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ENRICHMENT OF SPAGHETTI WITH GREEN ALGAE

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ABSTRACT

The drum dried green algae *Scenedesmus acutus* was evaluated chemically, it contain (49.81%) protein . Although it is deficient in sulfer amino acids (methionine and cystein) , yet, treptophan reached a concentration of (1.62 gm / 16 gm N) , which represent a good score of essential amino acid . There fore it could be used as asupplement of tryptophan to the semolina .

Chemical composition, cooking quality, as well as sensory and biological evaluation of supplemented spaghetti with ethanol extracted algae at levels 2,5,10 and 15% replacement were studied and compared with semolina spaghetti as control sample

The results indicated that the supplemented spaghetti samples with ethanol extracted algae showed more protein , fat , ash , fibre contents and nutritive value , but less total carbohydrates than the semolina spaghetti sample at all replacement levels .

Spaghetti processed from 100 % semolina showed the highest quailty characteristics (cooking quality, sensory paramentrs and consumer acceptability) followed by spaghetti samples supplemented with ethanol extracted algae at levels until 5%.

Keywords: Algae, green algae, decoloured algae, semolina, spaghetti.

INTRODUCTION

The shortage of food in general and of proteins in particular needs no emphasis. Recently, single cell protein has been considered as a good source of protein . A world wide attention is being given to develop a suitable technology to enable it to be used in feed and food . The majority of the developing countries used cereals as a major food constituent , but these are generally deficient in lysine and threonine . So "a lgae ", for being a good source of lysine , threonine and tryptophan , can improve the protein quelity of cereals when used as a supplement .

El- Fouly et al., (1984) produced high protein algae from the species *Scenedesmus acutus* , *Chlorella vulgaris* grown under the Egyptain out – door conditions .

Pasta products from a class of foods , which are economical , easy to prepare, shelf stable , and can be served in many different ways (Breen et al., 1977) . The wheat protein efficiency ratio is less than half of that of casein . Therefore , by the selective addition of protein to pasta , nutritional value can be improved and the protein content increased (Morad et al., 1980 , Shams El Din *et al* ., 1997) .

The green algae have been used as a food or feed ingredient (Powell et al., 1961) supplemented the uniceilular algae to human nutrition in a quantity of 30 to 40 gm per day Medvedeva et al., 1969; Soeder et al., 1970 and Nassar , 2001) used the algae amino acids as an addition to flour and

meat to improve the quality of bread protein, also the acceptability of the algae diet was agreable when added to bread checolate cakes and cookies.

This work was undertaken to evaluate the effect of defatted dried green algae (*Scenedesmus acutus*) addition to semolina flour on the chemical composition, sensory evaluation, cooking quality and nutritional value of produced paste.

MATERIAL AND METHODS

Material :-

The drum dried green algae *Scenedesmus acutus* was obtained from botony lab., National Res Center, Dokki, Egypt.
Semolina flour was obtained from capri macaroni campany Cairo, Egypt.

Analytical methods :-

Moisture, fibre, protein, fat and ash contents were carried out according to AOAC (1990). Total carbohydrates were calculated by difference.Total contents of phosphorus, potassium, magnesium, sodium, calcium, iron, manganese, zine and copper were determined according to the methods described by Chapman and Pratt (1978). Atomic absorption spectrophoto-meter(zeiss FMD3) was used for determination of magnesium,iron, manganese, zinc and copper. The flame photometer was applied for potassium, sodium and calcium determination while spectrophotometric method was used for determination of the phosphorus content of the tested samples. The algae nucleic acide was extracted by the method of Fabregas and Herrero (1986) and followed by aspectrophoto - metric assay. The absorbanse of RNA and DNA was assayed at 676 nm.

Ecxtraction of pigment from algae with different solvents :

Three solvents were used in this investigation for treatment of dried green algae (*Sc. acutus*) to reduce the denes colour of the product to make its used in foods more feasable and appealing . The solvents were . Ethyl alchol 95% . Aceton and commercial hexane.

The extraction was carried out in big soxhlet unit that will fit for extracting 24 grams of dried green algae according to the method of El–Ashwah et al., (1976), the amount of solvent applied were 1500, 1200 and 1500 ml for ethanol, acetone and hexane each run respectively. At the end of extraction run, thimbles containing the extracted algae were removed emptied and the contents thoroughly mixed. The extracted was dried by air drying to remove the residues of solvents applied. The air dried products were kept in tightly closed containers.

Amino acids analysis :-

The HPLC apparatus (waters Assoc. USA) was used for identifying the amino acids of the tested samples (modified PICO–TAG method , according to Millipore Cooperative (1987) Amino acid score (AAS) was calculated as the following equation :-
AAS % = (g amino acid of sample) / (g same amino acid of FAD/ WHO reference protein) X100

Processing of spaghetti samples :-

The spaghetti sample were prepared in the food technology lab. NRC, Cairo, Egypt, by using pasta matic 1000 Simac Machine corporation, Millano , Italy. The mixing time was 4-6 min at 30 rpm under vaccum value of 35 cm.Hg Spaghetti was hydrated under atmospheric air for 15 min., then dried in a cabinet dried in a cabinet dryer at 40 C for 14 hr . Dried samples were cooled enough at room temperature and packed in polyethylene pouches until analysis.

Preparation of defatted dried green algae and semolina flours blends :-

The dried green algae (nuclic acid free) were ground to fine flour to pass through a 60 mesh sieve . Lipids were removed by repeated extraction with petroleum ether (40 – 60) , and then air dried . The defatted dried green algae flour was blended with semolina flour at 2,5,10 and 15% levels .

Physical properties :-

Farinograph tests were performed according to A.A.C.C.(1962)

Cooking quality of cooked pasta :-

The cooking quality of spaghetti samples were determined according to the methods described by Seyam *et al* (1976) and Lorenz et al, (1979), as well as Vasiljevic and panasik (1980) as follows :-

Ten grams of spaghetti were broken into about 5cm lengths and cooked for 20 min in 300 ml boiling distilled water containing 1.0% Nacl. The sample was then rinsed thoroughly with distilled water in a buchner funel , drained for 2-5 min and weighed . The volume increase % (swelling %) was determined using petroleum naptha in measuring sample volume . The cooking loss was determined by collecting the cooking and rinse waters in a preweighed glass beaker. Beakers were placed in an air oven at 100 C and the water evaporated to dryness. The weight of residue was reported as a percentage of dry spaghetti.

***Organolyptic* test :-**

Was evaluated as reported by Matz(1959)which was modified the determine taste, colour, texture and overall acceptability . The maximum score is (10) for each parameter .

Biological evaluation of cooked spaghetti :-

The net protein utilization (NPU) of tested diets was determined by the method described by Miller and pender (1955). Thirty two sprague Dawely weaning rats with an average weight (50 – 55 g .)were divided into eight groups of 4 rats each . The animals were fed the following diets :

Group	Diet
1	Non – protein
2	Control (casein)
3	Spaghetti from 100% semolina .
4	Spaghetti containing 2% ethanol extracted algae
5	Spaghetti containing 5% ethanol extracted algae
6	Spaghetti containing 10% ethanol extracted algae
7	Spaghetti containing 15% ethanol extracted algae

The investigated diets had 10% protein and were completed with respect to all other nutrients . Food and water were given ad libitum for a period of 10 days, the scattered food and the uneaten food were weighed. Food consumption was calculated . Faeces were also collected, dried at 70 C for 24 hr and weighed . Animals were killed with chioroform, dissected the carcasses were dried in an air oven at 105c for 48hr , then ground . Nitrogen content of the carcasses, food consumed and faeces were determined by micro kjeldahl method according to A.O.A.C(1990) .

$NPU = [B-(B_k - I_k)] / I X100$

Where

B and B_k are the total body nitrogen of the animals fed the tested and non – protein diets , respectively .

I and I_k are the intakes of nitrogen of the two group .

$True\ digestibility\ (D) = (I - (F-F_k)) / I X 100$

Where :

F and F_k are faecal nitrogen values of the protein diets – respctively .

I is the intake of nitrogen of the protein diet

Biological value :

$(BV) = NPU / D X 100$

Feed efficiency ratios of the different tested diets were calculated according to the following equation

$$Feed\ efficiency\ ratio\ (FER) = \frac{Body\ weight\ gain}{Total\ food\ intake}$$

RESULTS AND DISCUSSION

Chemical analysis of dried and extracted algae:-

The dried samples of green algae *Scenedesmus acutus* befor and after extraction of pigments by different solvents (Ethanol 95 % , aceton and hexane) were chemically analyzed for their crude protein, fat, ash, fibre, and total carbohydrates , the results are presented in table (1) .

Table(1):Chemical analysis of dried and decolored green algae Scenedesmus acutus (on dry weigt basis)

	Crude protcin	Fat	Fiber	Ash	Carbohy drate
Dried green algae	43. 36	12.13	5.07	8.91	30.53
Decolored algae by					
Ethanol	49.81	2.09	2.78	7.31	34.59
Aceton	46.32	8.50	3.49	7.48	34.21
Hexan	44.80	8.80	3.35	7.66	35.30

Carbolydrarcs by difference

The crude protein increased by decolored this increasing indicated that used solvents caused the removal of other constituents than the crude protein of dried algae , it reach to 49. 81, 46. 32 and 44. 80% for ethanol, aceton and hexane extracted respectively. these results are agree with El-Ashwah *et al.*, 1976 and Nassar 2001, who showed the *scenedesmus obliquus* extracted by ethanal aceton and hexane the protein content was increased.

The chemical analysis of *Scenedesmus acutus* before and after extraction by ethanol resulted an increased of crude protein and decrcasing in fat content than other solvent extracting, these result are agree with El-Ashwah et al. , 1976, Dam et al., 1965 and Nassar 2001. Ethanol extraction resulted in ayellowish product being befor than acetone and hexane in pigment reduction. Besides, solvent extraction resulted with losses some consitituents i.e. fat and ash.

The results in table (2), it noticed that, the extracted ethanol algae had signifcanthy higher protein fat, ash and fibre contents, but lower carbohydrates percentage than the semolina flour. El-fouly et al., 1984, produced high protein algae from the species.

Scenedesmus acutus, chlorella vulgaris grown under the egyptain out – door conditions.

Table(2):chemical composition of ethanol extracted green algae (Scenedesmus acutus) and semolina flours (on dry weight basis)

Constituents	Ethanal extrd algae (scenedesmus acutus)	Semolina floure
Protein %		
Lipid %	49 81	12.19
Fibre %	2.09	1.12
Ash %	2.78	0.65
Carbohydrates %	7.31	0.71
Nucleic acid %	34.59	85 33
Element (mg / 100 gm)	3.42	
Phosphorus	1.02	124.13
Potassium	0.78	125.75
Mangesium	1.250	30.12
Sodium	5.78	2.56
Iron	2.10	1.17
Manganese	0.22	0.68
Zinc	1.85	0.88
Calcium	1.80	15.87
Copper	0.20	0.28

From the same table showed that the nucleic acids content were 3.42. It is a faet that purines are the building blocks of the nucleic acids and also purines are present in our food (Griebsch and zollner, 1970). Total nucleic content in the dried Scenedesmus cells was found to be 3.7 % (Jaleel and Soeder 1973) but this amount is low when compared with the other microorganisms, El-fouly, *et al* ., (1984) and Nassar (2001) , reported that the green algae content of nucleic acids ranged between 3.9 to 4.2%

Data in the same table indicated the ethanel extracted algae had lower contents of the determined minerals but higher sodium, Iron and zinc than the semolina flour. The values reported for the minerals content in ethanol extracted algae and semolina flour agree with those previously reported by Abd El magied 1976, and yaseen 1993.

Amino acid composition of ethanol extracted algae :-

The protein hydrolysis of ethanol extracted algae shows the presence of eighteen amino acids table (3). The essential amino acids pattern (FAO, 1980), as shown in table (4) . Results indicate that ethanol extracted algae *Scenedesmus acutus* is deficient in sulfur containing amino acids, i.e. methionine and cystine. The other essential amino acids compared well with the FAO pattern Tryptophan reached a concentration of 1.62 gm, while FAO pattern indicates 1.40 gm.

Table(3):Amino acid composition of algae protin Scenedesmus acutus (gm amino acid / 16gm tatal nitrogen)

Amino acids	Ethanol extracted algae(*Scendesmus acutus*)
Essential:-	
Lysine	2.62
Threonine	2.87
Valine	2.88
Methionine	0.65
Cystine	0.47
Isoleucine	1,73
Leucine	4.40
Phenylalanine	2.21
Tryptophan	1.62
Non-essntial :-	
Histidine	o.56
Arginine	2,85
Aspartic acid	4.89
Glutamic acid	5.73
Serine	2.49
Proline	1.93
Glycine	3.29
Alanine	5.20
Tyrosine	1.49

Table(4): Essential amino acids of ethanol extracted green algae (Scenedesmus acutus) protein Compared with FAO / WHO provisional amino Acids pattern.(gm / 16gm total nitrogen).

Amino acids	FAO/WHO 1980	Ethanol extrated algae *Scenedesmus acutus*	
		amino acid of protein	Score %
Lysine	5.5	2.62	47.64
Threonine	4.0	2.87	71.75
Valine	5.0	2.88	57.60
Methionine+cystine	3.5	1 12	32.00
Isoleucine	4.0	1.73	43 25
Leucine	7.0	4.40	62.86
Phenylalanine + Tyrosine	6.0	3.70	61 67
Trytophan	1.4	1 62	115.71

$$\text{Amino acid score \%} = \frac{\text{gm amino acid of protein}}{\text{gm amino acid of FAO / WHO reference protien}} \times 100$$

The score %, for tryptophan was calculated as 115.71%. There by, *Scenedesmus acutus* can be utilized to supplement the tryptophan deficient semolena protein. This finding agrees with several reports concerning the amino acids composition of various species of algae (Cook et al., 1963 and Abd El . Magied. 1976). Becker and vankataruman , 1976,.found that the essential amino acids. Content of *Scenedesmus acutus* was nearly the same as FAO pattern,while threonine and tryptophan content is high and comparable to egg protein.

Chemical composition of spaghetti :-

Table (5) shows the chemical composition of supplemented spaghetti with ethanol extracted algae. Ethanol extracted algae is characterized by its high protein content therefore, the protein content of the spaghetti samples was increased as result of the
addition of ethanol extracted algae.

Table(5): Chemical composition of produced spaghetti samples (on dry weight basis)

Constituents%	control	Spaghetl Supplemented With algae (*Scendesmus acutus*)			
	0 %	2%	5%	10%	15%
protein	12.42	13.83	16.22	18.45	20.60
Fat	1.25	1.43	1.51	1.59	1.68
Fibre	0.85	0 99	1.14	1.20	1.43
Ash	0.81	1.02	1.12	1.24	1.38
carbohydrate	84.67	82.73	82.01	77.52	74 91

Carbohyd rates calculated by diffrence

The same trend was also noticed for the fat, fibre and ash contents and probably could be explained by the fact that ethanol extracted algae contains higher levels of these constituents than the semolina flour. It was also noted that the addition of 15% ethanol extracted algae to semolina flour resulted in algae decrease in the carbohydrates content of the produced spaghetti samples.These results agreed with Yaseen (1993) .

***Rheological properties* of pasta (dough) Farinograph test :-**

Rheological properties of the resultant dough were investigated using Brabender Farinograph. Data in table(6) indicate that the addition of different levels increased the water absorption of the dough from (52.3to 60.80.) compared with the control (48%). Regarding to mixing time results cleared a decrease by Increasing the different levels. The results in Table(6) also show that addition of the high protein source to increase the stability of the dough . Weaking of the dough index has principal role in spaghetti production .

Table(6) clears that the addition of different levels tended to decrease dough weaking value .

Table (6): Effect the addition of ethanol extracted algae (Scenedesmus acutus)on the farinograph properties of pasta

Treatment	Water absorption	Mixing time (min)	Dough stability (min)	Dough weaking (B.U.)
Control(semolina 1CC%)	48	2.0	1.75	240
Se E A *S. acutus* at levels of				
2%	52.3	2.5	1 25	225
5%	56.4	3.0	1.00	220
10%	60.0	4.75	0.75	175
15%	60.8	9.5	0 75	80

B.u.=Brabinder units.
Se E A: Semolina supplemented with ethanol of extracted algae.

The effect of these additives has been attributed to proteins poor in sulfhydryl group (Compose *et al* ., 1978 and El-Farra *et al*., 1981) .

***Cooking quality* of spaghetti :-**

The spaghetti cooking quality properties (cooked increase , cooked volume and cooked loss) were affected by the addition of ethanol extracted algae at 2,5,10 and 15% replacement levels. Results presented in fable (7) indicated that the cooked weight and volume of supplemented spaghetti were decreased gradually with the increase of supplementation level, compared to the sample (100% durum semolina). Spaghetti samples and supplemented with ethanol extracted algae at levels 2,5,10% had a slight decrease in cooked weight and volume compared to control. These results could be attributed to the different in the protein quality and quantity of the supplemented flour.

Table(7): Cooking quality values of spaghetti supplemented with ethanol extracted – algae (Scenedesmus acutus)

Treatment	Change in					
	Cooking weight		cooked volume		cooked loss	
	%	Relative value	%	Relative value	%	Relative value
Control (Semolina 100%)	270.413	100	255	100	7 23	100
Sp E A (*S acutus*) at levels of						
2%	269 821	99 78	239	93 7	8 22	114 11
5%	265 736	98 27	210	82.4	8.65	119 64
10%	245.412	90.75	195	76 4	9.50	131 40
15%	215 226	79 59	172	67.5	9 95	137 62

Sp E A: Spaghetti supplemented with ethanol extracted algae.

Matsuo and Irvine (1970) reported that both protein quality and quantity affect spaghetti cooking quality properties. Hummel (1966), mentioned that good quality macaroni products should absorb water at least twice of their weight to swell to three or four times of their original volume. Results in the same table showed that the cooking loss of the supplemented spaghetti was increased gradually with increase the level of supplempentation compared to the control spaghetti which may be due to a high water soluble protein fraction .

Biological evalnation :-

The results of the biological assay experiments are presented in table (8 and 9). Rats receiving spaghetti tested diets consumed less food and gained lower body weight than the rats receiving the control diet (casein). The nitrogen in dry body weight, nitrogen intake and faecal nitrogen for rats receiving spaghetti tested diets were lower than casein diet (control). No death cases occurred when feeding on spaghetti tested diets. Feed efficiency ratios (FER) of supplemented spaghetti with ethanol extracted algae *Scendesmus acutus* diets were higher than that of semolina spaghetti diet , but lower than that of case in diet. Fortified spaghetti with ethanol extracted algae diets had higher NPU values from (60.23 to 67.40 %) than that of semolina spaghetti diet (57.80%) but lower than that of casein diet (72.10% .

Table (8): Food intake , body nitrogen , nitrogen intake , faecal nitrogen and body weight gain for experimental diets .

Diet	Food intake (gm)	Body nitrogen (gm)	Nitrogen intake (gm)	Faecal nitrogen (gm)	Body weight gain (gm)
Control (Casein)	389	8.16	6.20	0.87	+92
Non nitrogen	210	3.69	-	0.28	-50
Semolina spaghetti	312	6.58	5.00	.0.91	+42
Sp E A (S.acutus) at levels of					
2%	322	6.81	5.18	0.87	+45
5%	360	7.44	5.71	0.91	+50
10%	368	7.58	5.84	0.88	+55
15%	374	7.68	5.92	0.89	+68

Sp E A: Spaghetti supplemented with ethanol extracted algae.

The same trend was noticed for digestibility, as shown in table(9). These results are in agreement with these of Gonzalez (1972), Shams El Din , (1997) and Nassar (2001) .

The results indicated that the addition of ethanol extracted algae *Scenedesmus acutus* to semolina increased protein , fat , fibre, ash and nutritive valne but decreased total carbohydrate of investigated spaghetti at all replacment levels compared to semolina spaghetti (control) .

Ethanol extracted algae *Seenedesnus acutus* can be recommanded for spaghetti prepartion until 5% replacement level. further supplementation a bore this level improued FER , NPU, D and BV, but decreased weight volume of investigated spaghetti samples .

Table (9): Feed efficieng ratio (FER), net protein utilization (NPU) , digestibility (D) and biological value (BV) for experimental diets .

Diet	FER	NPU	D	BV
Control (Casein)	0.23	72.10	90.48	79 69
Semolina spaghetti	0.13	57.80	87 40	66 14
Sp E A (S acutus) at levels of				
2%	0 14	60 23	88.61	67 97
5%	0.14	65.67	88 97	73 81
10%	0 15	66.61	89.73	74.23
15%	0 18	67.40	89.70	75.14

Sp E A: Spaghetti supplemented with ethanol extracted algae.

Sensory evaluation:-

Sensory evalution data of supplemented spaghetti with ethanol extracted algae was statistically analyzed.

Means comparison for the parameters colour, Taste, Texture and overall acceptability used to evaluate the spaghetti are shown in table 10. Results showed that , there were higher significant differences ($P < 0.05$ and $P < 0.01$) for all parameters used among the prepared spaghetti. The acceptable spaghetti could be produced by substituting semolina up to 5% leads to reduction in the total scores of acceptability to 73.5% and 42% compared with 93.5% for control sample.

In general ,the results indicated that, ethanol extracted algae *Scenedesmus acutus* can be recommended for spaghetti preparation until 5% replacement level, further supplementation above.

This level improved FER, NPU, D and BV but decreased weight volume of investigated spaghetti samples.

Table (10): Statistical evaluation of sensory scores (L.S.D) of spaghetti supplemented with ethanol extracted algae

Sensory attributes	Spaghetti samples					F value	L.S.D
	Control	2%	5%	10%	15%		0.05
Colour	9.6	7.4	7.6	4.6	6.8	29.02	1.65
Taste	8.8	6.2	6.8	4.2	2.4	43.48	1.59
Texture	9.2	6.2	7.4	5.2	3.8	20.05	1.47
Overall acceptability	9.8	8.0	7.6	4.8	3.8	96.77	1.77
Total acceptability	93.5	69.5	73.5	47	42		

F Book: 0.05=3.01, 0.01=4.77

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تدعيم الاسباجيتى بالطحالب الخضراء

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تم تقيم الطحلب الأخضر *Scenedesmus acutus* كيميائيا حيث وجد أنه يحتوى على ٨١و٤٩% بروتين ومع افتقاد هذا الطحلب إلى الأحماض الامينية الكبريت (ميثيونين – سستين) إلا أنه يحتوى على نسبة عالية من الترتيوفان (٦٢و١جم/١٦ جرام نتروجين) والذى يعتبر من الأحماض الأمينية الأساسية ولهذا استخدم هذا الطحلب كمصدر للتربتوفان لتدعيم دقيق السمولينا كما تم استخدام بعض المذيبات مثل الإيثانول والأسيتون والهيكسان للإستخلاص اللون الأخضر من الطحالب .

وقد تم دراسة التركيب الكيماوى وخواص الطعم والتقيم البيولوجى والحسى للاسباجيتى المدعم بمستويات مختلفة (٢،٥،١٠،١٥%) من الطحلب المستخلص بالإيثانول ومقارنتها بالاسباجيتى الكنترول(دقيق سيمولينا١٠٠%)

وقد اوضحت النتائج أن عينات الاسباجيتى المدعم بالطحلب المستخلص بالإيثانول كان محتواها مرتفع فى البروتين والدهن والالياف الرماد والقيمة الغذائية وكانت منخفضة فى الكربوهيدرات فى جميع مستويات التدعيم المختلفة عن عينة الاسباجيتى الكنترول (سمولينا ١٠٠%). والإسباجيتي المصنعة من ١٠٠% سيمولينا كانت أعلى في صفات الجودة (جودة الطهي والخواص الحسية والقابلية للإستهلاك) تلاها العينات المدعمة بالطحلب المستخلص بالإيثانول حتى ٥%.