
EFFECT OF DRYING PROCESSES ON BIOCHEMICAL CONTENTS OF *SPIRULINA PLATENSIS* AS A PROTIEN SOURCE FOR FISH DIET

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ABSTRACT

The present work aimed at studying the effect of drying temperature on the biochemical contents of *Spirulina platensis* to choose the best temperature that can be used for drying without affecting its contents to be used as a fish diet, with a good quality, low price and to give the immunity to fish in fish farms. Open air and temperatures (60°C and 120°C) were applied for drying *Spirulina platensis*. The alga was cultivated on large scale in concrete ponds (25 and 50 m³) to be used as fish meal. The crude protein content of the dried matter in *S. platensis* biomass was 59.1, 63.2, and 64.8% in case of open air, 60°C and 120°C respectively. Also there are no significant differences measured of amino acids content on open air, 60 °C and 120 °C. The nutritional value of protein is due to its amino acids contents which were of 49 %, 49% and 50% for the dried matter at open air and 60°C and 120°C respectively. Also, the lipid contents, carbohydrates, fibers and ash were 0.74%, 20.78%, 0.18% and 8.8% respectively in open air, 0.78%, 18.9%, 0.22% and 8.8% at 60°C and 0.94%, 19.74%, 0.12% and 9.0% respectively at 120 °C. The results revealed that the concentrations of chemical contents (lipid, carbohydrates, fibers and ash) were higher at 120 °C than those at the other two procedures. The concentrations of crude protein (64.8 %) and amino acids (50%) were also higher at 120 °C. It was found that there is no difference between the three procedures of drying but drying at 120 °C saves time and therefore it is better to be used. Sixteen weeks of feeding was conducted to investigate the effect of fish meal replacement with *Spirulina* on

growth performance and body weight. About 20% increase of weight gain and with highly significant average daily gain 0.450 and 0.546 for diet1 (control) and diet 2 (*Spirulina* diet) respectively and feed conversion ratio were 1.189 and 1.333 for diet1 (control) and diet 2 (*Spirulina* diet). The use of the harvested alga *S. platensis* was to solve the problem of availability as protein source not only as fish feed but also saving aquatic environment.

INTRODUCTION

Spirulina (Arthrospira) plate 1 is a filamentous cyanobacterium easily cultivated and cheaply recovered by filtration from the medium. *Spirulina* is an attractive source of protein, carbohydrates, lipids, chlorophyll, vitamins, amino acids and pigments that it can be used in food, feed, pharmaceutical use and cosmetic industries. *Spirulina* contains about 60% highly digestible protein, essential and non-essential amino acid, more beta carotene than any other whole food and it is rich in vitamin B, minerals, trace elements and enzymes. *Spirulina* is one of the most frequently used microalgae in aquatic animal feeds due to its high contents of protein, vitamins, essential amino acids, minerals, essential fatty acids and antioxidant pigments such as carotenoids (Nakagawa and Montgomery, 2007). High protein content of *Spirulina* as well as its well- balanced amino acid profile compared with other plant protein sources makes it as potential fish meal replacer in aquafeed formulation (Hanel *et al.* 2007)

Spirulina platensis is needed to be used on a large scale for commercial source of nutrients due to its high contents of amino acids, vitamins and high protein. Therefore it is needed to produce *S. platensis* as supplementary feed for fish. Zarrouk (1966), Costa *et al.* (2001), Soletto *et al.* (2004) and Feng

and Wu (2006) were used different utilization purposes with different ratio of commercial nitrogen sources (urea, ammonium sulphate, human urine and nitrate) for *Spirulina platensis* production. Also El-Gamal (1998) produced protein and carbohydrates from *Spirulina platensis* cheaply cultivated on organic wastes supplemented by organic substrate molasses which stimulate the growth of this alga.

A great deal of researches has gone into methods of protecting the nutrients quality during the drying process. Freeze drying is the best way of drying but it is so expensive and complicated. Sun drying is the most popular among small producers, but requires a few precautions. Helene and Fabiola (2004) used a different process of drying as convective drying, freeze drying, infrared drying and spray drying. The biochemical and physical criteria of *Spirulina platensis* were studied and concluded that freeze drying showed the high retention of the analyzable protein and sugars. Abou El-Kheir *et al.* (2008) dried *Spirulina* in open air for 48 hours in shade and average temperature of 30-35 °C and produced *Spirulina platensis* powder with 61 % protein and 5.28 g/10g amino acids. Jenny *et al.* (1997) the effect of three drying methods sun-drying, oven-drying and freeze-drying on nutritional composition of seaweed *Sargassum hemiphyllum* were studied and concluded that sun-dried seaweed has lower values of ash, mineral and total vitamin C contents than the other procedures. It was mentioned that this might be due to the leaching effect and long exposure time to air drying (sun-drying) although oven-dried seaweeds have the greatest nutrient losses, probably mainly due to

the effect of high temperature during drying where it contained the highest mineral content.

Hilal and Ozlem (2014) reported another process for drying *Spirulina platensis* which was cultivated on chicken manure (by adding bicarbonate and urea). The biomass was dried at 50 °C for three days in incubator and the yield was preserved in the refrigerator also second drying process was applied at 65 °C for 45 min.

Guo Yao (2012) studied the effect of different drying process on the quality of *Spirulina platensis*. Four approaches of drying process, including microwave drying, freeze drying and hot air drying were investigated by measuring organoleptic qualities. The results indicated that freeze drying produce a green powder with strong fishly smell similar to that of the used fresh alga.

Aji and Mohamad (2012) concluded that the higher operation temperature, the faster drying time, at thickness of *Spirulina platensis* mass ranging from 1-3 mm and operational temperature below 70 °C. The quality of *Spirulina platensis* can fit the market requirements at 60-70 °C for drying time about 30-40 min. Tiburcio (2007) applied three low cost drying methods (sun and oven) to produce *Spirulina platensis* powder of optimal quality.

Phycocyanin is a natural blue pigment used in food and pharmaceutical industry and *Spirulina platensis* is an excellent source of phycocyanin. The protein fraction may contain up to 20% of phycocyanin which extracted from *Spirulina platensis* oven dried biomass (Vonshake, 1997 and Wanida *et al.* 2014). Srada *et al.* (1999) studied a number of drying methods (crossflow

dried, spray dried and oven dried) for *Spirulina platensis* which resulted in approximately 50% loss of phycocyanin.

The main objective of this work is to study the effect of temperature for drying *Spirulina platensis* to choose the best temperature that saves its biochemical contents so that it can be used as fish diet in aquaculture.

MATERIAL AND METHODS

Strain: In the present study *Spirulina platensis* strain as shown in plate 1 was obtained from stock available in National institute of oceanography and fisheries, Hydrobiology department, Cairo, Egypt.

Culture condition: Experiments were done in open air under green house at Wadi El-Natroun area, Keram fish farm, Behira governerate, Egypt. Modified Zarrouk's medium (Abou ElKheir, *et al.* 2008) was used as a basic culture medium which prepared by dissolving the following major salts in 1 m³ underground water:

10.0 kg commercial NaHCO₃, 250 g Urea, 50 ml H₃PO₄, 0.5 kg K₂SO₄, 1.0 kg NaCl, 200 g MgSO₄. and 10 g FeSO₄ (all chemicals are of commercial grade)

Procedure: The experiments were carried out in concrete ponds containing 25m³ and 50m³ (plates 3 and 4) of modified Zarrouk medium. 3% (v/v) inoculum of stock *Spirulina platensis* was added i.e for 1m³ medium 30 L inoculum stock of *Spirulina platensis* was added.

The measured temperature values were found to be between 25 °C and 32 °C and the pH values were found to be between 8.5 and 9.5 in the cultivation medium.

The biomass of *S. platensis* as represented by µg/L chlorophyll-a was measured daily by Eureka device Hydrolab Manta 2 water quality multiprob as shown in plate 2.

Harvest: The alga was harvested after 12 day of growth from the medium solution by plankton cloth of 20 µm mesh diameter (Plates 5 and 6)

Drying: The harvested biomass was subjected to air drying, oven drying 60 °C and oven drying 120 °C for six hours to select the best procedure for saving the chemical contents of *S. platensis* to be used as fish diet.

Biochemical contents:

-Crude protein content was measured and calculated according to A.O.A.C. (2000) by micro-kjeldahl method.

-Phycocyanine was extracted and estimated Spectrophotometrically Seigelman and Kycia (1978)

-Carbohydrates was determined by the phenol sulphuric acid method as described by Dubois *et al.* (1993)

-Lipids and fibers were determined according to A.O.A.C. (1980).

Ash was determined according to A.O.A.C. (1984).

-Amino acids were measured according to official method of Analysis A.O.A.C. (2000)

Feeding experiments: The design of the experiment was carried out to investigate the effect of two types of diets on growth performance of Nile

tilapia. Diet 1 (control) and Spirulina diet 2 (total replacement of fish meal by dried *Spirulina platensis*) for Tilapia feed. Nile tilapia fingerlings with initial weight average 5-6g were stocked at rate of 200 fish/ pocket. The experimental period was started from 4th July 2015 to 4th November 2015 and lasted for 120 days. According to the data of body weight, the parameters Average daily gain (ADG), Weight gain (WG) and Feed conversion ratio (FCR) were calculated according to (Osman *et al.*, 2003)

RESULTS

Table 1 shows the chemical components and their concentrations in underground water used in the production of *Spirulina platensis*. The results revealed that the carbonate was below the detection limit. The concentrations of bicarbonate, calcium and magnesium were measured in mg/l and the concentrations of ammonia, nitrite, nitrate and Orthophosphate were measured in µg/l. The hardness of water (CaCO₃ and MgCO₃) was 48.1 and 29.3 mg/l respectively. The concentrations of limiting nutrients ammonia, nitrite, nitrate and orthophosphate were 282.9, 151.7, 365 and 178.2 µg/l respectively.

The chemical components for this type of water revealed that it was suitable for production of *Spirulina platensis* where all these concentrations were below the limit of production media for *Spirulina platensis*.

Table 1 The main chemical contents and their concentrations in Underground water

Content	Concentration
Carbonate mg/l	B.D.L
Bicarbonate mg/l	215
Ammonia $\mu\text{g/l}$	282.9
Nitrite $\mu\text{g/l}$	151.7
Nitrate $\mu\text{g/l}$	365.0
Orthophosphate $\mu\text{g/l}$	178.2
Calcium mg/l	48.1
Magnesium mg/l	29.3

B.D.L below detected limit

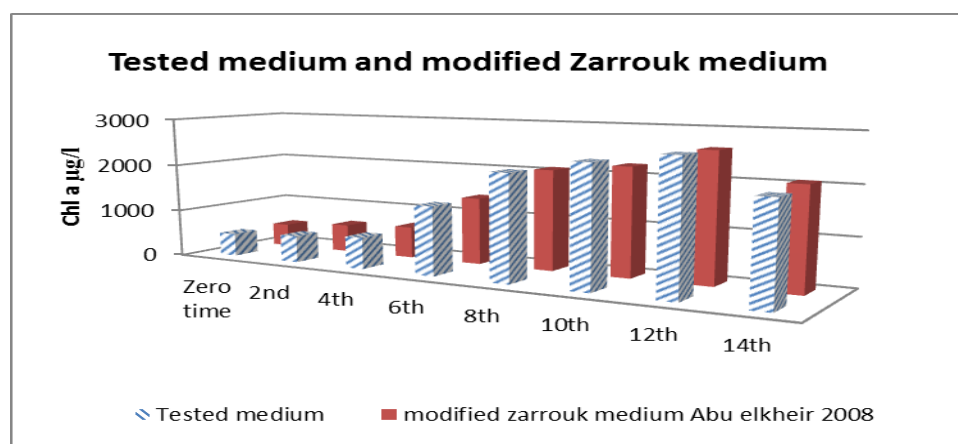


Figure 1. Concentration of Chl a $\mu\text{g/l}$ as a function of *Spirulina platensis* growth in tested medium and modified Zarrouk medium (Abou-El-kheir 2008) incubation period for 14 days

Results in Figure 1 revealed that the maximum value of chl-a was at 12th day at when harvest started.

The results presented in Figure 2 revealed that phycocyanine concentrations have the highest value of 100 % in fresh *S. platensis* and decreased with the increase of the drying temperature. In oven dried processing , the phycocyanin content was 64% and 38% at 60 °C and 120 °C respectively while in open air it was 86%. There was considerable loss of phycocyanin in the dried samples.

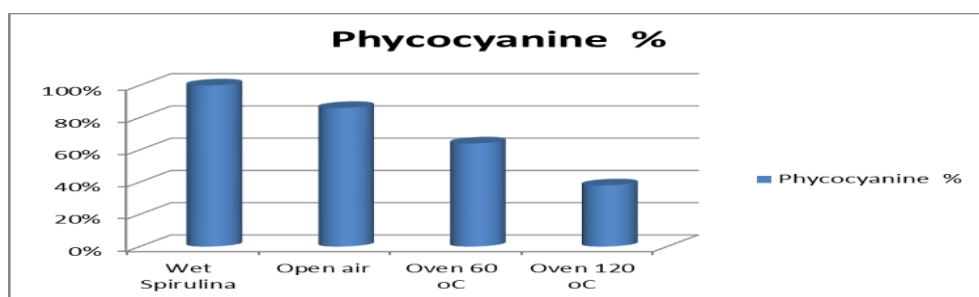


Figure 2: Effect of drying temperatures on the Phycocyanine % extracted from *S. platensis*

The biochemical contents of *S. platensis* powder (crude protein, carbohydrates, lipids, ash, fibers and moisture) were detected by percentage (%) as shown in Table 2, regarding the three drying processes.

Table 3: Biochemical contents of *S. platensis* dried in open air, oven dry 60 °C and 120 °C

Drying processes	Crude Protein %	Carbohydrates%	Lipids %	Ash %	Fibers %	Moisture %
Open air	59.1	20.78	0.74	8.8	0.18	10.4
Oven 60 oC	63.20	18.9	0.78	8.8	0.22	8.10
Oven120 oC	64.8	19.74	0.94	9.0	0.12	5.4

The results in Table 3 revealed that the crude protein were 59.1, 63.2 and 64.8 % at open air, 60 °C and 120 °C drying temperature respectively. Carbohydrates concentration 5.4 %. The protein contents slightly higher at 120 °C. Carbohydrates values were 20.78, 18.9 and 19.74 as they slightly the same percent. lipid concentrations were 0.74, 0.78 and 0.94 % at open air, 60 °C and 120 °C respectively , the results of lipids were low. The ash content % ranged from 8.8 – 9.0 for the three types of drying. The fibers contents were 0.18, 0.22 and 0.12 % at open air, 60 °C and 120 °C respectively.

The percentage of essential and non-essential amino acid contents of dried *S. platensis* in open air, 60 °C and 120 °C are shown in table 4.

Table 4 :Hydrolyzed amino acid composition of *S. platensis* dried at open air, 60 °C and 120 °C (mg/g)

Amino acid		Amino acids mg/g		
		Open air	60 oC	120 oC
Essential Amino Acids	Leucine LEU	4.22	4.19	4.34
	Isoleucine ILE	2.76	2.69	2.81
	Phenylalanine PHE	2.38	2.54	2.42
	Valine VAL	3.27	3.24	3.39
	Therionine THR	2.5	2.52	2.56
	Lysine LYS	2.21	2.19	2.22
	Histidine HIS	0.84	0.8	0.84
	Methionine	1.09	1.05	1.04
Non -Essential amino acids	Aspartic ASP	4.99	5.03	5.11
	Serine SER	2.32	2.29	2.32
	Glutamic GLU	7.61	7.67	7.98
	Proline PRO	1.91	1.76	1.92
	Glycine GLY	2.49	2.54	2.62
	Alanine ALA	4.09	4.02	3.96
	Tyrosine TYR	2.01	2.22	2.11
	Arginine ARG	3.55	3.46	3.54
	Cystine CYS	0.42	0.38	0.47
TOTAL		48.66	48.59	49.65

The results of the total amino acids contents of *S. platensis* dry matter were 48.66, 48.59 and 49.65 mg/g in open air, 60 °C and 120 °C respectively.

The results in Table 4 showed the amino acids contents % of dried *Spirulina platensis* in 120 °C was higher than that in open air or in 60 °C for all amino acids except phenylalanine, methionine, alanine, tyrosine and arginine were slightly lower. The highest values of essential amino acid were

4.22, 4.19 and 4.34% is leucine LEU in open air, 60 °C and 120 °C respectively.

The highest values of non-essential amino acid (glutamic acid) were 7.61, 7.67 and 7.98 % in open air, oven 60 °C and oven 120 °C respectively.

Table 5 :Growth performance of Nile tilapia fingerlings

Growth performance					
Diets	Initial Body weight (g)	Final Body weight (g)	WG (g)	ADG (g)	FCR
diet 1 (control)	4.763a	58.763b	54.000b	0.450b	1.189a
diet 2 (Spirulina)	5.056a	70.66a	65.603a	0.546a	1.333b
SE	0.110	0.712	0.627	0.005	0.011
Pr	0.1345	0.0003	0.0002	0.0002	0.0228

SE standard error

Pr probability

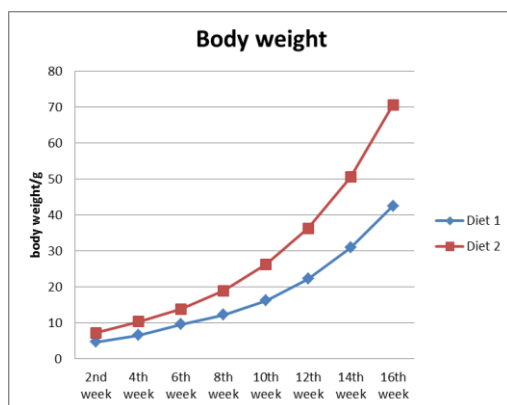


Figure 4: Body weight g/fish every 2 weeks for 16 weeks

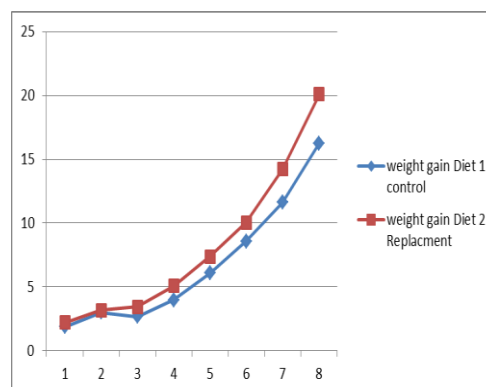


Figure 5 weight gain of the two groups fed on Diets

The growth performance of Nile tilapia fingerlings fed on the experimental diets (control diet and *Spirulina* diet) is presented in Table 5. Table 5 shows that there was highly significant growth performance for the two types of diets. Results showed that the higher value of weight gain was 65.603 for group fed on diet 2 (*Spirulina* diet) with total replacement of *Spirulina platensis* than that fed on diet 1 (control). Also with higher average daily gain of 0.546 for the group that fed on diet 2 (*Spirulina* diet) was higher than that group fed on diet 1 (control). Figures 4 and 5 show that the body weight and weight gain for the group of fish that fed on diet 2 were higher than the group fed on diet 1 (control) during the duration of the experiment.

DISCUSSION

The results of analysis in Table 1 revealed that the concentrations of the chemical components for the used underground water were suitable for production of *Spirulina platensis* where all these concentrations were below the limit of production media.

The recent researches aimed to change the source of water from distilled water as in Zarrouk medium to other available sources. There are different types of water can be used for production of *Spirulina platensis* starting from distilled water as in Zarrouk's medium (1966), fresh water as in Jorge *et al.*, medium (2003), brackish water as in Richmond medium (1988) and alkaline water as Vonshake (1978), Aly (2000) and Goldstein (1986) media, Tolga (2007) studied the growth of *Spirulina platensis* using drinking water for preparation of Zarrouk medium, Abou El-Kheir *et. al.*, (2008) used a boiled tap water for commercial production of *Spirulina platensis*, Fox (1999) and

Sandeep *et. al.*, (2015) used sea water for large scale production of *S. platensis*. All the above researches tried to adapt the growth of *S. platensis* by using to the available sources of water as cost effective.

The results presented in Table 2 revealed that phycocyanin concentration produced from the used dried *S. platensis* in open air, 60 °C and 120 °C decreased with the increase of drying temperature, taking in consideration that phycocyanine concentration of 100% was in the fresh *S. platensis* sample. Therefore, fresh biomass is the best for phycocyanin content. Srada *et. al.* (1999) studied a number of drying methods for *Spirulina platensis* (crossflow dried, spray dried and oven dried) where phycocyanin loss was approximately 50%. This results were not in agreement with the present study in case of open air dry and 60 °C where the loss of phycocyanin was 14% and 36% respectively but in agreement with that in 120 °C where the loss was 62%.

The present results Table 3 revealed that the modified medium produce *S. platensis* containing a crude protein of 59.1%, 63.2% and 64.8% in open air, 60 °C and 120 °C respectively. These results were in agreement with the results obtained by Aly and Aumar (2003) and Abou El-kheir *et. al.* (2008) who obtained a crude protein from *S. platensis* ranged from 50-70% by using modified Zarrouk's medium by replacing other nutrients such as ammonium sulphate, potassium nitrate, urine and commercial fertilizers in the medium. Due to the quality of protein produced from *Spirulina platensis*, it is used as protein source in diets with different ratios.

The valuable protein is due to its availability and proportion of amino acids (essential and non- essential). The term essential amino acid includes amino acids that are required for optimum growth of the heterotrophic organisms and the non-essential amino acids also important for growth but they can synthesize by heterotrophic organisms.

The present results showed that the values of amino acids were 49%, 49% and 50% at open air , 60°C and 120 °C respectively which was in agreement with the results obtained by Dillon and Phan (1993) who reported that essential amino acids were 47% of protein and 50% total amino acids. Also Abou El-kheir *et. al.* (2008) reported that amino acids were 52% of crude protein produced by using commercial medium. According to the used medium and its components, the change of proportion of amino acids and ratios of biochemical contents of *S. platensis* gave the high nutritional value of *Spirulina* compared with the other plant foods. The present results were in agreement with results obtained by Mephram (1997) and Abou El-kheir *et. al.* (2002) who reported that *S. platensis* was commercially productive alga which was a remarkable rich source of pigments and amino acids.

The results revealed that the growth performance of Nile tilapia fingerlings fed on diet 2 (*Spirulina* diet) with replacing *S. platensis* as a source of protein gave a highly significant increase in the weight gain and body weight with about 20% and an increase in food conversion ratio. So *Spirulina* can be used as a good replacer for fish as protein source. The results are in agreement with that obtained by Hanel *et. al.*, (2007) who concluded that due to high protein contents of *S. platensis* and its essential amino acids

makes it as potential fish meal (FM) replacer in aquafeed formulation. In the present study *S. platensis* was used as complete replacement for protein source which was in agreement with the results obtained by Dernekbası *et. al.*, (2010) and Tongsirı *et. al.*, (2010)

Plates

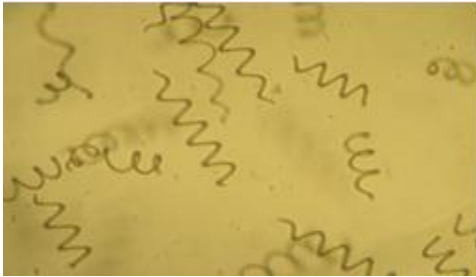
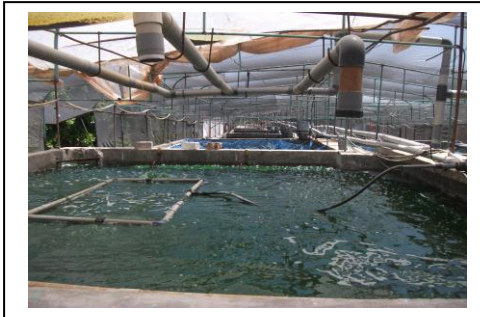


Plate 1 *Spirulina platensis* (Nords, Geitler.) (microscopic photo 40 X)



Plate 2 Manta 2 Water quality Sonde (Eureka)



25 m³



50m³

Plates (3 and 4): Cultivation of *S.platensis* on modified Zarrouk Medium in concrete ponds 25 m³ and 50m³



plates (5 and 6): The Harvested *S. platensis* from pond by 20 um mesh diameter plankton cloth

CONCLUSIONS AND RECOMMENDATIONS

From the present study the biochemical contents for *S. platensis* were the best in oven dry 120 °C for large scale production however it is difficult to be applied in a small scale (field or aquaculture production), so open air drying is effective and available procedure in field and aquacultures where some precautions must be done.

- 1- Direct open air drying must be very quick otherwise the chlorophyll a will be destroyed and the dry product will appear bluish
- 2- The drying process at 120 °C is method saving time for drying *Spirulina platensis*, and saving the biochemical contents.
- 3- The total duration of drying should not exceed few hours and drying temperature should be limited up to 120 °C
- 4- *Spirulina* is a good replacer source of protein in aquafeed culture.

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تأثير طرق التجفيف على المحتوى الكيميائي لطحلب اسبيرولينا بلاينسيس كمصدر للبروتين فى عليقة الاسماك

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المستخلص

يهدف البحث الحالى الى دراسة تأثير درجات الحرارة على المحتوى الكيميائى لطحلب اسبيرولينا بلاينسيس عند تجفيفها لاختيار افضل درجة حرارة للتجفيف دون التأثير على محتواها الكيميائى لاستخدامها كعليقة للاسماك ذات محتوى بروتينى بجودة عالية وفيتامين لتكوين عليقة اسماك بجودة عالية و تكلفة أقل من العلائق التجارية الأخرى لتعطى انتاجا أفضل ومناعة للاسماك.

تم تجفيف طحلب (اسبيرولينا بلاينسيس) فى الهواء الجوى- عند درجة حرارة ٦٠ درجة مئوية -درجة حرارة ١٢٠ درجة مئوية وتم قياس المحتوى الكيميائى للطحلب (بروتين- كربوهيدرات- دهون- الياف- رماد- احماض امينية)، تم زراعة هذا الطحلب على نطاق واسع فى احواض أسمنتية (٢٥ م^٢ و ٥٠ م^٢) لاختيار انسب طرق التجفيف.

احتوت الكتلة الحيوية من طحلب اسبيرولينا بلاينسيس على نسبة بروتين خام على الجودة من المادة الجافة وهى 59.1 ، 63.2 ، 64.8 % عند التجفيف فى الهواء، درجة حرارة ٦٠ درجة ودرجة حرارة ١٢٠ درجة على التوالي . وترجع القيمة الغذائية للبروتين على مدى احتوائية من الاحماض الامينية والتي كانت ٤٩%-٤٩% و ٥٠% من المادة الجافة عند التجفيف فى الهواء، درجة حرارة ٦٠ درجة مئوية و درجة حرارة ١٢٠ درجة مئوية على التوالي وكذلك تم تقدير نسبة الدهون - الكربوهيدرات -الياف والرماد وكانت ٠.٧٤ ، 20.78 ، ٠.١٨، ٨.٨% على التوالي عند التجفيف فى الهواء . و على ٠.٧٨ ، 18.9 ، ٠.٢٢ ، ٨.٨ %

على التوالي عند التجفيف في ٦٠ درجة مئوية. و ايضا على ٠.٩٤ ، ١٩.٧٤ ، ٠.١٢ ، ٩.٠٠% على التوالي عند التجفيف في ١٢٠ درجة مئوية. اظهرت النتائج ان المحتوى الكيميائي للمادة الجافة (دهون- كربوهيدرات - الياف و رماد) كانت أعلى عند تجفيف ١٢٠ درجة مئوية عنها عند التجفيف في الهواء او عند درجة ٦٠ درجة مئوية وكذلك كانت نسبة البروتين الخام (٦٤.٨%) والاحماض الامينية (٥٠%) عند درجة حرارة تجفيف ١٢٠ درجة مئوية. كما اظهرت النتائج انه لا يوجد فرق معنوي في تأثير درجات الحرارة على تركيز الاحماض الامينية، تم تغذية اصباغيات اسماك البلطي النيلى لمدة ١٦ أسبوع لمعرفة تأثير استبدال كامل لطحلب اسبيرولينا بلاثينسيس كمصدر للبروتين في عليقة الاسماك على معدلات النمو و الوزن الحيوى. وأظهرت النتائج زيادة ٢٠% فى الوزن تقريبا بالاضافة لوجود فرق معنوي فى النتائج على متوسط الزيادة اليومية للسمة بقيمة ٠.٤٥ جم ، ٠.٥٤٦ جم لكل من عليقة الكنترول وعليقة الاسبيرولينا على التوالي وكذلك معدل التحول ١.١٨٩ و ١.٣٣٣ لكل من عليقة الكنترول وعليقة الاسبيرولينا على التوالي، واتضح ان استخدام محصول هذا الطحلب كمصدر للبروتين فى عليقة الاسماك يوفر الحماية للبيئة المائية.