

PROCEEDINGS

16th ASEAN Regional Symposium on Chemical Engineering

“Chemical Engineering at the
forefront of Global Challenges”

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Manila Hotel
Manila, Philippines

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The Catholic University of the Philippines

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FOREWORD

It is with great pleasure that the Chemical Engineering Department of the University of Santo Tomas (UST), on the occasion of its 75th Foundation Anniversary, hosts the 16th ASEAN Regional Symposium on Chemical Engineering (RSCE), in cooperation with the Philippine Institute of Chemical Engineers (PICHÉ) and the PICHÉ-Metro-Manila Academe Chapter. As UST prepares to celebrate its Quadricentennial in 2011, it opens its doors to the members of the Chemical Engineering Scientific and Professional Community in the ASEAN and Asia-Pacific regions in the spirit of international cooperation and collaboration in order to bring forth technological solutions to pressing global challenges. Thus, the theme of the 16th ASEAN RSCE is *"Chemical Engineering at the Forefront of Global Challenges"*.

As this is the first time that the Chemical Engineering Department is hosting an international event as prestigious as the 16th ASEAN RSCE, it is keen in keeping the Symposium as a venue for meaningful scientific and professional exchanges among its participants and ensuring that such exchanges become a catalyst of collaboration and innovation in the ASEAN Region. Therefore, in addition to the customary Technical and Poster Sessions, four fora were included in the Scientific Programme where topics that are deemed apt in addressing current regional and global challenges are tackled. It is hoped that this format will accommodate Academic, Industrial and Governmental viewpoints, both in highly scientific discussions and more informal exchanges of ideas, and more effectively synthesize sustainable solutions to pressing global challenges. This Proceeding is a testament of such exhilarating discussions and exchanges.

The Organizing Committee recognizes how the Chemical Engineering discipline is evolving into an almost basic science-like discipline on which allied fields anchor to create new branches of technological disciplines of highly specific applications. It is viewed that this evolution reiterates the fact that Chemical Engineering will continue to be a highly relevant discipline in responding to global challenges that involve science and technology. Therefore, this year, rather than dividing the Technical Sessions according to Chemical Engineering Principles, the Organizing Committee divided the Scope of the Symposium into five areas where different Chemical Engineering principles may be used to address specific issues that require technological intervention: (1) Emerging Technologies, (2) Materials Innovation, (3) Processing: State of the Art, (4) Product Innovation and Entrepreneurship, and (5) Advances in Chemical Engineering Education. More than 100 paper and poster presentations with authors from 15 different countries are showcased in the 16th ASEAN RSCE. We are grateful for their participation and the opportunity to host them. Likewise, the Organizing Committee thanks the invited speakers, Technical Session Chairs and all the attendees. The Organizing Committee also wishes to thank the 16th ASEAN RSCE sponsors, donors and exhibitors, without whose generosity, holding this event will not be possible.

The historical Manila Hotel was chosen as a venue because of its long history of catering to people and events that had significant impact to the Philippines. The 16th ASEAN RSCE, being a milestone for the UST Chemical Engineering Department, deserves to be held in a storied venue such as the Manila Hotel.



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University of Santo Tomas

December 2009

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UST History

The University of Santo Tomas (UST) is the oldest existing university in Asia and in terms of student population, the largest Catholic University in the world located in one campus. It was through the pioneering desire of Bishop Miguel de Benavides, O.P., (1550-1605), the third Archbishop of Manila, to establish an educational institution and the donation he bequeathed, that the University of Santo Tomas was founded on April 28, 1611. It was originally conceived as a school to prepare young men for the priesthood. Originally located within Intramuros, the Walled City, UST was first called Colegio de Nuestra Señora del Santisimo Rosario and was later renamed Colegio de Santo Tomas, in memory of the foremost Dominican theologian, St. Thomas Aquinas.

The University holds three distinct titles: 'Royal' granted by King Charles III of Spain in 1795, 'Pontifical' bestowed by Pope Leo XIII on September 17, 1902, and 'The Catholic University of the Philippines' conferred by Pope Pius XII in 1947.

It transferred to its present location in España, Sampaloc, Manila in 1927.



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Effect of Time and Temperature on Reflux Extraction of Testosterone from Sea Cucumber (*Holothuria scabra* J) as Source of Natural Testosterone

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ABSTRACT: Testosterone, the steroid hormone, is not only produced by sea cucumber but a lot of other organisms as well, which is used to sex reversal and aphrodisiac. This research aimed to invent extraction method, conventional (maceration, reflux and percolation), which is capable to yield highest steroid of sea cucumber extract. Raw material shown characteristic as sea cucumber which is contain water rate 88.99%, protein rate 38.96%, fat rate 4.18% and dusty rate 31.43%. Effect of temperature (40, 50 and 60°C) and time (0, 30 up to 240 minutes) on scale-up reflux extraction also studied. In scale-up reflux extraction of 3000 ml showed increasing temperature will increase sea cucumber steroid extract (%). The influence of extraction duration at temperature 40, 50 and 60°C showed result of 0.68, 0.69, and 0.84% content, respectively.

Keywords: extraction, steroid, refluxs, testosterone

INTRODUCTION

Sea cucumber, as one of marine resources, which is also known as teat fish, sandfish and sea ginseng, preconceived contain testosterone steroid, since it can increase men's vitality. High protein content (39.09%) (Anonim. 2005) as enzyme in sea cucumber is such as alkaline protease (Xue-Yan Fu *et al.* 2005a), arginine kinase, bromelain and alcase (Xue-Yan Fu *et al.* 2005b). Kustiariah (2006) successful identify testosterone's steroid from sea cucumber and applies it on chicken. Utilization of extraction as aphrodisiac on human has been done and tested on mice (Nurjanah, S. 2008). Protein as antibody can be seen from contents of the active compound, as antibacterial (Villasin and Christopher M.P. 2000, Ridzwan, B.H. *et al.* 2005, Haug T. *et al.* 2002), antifungal (Muray, A. P. *et al.* 2002) and anticoagulant (Mulloy, B. *et al.* 2000). Protein in sea cucumber has complete amino acids both essential and non-essential amino acids. Amino acid is very useful in protein synthesis in muscle and androgen hormone formation, which is testosterone functioning in reproduction process both libido enhancer and spermatozoa formation. Fat contents of fresh sea cucumber (4.35%) consist of saturated and unsaturated fatty acids (Fredalina, B. H. *et al.* 1998). Besides, there are 60 types of free sterol in sea cucumber

The potential to use sea cucumber extract as natural testosterone is promising, but the extraction method that is able to produce high purity testosterone is not achieved yet. While temperature influence and time of extraction not yet been checked. Therefore, further research of temperature influence and time of extraction are required. This paper investigates influence of temperature and extraction time of reflux extraction for the determination of natural testosterone in sea cucumbe. Reflux extraction is known from previous study as extraction method of sea cucumber that yields the highest steroid testosterone. The studied including types of solvent i.e. chloroform methanol mixture and also the ratio of substance: solvent 1:2 w/v.

MATERIAL AND METHODS

Raw material used was matured sea-cucumber (*Holothuria Scabra* J), which acquired of fisherman haul at Bengkulu Province, Indonesia. The characteristics of sea cucumbers used in the research were the same as the characteristics of the steroid producing sea cucumbers identified by Riani, *et al* (2007) and

also the same as sea cucumbers used by Kustiariah (2005) and Nurjanah (2008).

Equipments used in this research is instrumentals of raw material preparation, namely grinder tool, dryer, weighing digital (AB's Toledo Mettle 204 s), chemical material for proximate analysis. Chemicals to identify steroid, chemicals at color test (acetate anhydrate, condensed H₂SO₄), equipment of conventionally extraction, centrifuge and vacuum evaporator and the quantitative test use spectrophotometer UV-VIS,

The sea cucumbers to be extracted were characterized on the species and age based on weight and length of sea cucumbers. This is important because the weight and length of sea cucumber determine the existence of testosterone. Testosterone exists in matured sea cucumber with weight about 200-500 g and length about 25-35 cm. A suitable sea cucumber was cleaned; the flesh was separated from the entrails, milled and then analyzed proximately

Research divided into three stages, that is 1) characterization and chemical analysis of sea-cucumber, 2) reflux extraction at scale 3000 ml and 3) qualitative and quantitative analysis of testosterone from extract result. Reflux extraction by using methanol chloroform solvent 1:2 v/v and ratio of substance: solvent 1:2 b/v are used at this stage, because as shown in the previous studied, the condition of this extraction yield the highest testosterone. This step intent to determine temperature and extraction time which gives the highest testosterone. Sea cucumber flesh fresh which already been milled is weighed as much 1000 g, entered into extractor, added by solvent as much 2000 ml. Extraction conducted at various temperature (40, 50 and 60°C) and extraction time (30, 60, 90, 120, 150, 180, 210 and 240 minutes). Squealer remains to be done during extraction, to increase contact between material and solvent. Result of extraction is taken each 30 minutes as much 10 ml, then centrifuge, and dissociated between liquid phase/supernatant and precipitant/residue. Supernatant evaporated by using rotary vacuum evaporator until all solvent condense and then weighed.

RESULTS AND DISCUSSION

Chemical Analysis of Raw Sea Cucumber (*Holothuria scabra* J)

Chemical analysis of the sea cucumbers used to determine water content, fat, protein and ash of the fresh and the powder of the sea cucumbers as raw material. Results of the analysis are

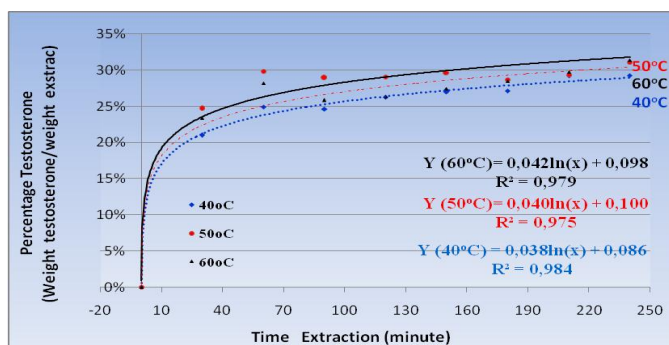


Figure 1. Testosterone weight of reflux extraction, scale 3000 ml

shown in Table 1. Highest ash content (31.43% of fresh and 42.43% of the powder) of the sea cucumbers was due to the lime covering the whole body of the sea cucumbers. Sea cucumber is one of the animals with spikes on the skin or *Echinoderm*. The spikes are distributed in epidermis layer which are microscopic lime particles

Table 1. Proximate Analysis Results

Component	Result of a fresh sea cucumber	Result of the powder of the sea cucumbers
Water (%)	88,99	10,81 ± 2,01
Ash (%)	31,43	42,43 ± 4,24
Fat (%)	4,18	1,83 ± 0,27
Protein (%)	38,96	29,81 ± 0,29

Conventional Extraction of Sea Cucumber

Further testing use of chloroform solvent equal to 7.9547 mg did not cause significant difference compare to mixture of methanol-chloroform solvent which is equal to 7.6142 mg. While influence of ratio showed that when more solvent was used, testosterone extraction was higher, however ratio 1:2 not significance differ from ratio 1:3. Extraction by reflux show highest result obtained by using chloroform solvent, then mixture methanol-chloroform, acetone and lowest by methanol, respectively.

Influence of Temperature

Sea cucumber extraction in larger scale is done bases on method, solvent and ratio of material and solvent, which chosen at previous step, namely reflux extraction method by chloroform methanol solvent and ratio of material vs. solvent is 1:2 (w/v). Hereafter conducted chosen temperature and extraction time which can yield highest testosterone, namely 40, 50 and 60°C, at extraction up to 4 hours (240 minutes). Instillation or Warm-Up on extraction reflux constitutes very assistive condition process. Temperature step-up will increase solubility, so that dissolve quicker testosterones.

Temperature influence to testosterone weight shows that extraction temperature step-up of 40°C until 60°C increase testosterones weight. This result figures that temperature step-up will increase solubility and diffusivities on extraction; in line with Aguilera (2003) one that declares that extraction constitutes solvent synergist work through diffusion process of solvent and solubility, whether through cell network as well as through capillary. Testosterone weight can be seen on Figure 1. Result of equation point out extraction temperature step-up of 40°C as 50°C will increase average weight testosterone from 6,349 becoming 7,905 mg/100 g dry weight (dw) of fresh sea-

cucumber, but then succeeding temperature step-up give weight testosterone which not differ (7,746 mg/100 g dw). This result in line with theory expressing that temperature have an effect to extraction rate, where the higher temperature, extraction result gets to increase. But the improvement limited by level of solvent evaporation (Tzia, C. and George L. 2003).

Besides amount of high testosterone become base of election of extraction temperature, it is important to know testosterone percentage, namely percentage of testosterone weight to crude extract weight which obtained by effect of distinctive extraction temperature. Extraction temperature step-up (40, 50 and 60°C) will raise level of testosterone percentage (testosterone percentage) alternately 0,689%, 0,692% and 0,776%

Duration of extraction also influences weight and percentage of obtained testosterone, namely the longer extraction, testosterone weight and percentage progressively increases. Increasingly temperature, on same duration of extraction, the weight and percentage of testosterones also becoming increases until temperature 50°C, but that step-up not happening on temperature 60°C. Percentage of testosterone obtained visible in detail at Figure 2

Temperature step-up also influence on extraction time. This observational result point out increasingly temperature, extraction time progressively shortens.

Temperature step-up of 40 to 50°C in result testosterones weight ± 6,347 taking a short cut extraction time from 240 minute become 120 minute. The small difference testosterone percentage due to extraction temperature step-up not only increase testosterone solubility, but also increases other component solubility. It can be seen on extracts yielding step-up.

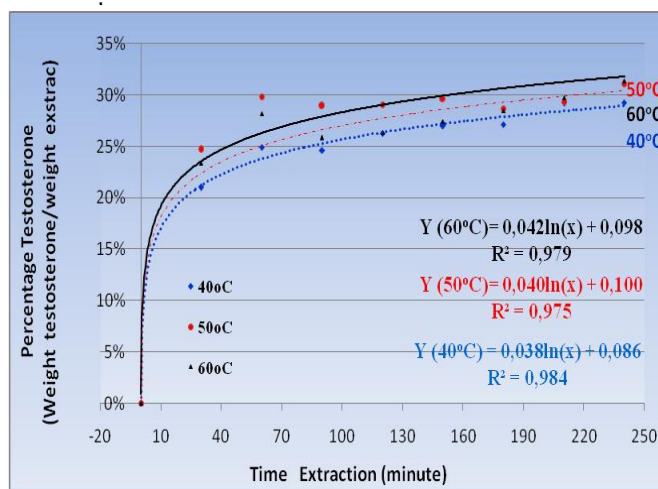


Figure 2. Testosterone percentage at various temperature of extraction

CONCLUSIONS

Study of reflux extraction of scale 3000 ml has been conducted. From this research we had some conclusion:

- Temperature that results highest testosterone is 50°C. Temperature step-up of 40 to 50°C increase testosterone weight of 6.349 to 7.905 mg/100 g dw fresh sea cucumber, meanwhile succeeding temperature step-up doesn't point out testosterones weight significance difference. But then, temperature step-up (40, 50 and 60°C) not influential to testosterones percentages (testosterone weight/ weight extracts crude) namely equal to 0,689, 0,692 and 0,776%

b. The highest testosterone found at 180 minute on all extraction temperature. Succeeding extraction time step-up doesn't point out testosterone's weight difference. Progressively increasing of extraction temperature (40 to 50°C), extraction time required for same result of testosterone's weight (6.349 mg 100 g dw⁻¹ fresh sea cucumber), progressively shorten (from 240 becoming 120 minutes).

SUGGESTIONS

Need further research in larger scale (pilot plant), so that the extraction conditions are close to industrial production scale.

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CERTIFICATE

This is to certify that

Kurnia Harlina Dewi

has participated as

**presenter, POSTER SESSION 4 : " Comparison of different extraction techniques for isolation of testosterone from sea cucumber
(*Holothuria scabra* J)"**

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