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Original Research Article

Production and Utilization of Microalgae

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Abstract

This study was conducted to culture, process and utilize the rare and exceptional nutrients from *Spirulina sp.*, a local micro-algae considered to be a primeval nutrition and complete protein food. The processed *Spirulina* was used in the enrichment of the nutrient composition of junk foods that are considered popular snacks among youngsters. The study is composed of the following: culture and propagation of *Spirulina*, processing of *Spirulina*, product formulation and fortification of snack chips and efficacy test. The pH and salinity composition of the *Spirulina* culture media was determined. Results showed that *Spirulina* thrived best in an alkaline solution with a pH level of 10 and saline solution with a concentration of 10% weight over volume. The chemical composition of fresh *Spirulina* cultured in the laboratory was analyzed. This significantly registered high crude protein 56.5%, crude fat 10.3%, crude fiber 0.5%, total ash 8.8% and moisture content 16.2%. Results of the chemical analysis showed that *Spirulina* fortified snack chips have higher crude protein of 70%, total ash of 8%, crude fiber of 1.67%, moisture content of 7.26% and lower fat of 16.66% necessary for human nutrition and as compared to unfortified snack chip which recorded a crude protein of 21.0%, a very high crude fat 29.83%; crude fiber 3.33%, moisture content 7.26% and total ash 3.16%. Preliminary study on the result of the efficacy test showed that there was greater and significant increase in weight and arm circumference of pre-schoolers fed with *Spirulina*-fortified snack chips within a short range of time than those who were fed with commercial snack chips.

Keywords

Fortified snack chips

Human nutrition

Pre-schoolers

Spirulina

Introduction

In the Philippines, almost 70% of the population is suffering from malnourishment. About 967,000 pre-schoolers are underweight and for every 2.16 seconds, a child is dying from malnutrition. Many people preferred fast conventionally grown and over-processed foods which are usually rich in fats, carbohydrates and sugar but low in nutritional value loaded with chemicals. These foods increase body weight, raise cholesterol levels and worsen the digestive system. Most school children choose junk

foods which lack essential vitamins and minerals necessary for their growth and nutritional requirements. This scenario may result to susceptibility to infections and impaired physical and mental health triggered by malnutrition and other nutrition-related diseases. To address this problem, the Department of Health embarked on a three-prong strategy of supplementary feeding nutrition education and food fortification especially to snack foods. Based on studies, food fortification is the most-effective sustainable strategy to solve malnutrition, one of the major problems in the Philippines particularly with reference to vitamin A, iron and protein deficiency (www.doh.gov.ph; Ciferri, 1983; Guevarra, 2000; Grecia, 2004). Evidently, the utilization of microbial protein for food fortification can offer a quick solution to malnutrition. *Spirulina* has been considered as the best alternative future source of food. This microalgae has been studied in many countries. However, there was a dearth of information and researches on local *Spirulina* considering the high nutritional value of *Spirulina* of this species. This research was conducted to propagate, process and utilize the local species of *Spirulina* for food to improve the growth and health of school children who are prone to diseases and malnutrition (Karyadi, 1977; Jelliffe, 1966; Javier, 2000; Pambid, 2004).

This study was conducted to propagate, process and utilize *Spirulina* with the following objectives:

1. To determine the pH level and salinity of the freshwater medium of *Spirulina*.
2. To determine the chemical composition of *Spirulina* under laboratory cultures.
3. To process snack chips fortified with food nutrients from *Spirulina*.
4. To compare the chemical composition of the snack chips before and after fortification with *Spirulina*.
5. Evaluate the effect of *Spirulina*-fortified snack chips and commercial snack chips in the health growth of pre-schoolers.

Materials and methods

Experimental design and treatments

The study used a Completely Randomized Design (CRD). In chemical characterization, four (4) treatments with 4 replicate each were used whereas in processing snack chips, two (2) treatments with three replicates were employed. In the efficacy test, two (2) treatments with 13 replicates were used.

The study consisted of the following: determination of the chemical characterization of *Spirulina* culture media, chemical composition of *Spirulina* cultured in laboratory; chemical analysis of *Spirulina*-fortified snack chips and feeding of *Spirulina* – fortified snack chips.

The treatments were:

Chemical characterization of *Spirulina* culture media

A. pH Determination

T_1 - 8
T_2 - 9
T_3 - 10
T_4 - 11

B. Salinity determination

T_1 – 5% (w/v)
T_2 – 10% (w/v)
T_3 – 15% (w/v)
T_4 – 20% (w/v)

Processing of fortified-snack chips

T_0 - commercial snack chips
T_1 - *Spirulina*-fortified snack chips

For efficacy test:

T_0 - pre-schoolers fed with commercial snack chips
T_1 - pre-schoolers fed with *Spirulina*-fortified snack chips

Laboratory culture of *Spirulina*

The medium was prepared by stirring in dried waste matter into the water. The proportion of water to waste ranged from 0.5 to 3% weight over volume (w/v). Adjustments were made to attain a pH level of 11and optimum salinity of 15 percent weight over volume (w/v). Addition of sodium bicarbonate raises the pH level while sodium chloride adjusts salinity.

Preparation of inoculum

The inoculum was cultivated in freshwater culture media under a controlled supply of oxygen (PR 7500), temperature at 30°C and light source of 6.0 k lux. The culture period from juvenile to mature *Spirulina* was from 2-3 weeks.

Harvesting, drying and storage

Harvest was done by skimming the algae scum with the use of a fine cloth or wire mesh. When thoroughly rinsed, the algae paste was spread out on a piece of cloth and laid out on a concrete to dry in the sun. When dried, the algae biomass appears as a smooth, blue-green parchment-like material. When using cloth for drying, removal of the algae cake was done by crumpling and slight rubbing either with both hands or with the use of a spatula. The end product was in the form of *Spirulina* flakes. Dried *Spirulina* was stored in brown paper bag at room temperature.

Processing of *Spirulina* and fortification of snack chips

1. About 5 kilograms macaroni chips were cleaned and placed in a casserole.
2. About 15 liters of water, a pinch of salt were added. Then stirred and drained until the macaroni became soft.
3. The macaroni was sun dried for three days.
4. The parched macaroni were deep fried.
5. About 10 g of macaroni snack chips were rolled to 22 mg of *Spirulina* powder.

Proximate chemical analysis

The chemical composition of fresh cultured *Spirulina* algae, *Spirulina* fortified macaroni snack chips were analyzed for moisture content, crude fiber, crude fat, crude protein and total ash using standard procedure.

Data gathered

The data gathered in the study were:

1. pH level
2. Salinity level
3. Moisture content
4. Crude fiber
5. Crude fat
6. Crude protein
7. Ash content

Statistical analysis

The gathered data in chemical characterization was analyzed and computed using the single-factor Analysis of Variance at 5% level of significance while the data in processing snack chips was analyzed and computed using t-test to determine the chemical composition of macaroni snack chips before and after fortification.

The mean increase in weight and arm circumference after every 15 days until the 45th day of feeding of 26 pre-schoolers randomly divided into two groups were analyzed using the single-factor Analysis of Variance (ANOVA) with two groups.

Results and discussion

Chemical characterization of *Spirulina* liquid medium

Table 1 shows the pH level of the freshwater medium where *Spirulina* can thrive best and produce optimum yield. Based from the results, pH 10 registered the greater yield of algae biomass while pH 8 gave the lowest yield. This indicates that *Spirulina* can thrive at an alkaline medium and can give an optimum yield at pH 10.

Table 1. Means of dry weight of *Spirulina* in different pH concentrations.

Treatments	Weight (kg)
T1 – pH 8	0.302 *c*
T2 – pH 9	0.46 *b*
T3 – pH 10	0.61 *a*
T4 – pH 11	0.50 *b*
Means followed by common letter are not significantly different at 5% level of significances of DMRT	

Based on Table 2, *Spirulina* can be cultured in a saline freshwater medium. Results show that the salt (sodium chloride) concentration where *Spirulina* can best produce optimum yield is at 10% weight over volume. It also indicates that as salt (sodium chloride) concentration is getting the higher, *Spirulina* biomass decreases.

Table 2. Means of dry weight of *Spirulina* in different salt (sodium chloride) concentrations.

Treatments	Weight (g)
T1 – 5% (w/v)	1.57 *b*
T2 – 10% (w/v)	2.37 *a*
T3 – 15% (w/v)	1.1 *c*
T4 – 20% (w/v)	0.87*c*
Means followed by common letter are not significantly different at 5% level of significances of DMRT.	

Chemical composition

Table 3 presents the chemical composition of the cultured *Spirulina*. It significantly registered very high crude protein, 56.5%; crude fat, 10.3%; crude fiber, 0.5%; moisture content, 16.2%; and total ash, 8.8%. This shows that *Spirulina* is a perfect protein factory. Harnessing the potential of *Spirulina* will help prevent the over exploiting of the area and agricultural land sources.

Table 3. Chemical composition of the cultured *Spirulina*.

Cultured	Crude protein %	Crude fat %	Crude fiber %	Moisture content	Total ash
Spirulina	56.5	10.3	0.5	16.7	8.8

Proximate chemical analysis of the *Spirulina* fortified and commercial snack chips

Based on Table 4, *Spirulina*-fortified snack chips registered a higher moisture content, 7.26%, crude ash, 8.0%; and remarkably high crude protein content, 70.0%. The moisture content is just ideal for processed *Spirulina* chips needed to prolong its shelf life and minimize the attack of molds. The high ash content of *Spirulina* fortified snack chips had indicated the presence of essential vitamins and minerals fitted for human consumption and nutrition. *Spirulina* snack chips contain very high crude protein which makes them more nutritious, easily assimilated and enlivens metabolism in the body than commercial snack chips. The crude fat component of *Spirulina*-fortified snack chips are essential fatty acids which are less in cholesterol. The commercial snack chips contain fats laden with cholesterol which can increase the risk of obesity and other cardio-vascular diseases.

The crude fiber content of fortified snack chips is 6.67% while a commercial snack chip is 3.33%. This indicates that *Spirulina*-fortified snack chips are digestible and absorbable in human metabolism and can easily stimulate the growth of beneficial microorganisms in the large intestines which facilitate the process of human digestion.

Table 4. Proximate chemical analysis of *Spirulina*-fortified snack chips and commercial snack chips.

Analysis (%)	*Spirulina* fortified snack chips	Commercial snack chips
Moisture	7.26	6.43
Total ash	8.0	3.16
Crude protein	70.0	21.00
Crude fats	16.66	29.83
Crude fiber	1.67	3.33

Efficacy test

Table 5 shows the mean of weight and arm circumference increases of pre-schoolers fed with *Spirulina*-fortified and commercial snack chips after 45 days. Both treatments registered a minimal increase in weight (except for treatment 1) and arm circumference.

Table 5. Means of weight and arm circumferences increases of pre-schoolers fed with snack chip after fifteen days.

Treatments	Weight (kg)	Arm circumference (inches)
T_0 Pre-schoolers fed with commercial snacks chips	0.231 a	0.002 a
T_1 Pre-schoolers fed with *Spirulina* fortified snacks chips	0.446 a	0 a
Means followed by common letter are not significantly different at 5% level of significances of DMRT.		

Table 6 presents the mean of weight and arm circumference increases of pre-schoolers fed with commercial snack chips and *Spirulina*-fortified snack chips after 30 days. Treatment 1 achieved greater increase in both weight and arm circumference by 0.753 kg and 0.307 kg respectively than in Treatment 0. The feeding of snack chips to pre-schoolers seem to affect significantly their health growth with those fed with *Spirulina*-fortified snack chips with the greatest increase in anthropometric measurements (weight and arm circumference).

Table 6. Means of weight and arm circumference of pre-schoolers fed with snack chips after 30 days.

Treatments	Weight (kg)	Arm circumference (inches)
T_0 Pre-schoolers fed with commercial snacks chips	0.462 a	0.062 a
T_1 Pre-schoolers fed with *Spirulina* fortified snacks chips	1.215 b	0.369 b
Means followed by common letter are not significantly different at 5% level of significances of DMRT.		

Table 7 shows the mean of weight and arm circumference increases of pre-schoolers fed with commercial snack chips and *Spirulina*-fortified snack chips after 45 days. It shows highly significant differences between the treatments in both weight and arm circumference increases. The longer the time of consumption of the *Spirulina*-fortified snack chips by the pre-schoolers resulted to greater increase in weight and arm circumference than in feeding with commercial snack chips. This can be attributed to the higher nutritional composition of *Spirulina*-fortified snack chips than the commercial snack chips as proven from the chemical analysis.

Table 7. Means of weight and arm circumference increases of pre-schoolers fed with snack chips after 45 days.

Treatments	Weight (kg)	Arm circumference (inches)
T_0 Pre-schoolers fed with commercial snacks chips	0.692 a	0.115 a
T_1 Pre-schoolers fed with *Spirulina* fortified Snacks chips	1.770 b	0.523 b
Means followed by common letter are not significantly different at 5% level of significances of DMRT.		

Summary and conclusions

Based on the results of the study, the following conclusions were established.

1. Processing and fortification of snack chips with a ratio of 10 grams of snack chips to 22 mg. *Spirulina* powder in effective in increasing the weight and arm circumferences of pre-schoolers.
2. *Spirulina*-fortified snack chips contain higher and useful biochemical composition in terms of fiber, protein, and moisture content.
3. Utilization of *Spirulina*-fortified snack chips as a food supplement to pre-schoolers improves their health growth and increases their arm circumference and weight greater than commercial snack chips.
4. Significant mean increase in weight and arm circumferences is seen in the longer consumption of *Spirulina*-fortified snack chips than commercial snack chips.

Recommendations

Based on the findings of study, the following recommendations were formulated:

1. Use of *Spirulina* in food fortification.
2. Follow-up studies must be conducted to confirm the acceptability of *Spirulina* to abate malnutrition and other health related problems.
3. Feeding of *Spirulina*-fortified snack chips must be administered with malnourished children in a controlled condition (i.e. same nature, amount of food and frequency of eating)
4. Application of higher amount of *Spirulina* powder in fortifying snack chips and longer consumption of the product to youngsters to verify if there is greater improvement in their health growth.
5. Conduct more studies on the processing of *Spirulina* as feed additive in poultry or piggery.
6. Conduct studies on different marine microalgae specifically blue-green algae aside from *Spirulina* to utilize their nutritive components beneficial to mankind.
7. Determine the chemical composition of *Spirulina* as affected by pH and salinity.
8. Determine the range of salinity where *Spirulina* can give an optimum yield.

Implications

1. *Spirulina* is an effective source in fortifying foods.
2. If the weight and arm circumference of the malnourished children increased, it can also increase the weight of animals if it will be used as feed additives.
3. *Spirulina* can improve the health of the public.

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Appendices

Table 1. Analysis of variance on the dry weight of *Spirulina* at difference pH concentrations.

Source of variation	Degree of freedom	Sum of squares	Mean square	F value	P-value	F crit.
Between groups	3	0.14	0.05	9.38 *	0.005	4.066
Within groups	8	0.04	0.005			
Total	11	0.18				
* = significant						

Table 2. Analysis of variance on the dry weight of *Spirulina* at different salt concentrations.

Source of variation	Degree of freedom	Sum of squares	Mean square	F value	P-value	F crit.
Between groups	3	0.16	0.54	6.34 *	0.005	4.066
Within groups	8	0.69	0.008			
Total	11	0.23				
* = significant						

Table 3. Percent moisture content of the *Spirulina*-fortified and commercial snack chips

Snack chips	Dish no.	Wt. of dish (g)	Wt. of dish + sample (g)	Wt. of sample (g)	Wt. of dish + dried sample (g)	Moisture %	Mean
Spirulina-fortified	1	20.68	22.40	1.72	22.29	6.52	6.46 a
	2	20.74	22.42	1.68	22.32	6.38	
	3	16.73	18.76	2.04	18.63	6.47	
Commercial snack chips	1	21.11	23.06	1.95	22.94	5.76	6.00 b
	2	21.21	23.02	1.81	22.91	6.16	
	3	21.07	23.10	2.03	22.97	6.07	
Legend: Means with the same letter are not significantly different at 5% level.							

Table 4. Percent crude fiber contents of *Spirulina*-fortified and commercial snack chips.

Snack chips	Crucible code	Sample wt. (g)	Wt. after drying (g) (cruc.+ sample)	Wt. after ashing (g) (cruc. + sample)	Crude fiber %	Average %
Spirulina-fortified	A	0.2013	30.0968	30.0960	0.40	0.40 a
	B	0.2018	31.0750	31.0744	0.30	
	C	0.2012	30.7505	30.7495	0.50	
Commercial snack chips	D	0.2002	30.1960	30.1956	0.20	0.20 a
	E	0.2010	30.1742	30.1737	0.25	
	F	0.2003	31.7938	31.7935	0.15	
Legend: Means with the same letter are not significantly different at 5% level.						

Table 5. Percent crude fat of *Spirulina*-fortified and commercial snack chips.

Snack chips	Beaker code	Sample wt. (g)	Wt. of beaker (g)	Wt. of beaker + fat (g)	Fat %	Mean
Spirulina-fortified	A	2.809	67.908	68.760	30.26	29.77 a
	B	2.670	68.061	68.817	29.45	
	C	2.872	67.982	68.832	29.61	
Commercial snack chips	D	2.756	67.354	68.130	28.14	28.69 a
	E	2.681	67.170	67.945	28.93	
	F	2.736	67.641	68.435	28.99	
Legend: Means with the same letter are not significantly different at 5% level.						

Table 6. Percent crude protein of *Spirulina*-fortified and commercial snack chips.

Snack chips	Flash ID	Wt. of sample (g)	Volume of HCl (ml)	Crude protein %	Average
Spirulina Fortified	B1	0.5095	4.2	7.26	7.26 a
	B2	0.5099	4.2	7.25	
	B3	0.5093	4.2	7.26	
Commercial Snack Chips	B4	0.5065	4.1	7.13	7.10 b
	B5	0.5051	4.1	7.14	
	B6	0.5005	4.1	7.03	
Legend: Means with the same letter are not significantly different at 5% level.					

Table 7. Ash content determination of *Spirulina*-fortified and commercial snack chips.

Snack chips	Crucible No.	Wt. of crucible	Wt. of sample	Wt. of crucible + ignited sample (ash)	Ash %	Mean
Spirulina Fortified	1	25.3502	1.9821	25.4292	3.99	3.98 a
	2	25.8863	1.9252	25.9636	4.02	
	3	25.2923	2.0209	25.372	3.94	
Commercial Snack Chips	4	22.8781	2.0051	22.9491	3.54	3.60 b
	5	22.8868	2.0835	22.9621	3.61	
	6	25.8383	1.9325	25.909	3.66	
Legend: Means with the same letter are not significantly different at 5% level.						

Table 8A. Analysis of variance on the weight increase (kg) of pre-schoolers fed with snack chips after 15 days.

Source of variation	Degree of freedom	Sum of squares	Mean square	F value	Table F	
					5%	10%
Between groups	1	0.30	0.30	1.11 ns	4.26	7.82
Within groups	24	6.36	0.27			
Total	25	6.66				
ns = non-significant						

Table 8B. Analysis of variance on the arm circumference increase (kg) of pre-schoolers fed with snack chips after 15 days.

Source of variation	Degree of freedom	Sum of squares	Mean square	F value	Table F	
					5%	10%
Between groups	1	1.54×10^{3}	0.08 ns	1.11 ns	4.26	7.82
Within groups	24	0.40	0.02			
Total	25	0.40				
ns = non-significant						

Table 9A. Analysis of variance on the weight increase (kg) of pre-schoolers fed with snack chips after 30 days.

Source of variation	Degree of freedom	Sum of squares	Mean square	F value	Table F	
					5%	10%
Between groups	1	3.69	3.69	6.15 *	4.26	7.82
Within groups	24	14.47	0.60			
Total	25	18.16				
* = significant						

Table 9B. variance on the arm circumference increase (kg) of pre-schoolers fed with snack chips after 30 days.

Source of variation	Degree of freedom	Sum of squares	Mean square	F value	Table F	
					5%	10%
Between groups	1	0.61	0.61	8.71 **	4.26	7.82
Within groups	24	1.78	0.07			
Total	25	2.39				
** = highly significant						

Table 10A. Analysis of variance on the weight increase (kg) of pre-schoolers fed with snack chips after 45 days.

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	F Value	Table F	
					5%	10%
Between groups	1	7.54	7.54	9.67 **	4.26	7.82
Within groups	24	18.83	0.78			
Total	25	26.37				
** = highly significant						

Table 10B. Analysis of variance on the arm circumference increase (kg) of pre-schoolers fed with snack chips after 45 days.

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	F Value	Table F	
					5%	10%
Between groups	1	1.08	1.08	13.5 **	4.26	7.82
Within groups	24	1.86	0.08			
Total	25					
** = highly significant						