralized alcohol and dissolved the sample on hot plate. Added 2-3 drops of phenolphthalein indicator and noted the amount of 0.1M sodium hydroxide required to completely neutralize the hydrolyzed sample solution as performed by Serri, et at 2008 [8].

Degree of hydrolysis "X" calculated by using the following formula.

$$X\% = \frac{\text{Vol. ofNaOHXMolarityofNaOHXMr. ofRespectiveFattyacid}}{10\ \text{XWeightofthesample}}$$

ACID VALUE

1g of hydrolyzed sample was weighed in 500 ml Erlenmeyer flask and added with 100 ml of freshly neutralized alcohol. Transferred the flask to hotplate until sample was dissolved. Then added 0.5 ml of phenolphthalein indicator solution and titrated this sample against 0.1N sodium hydroxide to faint pink color which persisted for 30 seconds. Noted the volume of 0.1N NaOH required to completely neutralize the sample and determined the Acid value by using the following formula.

$$\text{AcidValue} = \frac{\text{Vol. ofNaOHXNormalityofNaOHX56.10}}{\text{Weightofthesample}}$$

SAPONIFICATION VALUE

Weighed 1g of hydrolyzed sample in 500 ml Erlenmeyer flask and added 30 ml of Ethanolic KOH and boiled under air condenser gently on hotplate for 30 mins to ensure the complete Saponification of the sample. Added 1 ml of phenolphthalein indicator after the sample was cooled and titrated against 0.2 M HCl. Using the same reagents a blank titration was carried out and saponification value calculated by using the following formula.

$$\text{SaponificationValue} = \frac{(S - B)\text{XMolarityofHClX56.10}}{\text{Weightofthesampleingrams}}$$

S= sample titre value, B = Blank titre value, 56.10 = Mr of KOH

FREE FATTY ACID VALUE

Weighed carefully 1g of hydrolyzed sample in 250 ml conical flask and added 100 ml of neutralized alcohol. Heated the mixture of hydrolyzed sample and alcohol on hotplate until it almost boiled. Then added 2-3 drops of phenolphthalein indicator solution and titrated with 0.1M NaOH till the end point, a faint pink color, reached. Noted the volume of 0.1M sodium hydroxide required to completely neutralize the hydrolyzed sample solution and calculated the FFA value of the sample by using the following formula.

$$\%\ \text{Free Fatty Acid (as Stearic acid)} = \frac{\text{T X M X 28.4}}{\text{W}}$$

T= sample titre value, M = molarity of NaOH, W = weight of sample

ESTER VALUE

Ester value was determined by subtracting Acid Value from Saponification Value.

RESULTS AND DISCUSSION

Extraction of lipids

Extraction of lipids is the key factor in determining the percentage efficacy of the process. In the present work both cold and hot solvent extractions were performed side by side. The following Table-I predicts the results of both cold and solvent hot extractions using n-Hexane as the extraction media.

Table-1 Comparative results of cold & hot solvent extractions

Sr. No.	Extraction Method	Mass of extract (g)
1	Cold extraction	15.77g
2	Hot extraction	47.21g

The present experimental data shows that hot solvent extraction yields high amounts of lipid extracts from edible tallow (animal fat) as compared to cold solvent extraction performed on the same kind of sample. However this high yield of the process is achieved at the cost of valuable energy.

ACID HYDROLYSIS OF EXTRACTED LIPIDS:

Acid hydrolysis is the process of releasing free fatty acids and glycerol from their respective triglycerides. A wide number of methods have been studied throughout the history to get commercial amounts of free fatty acids from their triglycerides. However glycerol obtained was although not the aimed goal of any hydrolysis mechanism but a valuable by product to be sold commercially.

In the present work variable concentrations of HCl (2 M, 8 M and 10 M) have been employed for varying period of time (1h, 1.5h, 3h, 6h and 12h) and results are expressed and compared in terms of Degree of hydrolysis, Acid value, Saponification value, Free Fatty acid value and Ester Value.

DEGREE OF HYDROLYSIS

Degree of hydrolysis helps to determine the percentage release of fatty acids from their triglycerides and develop a typical hydrolysis profile of respective sample. In the present work lipids extracted from edible tallow have been hydrolysed at a constant temperature of 80 °C and effect of hydrolyzing acid strength and reaction time has been studied.

EFFECT OF ACID STRENGTH ON DEGREE OF HYDROLYSIS

Effects of acid strength on degree of hydrolysis of lipids, extracted from edible tallow, at constant temperature (80 °C) and reaction time of 1h can be predicted from Table-2.

Table-2: Effects of acid strength on degree of hydrolysis

Sr. No.	Strength of HCl (M)	Degree of Hydrolysis (%)
1	2M	0.28
2	8M	4.2
3	10M	4.80

It is obvious from the results that acid strength has pronounced effects on the degree of hydrolysis of lipids, extracted from edible tallow, at constant temperature (80 °C) and reaction time of 1h [9]. Initially at the concentration of 2M HCl only a small amount of lipids were hydrolyzed by acid hydrolysis however greater acid strength resulted greater hydrolysis of lipids for the same set of conditions.

Reaction time is another key factor in determining the successful separation of fatty acids from their triglycerides [9]. Here the effect of reaction time on acid hydrolysis of edible tallow lipids has been investigated at a constant reaction temperature of 80 °C for two set of acid strengths (8M and 10M) for varying time periods i.e. 1h, 1.5h, 3h, 6h

and 12h.

Table-3: Effect of reaction time on degree of hydrolysis

Sr. No.	Strength of HCl (M)	Degree of hydrolysis of lipids (%)				
		1h	1.5h	3h	6h	12h
1	8M	4.2	5.1	6.5	22	28
2	10M	4.8	5.4	7.6	32	58

The present data shows the extent of hydrolysis achieved at various reaction times at two concentrations, 8M and 10M, of hydrolyzing agent (HCl). The results show that there is almost a linear relation between degree of hydrolysis and reaction time. The maximum degree of hydrolysis is achievable at highest reaction time [9].

Effect of reaction time on other parameters i.e. acid value, saponification value, free fatty acid value and ester value has also been investigated.

Table-4: Effect of reaction time on: Acid value, Saponification value, Free fatty acid value and Ester value for 10 M HCl

Sr. No.	Time (h)	Acid Value	Saponification Value	% Free Fatty Acid Value	Ester Value
1	1	25.01	185.91	13.82	160.90
2	1.5	31.51	185.31	17.42	153.80
3	3	44.35	186.21	24.52	141.86
4	6	60.55	186.13	33.47	125.58
5	12	122.29	186.18	67.60	63.84

CONCLUSIONS

The present acid hydrolysis of meat tallow at a constant temperature of 80 °C in the presence of hydrolyzing acid (HCl) alone at its variable concentrations of (2 M, 8 M and 10 M) have been employed for varying period of time (1h, 1.5h, 3h, 6h and 12h) and process is characterized by Degree of hydrolysis, Acid value, Saponification value, Free Fatty acid value and Ester value.These entire characterization tests agree on the point that increasing the strength of hydrolyzing acid increase the percent release of fatty acids from their triglycerides [10]. Increasing the time duration for hydrolysis also makes it possible to achieve high yields of stearic acid from its natural source "tallow" [11]. The factor of hydrolyzing acid strength is important and a controlling factor, but high yields can only be achieved at high reaction time.

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