

Seaweed Processing Study

Full Report

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Preamble

Alaria esculenta is a seaweed with olive or yellow-brown fronds to 4 m long and 25 cm wide, more often about 1 m and 7.5 cm wide. Attached by a root-like holdfast at the base from which a narrow flexible stipe arises which continues into the leafy part of the plant as a distinct mid-rib, generally with a yellow-brown colour. This is the only kelp-like plant in Ireland and Britain with a distinct midrib and is the only one with sporangia borne at the base of the frond in special leaflets called sporophylls. *Alaria esculenta* is an attractive alga, whose name literally means 'edible wings'. It usually grows on rock in wave-exposed places, often forming a band at low water and in the shallow subtidal, but also occurring in tidal pools in the lower shore. In some areas, the plants are harvested during a narrow window in early summer, after they have put on reasonable growth but before the crashing waves shred the thin leaves; harvesting is often done by hand and knife at low tide. Juvenile plants should remain uncut.

Signed:


Dan Duff, M.Sc.
Laboratory Manager.

Signed:


F. J. Bloomfield, Ph.D.
Scientific Director.

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Alaria esculenta can be used for a variety of purposes from human consumption and alginate production to fodder and body-care products. It is rapidly gaining popularity in the natural foods market. It can be ordered from many sellers as whole, flaked, milled, or powdered. It is used for anti-aging body creams, foot creams, bath soaks, body and face masks, body polish, UV-protecting facial moisturizers, self-tanning lotions, lip balm, day- and night-creams, and nutritional supplements. It is high in calcium, Vitamins A, B2, B6, B12, K, iodine, and bromine, and also contains Vitamin C, nitrogen and trace elements. It is rich in sugars, proteins, vitamins and contains up to 42% alginic acid.

Consumer awareness in diet and health issues has increased dramatically in recent years. There is a huge demand for healthy alternatives with consumers more informed on the association of diet with health problems such as obesity, diabetes and heart disease. They are actively looking for more information on what nutrients are present in food products in order to make a more informed choice when purchasing. Coming from the sea, *Alaria* is a rich source of organic trace minerals in a form that is very easily assimilated. Iodine deficiency is commonplace and land plants typically do not supply the amount of iodine needed to meet our needs.

Alaria, as do most seaweeds, inhibit cancer cell growth in animal studies. It is considered anti-mutagenic, and anti-tumor (1). It has been postulated that the low rate of breast cancer and lung cancer in Japan is associated with seaweed consumption (2). Seaweeds can lower serum cholesterol levels as well as blood pressure and help remove arterial plaque as well as decrease platelet stickiness (3). *Alaria* and certain other seaweeds can also lower raised uric acid levels and can be anti-bacterial, anti-fungal, and anti-viral (4).

Alaria can be very effective in preventing damage due to radiation exposure from power plants, nuclear tests etc. Nuclear facilities release radioactive Iodine 131 into the atmosphere. Iodine 131 is heavier than natural iodine 127 and is implicated in the increasing rates of thyroid cancer and other thyroid disorders (5). The presence of iodine 127, which is naturally found in seaweed is believed to protect against iodine 131 exposure. Research has also shown that seaweeds also help remove radioactive strontium from the body (6).

Estrogen levels are rising in individuals due to higher levels of xenoestrogens from diet and chemical exposure. *Alaria* has been shown to favorably alter both estrogen and phytoestrogen levels in the body (7). In addition to helping return hormone levels to normal, it may also play a role in helping to prevent breast cancer in post-menopausal women.

Alaria contains algin, which is a binder of heavy metals from the gastrointestinal tract (8). It also contains Fucoidan, which is anti-inflammatory. It is an immune stimulant (9) and can speed up the healing response after physical trauma such as surgery (10).

Brown seaweeds such as *Alaria* also have been shown to assist in the excretion of dioxins and PCBs (11).

Objectives

The Chemical Analysis Laboratories Ltd (CAL) was commissioned to undertake a study on *Alaria esculenta*, whereby the protocol required methods utilised by Japanese seaweed processors for preparation of post-harvest product for further use and consumption (12).

The protocol was designed by Ms Watson at BIM, in order that CAL could process laboratory batches of *Alaria*, after which tests were carried out to measure the total viable bacterial counts (TVC) and Group 1 nutritional parameters to include Fat, Protein and Carbohydrate (CHO). The objectives were to ascertain the levels of TVC present in each batch after processing and also to study the effects of processing on the Fat, Protein and CHO content, following processing by different methods.

Sample Receipt

Approx. 70kg of *Alaria esculenta* was received at CAL from Ms Watson on 04/04/17. It was harvested at Bantry Bay on 03/04/17 – 04/04/17 and delivered in 10-12kg batches in plastic sacks. The samples were signed into Reception and stored at approx. 10°C prior to processing. The amount of seaweed required for further testing was 500g. the analysis reported hereunder utilised 10g for TVC analysis and 150 – 200g for Group 1 nutritional analysis. The remaining samples were frozen at -18°C for further testing in the future. When the protocol necessitated drying of the seaweed at different temperatures, the amount of these batches was increased tenfold to 5kg, to take into account that there would be around 90% reduction in weight following drying. In these cases, 500g portions were processed ten times. so that the amounts were the same as those that did not require drying. The ten samples were combined in each case prior to drying.

Experimental Design

As stated above, the protocol employed methods to precisely mirror the Japanese seaweed processing procedures on a laboratory scale. The following 18 processing methods (M1 – M18) were employed for every 500g batch prepared for further testing. It can be seen from the methods hereunder, that M1, 2, 12-14, only required a single 500g batch to be prepared, whereas M3-11, 15-18 required 10 x 500g batches, as they were dried at different temperatures. It should be noted that the batch dried at 40°C to 12% Moisture (M17 below) was employed as the Control sample for comparative purposes. It was also shown that the seaweed samples that were not dried had a moisture content of 66 – 85% with a mean value of 71%. To standardise the results, the data for Nos. M1, M2 and M12-M14 were reported at 12% Moisture and on a Dry Matter Basis (DMB). Where salting was required, the amount of salt used in each case was 150g.

The seawater was collected from Sandycove Bay at high tide, for use in seawater baths. Analysis for total bacterial counts (TVC) showed that there was none detected (<10cfu/ml).

- M1.** a). Blanch 500g at 90°C for 1 minute.
b). Wash in seawater bath at ambient temperature for 2 minutes.
c). Press lightly to remove excess seawater.
d). Salt (30 parts salt/100 parts seaweed) for 24 hours.
e). Press with weights (25kg) for 24 hours.
- M2.** a). Wash 500g in seawater bath at ambient temperature for 2 minutes.
b). Press lightly to remove excess seawater.
c). Press with weights (25kg) for 24 hours.
d). Salt (30 parts salt/100 parts seaweed) for 24 hours.
- M3.** a). Blanch 500g x 10 at 90°C for 1 minute.
b). Wash in seawater bath at ambient temperature for 2 minutes.
c). Press lightly to remove excess seawater.
d). Salt (30 parts salt/100 parts seaweed) for 24 hours.
e). Press with weights (25kg) for 24 hours.
f). Desalted by wash in seawater for 2 minutes.
g). Chop seaweed with carving knife.
h). Dry in Drying Oven to 12% at 40°C in a Kerres S oven.
- M4.** a). Blanch 500g x 10 at 90°C for 1 minute.
b). Wash in seawater bath at ambient temperature for 2 minutes.
c). Press lightly to remove excess seawater.
d). Salt (30 parts salt/100 parts seaweed) for 24 hours.
e). Press with weights (25kg) for 24 hours.
f). Desalted by wash in seawater for 2 minutes.
g). Chop seaweed with carving knife.
h). Dry in Drying Oven to 12% at 70°C in a Binder FD oven.

- M5.**
- a). Blanch 500g x 10 at 90°C for 1 minute.
 - b). Wash in seawater bath at ambient temperature for 2 minutes.
 - c). Press lightly to remove excess seawater.
 - d). Salt (30 parts salt/100 parts seaweed) for 24 hours.
 - e). Press with weights (25kg) for 24 hours.
 - f). Desalted by wash in seawater for 2 minutes.
 - g). Chop seaweed with carving knife.
 - h). Dry in Drying Oven to 12% at 90°C in a Binder FD oven.
- M6.**
- a). Blanch 500g x 10 at 90°C for 1 minute.
 - b). Wash in seawater bath at ambient temperature for 2 minutes.
 - c). Press lightly to remove excess seawater.
 - d). Press with weights (25kg) for 24 hours.
 - e). Chop seaweed with carving knife.
 - f). Dry in Drying Oven to 12% at 40°C.
- M7.**
- a). Blanch 500g x 10 at 90°C for 1 minute.
 - b). Wash in seawater bath at ambient temperature for 2 minutes.
 - c). Press lightly to remove excess seawater.
 - d). Press with weights (25kg) for 24 hours.
 - e). Chop seaweed with carving knife.
 - f). Dry in Drying Oven to 12% at 70°C.

- M8.**
- a). Blanch x 10 500g at 90°C for 1 minute.
 - b). Wash in seawater bath at ambient temperature for 2 minutes.
 - c). Press lightly to remove excess seawater.
 - d). Press with weights (25kg) for 24 hours.
 - e). Chop seaweed with carving knife.
 - f). Dry in Drying Oven to 12% at 90°C.
- M9.**
- a). Wash 500g x 10 in seawater bath at ambient temperature for 2 minutes.
 - c). Press lightly to remove excess seawater.
 - c). Salt (30 parts salt/100 parts seaweed) for 24 hours.
 - d). Press with weights (25kg) for 24 hours.
 - e). Desalted by wash in seawater for 2 minutes.
 - f). Chop seaweed with carving knife.
 - g). Dry in Drying Oven to 12% at 40°C.
- M10.**
- a). Wash 500g x 10 in seawater bath at ambient temperature for 2 minutes.
 - c). Press lightly to remove excess seawater.
 - c). Salt (30 parts salt/100 parts seaweed) for 24 hours.
 - d). Press with weights (25kg) for 24 hours.
 - e). Desalted by wash in seawater for 2 minutes.
 - f). Chop seaweed with carving knife.
 - g). Dry in Drying Oven to 12% at 70°C.

- M11.**
- a). Wash 500g x 10 in seawater bath at ambient temperature for 2 minutes.
 - c). Press lightly to remove excess seawater.
 - c). Salt (30 parts salt/100 parts seaweed) for 24 hours.
 - d). Press with weights (25kg) for 24 hours.
 - e). Desalted by wash in seawater for 2 minutes.
 - f). Chop seaweed with carving knife.
 - g). Dry in Drying Oven to 12% at 90°C.
- M12.**
- a). Blanch 500g at 90°C for 1 minute.
 - b). Wash in seawater bath at ambient temperature for 2 minutes.
 - c). Press lightly to remove excess seawater.
 - d). Salt (30 parts salt/100 parts seaweed) for 24 hours.
 - e). Press with weights (25kg) for 24 hours.
 - f). Freeze at -20°C.
- M13.**
- a). Blanch 500g at 90°C for 1 minute.
 - b). Wash in seawater bath at ambient temperature for 2 minutes.
 - c). Press lightly to remove excess seawater.
 - e). Press with weights (25kg) for 24 hours.
 - f). Freeze at -20°C.

- M14.** a). Wash 500g in seawater bath at ambient temperature for 2 minutes.
- b). Press lightly to remove excess seawater.
- c). Press with weights (25kg) for 24 hours.
- d). Salt (30 parts salt/100 parts seaweed) for 24 hours.
- f). Freeze at -20°C.
- M15.** a). Blanch 500g x 10 at 90°C for 1 minute.
- b). Wash in seawater bath at ambient temperature for 2 minutes.
- c). Press lightly to remove excess seawater.
- d). Dry in Drying Oven to 12% at 40°C.
- M16.** a). Blanch 500g x 10 at 90°C for 1 minute.
- b). Wash in seawater bath at ambient temperature for 2 minutes.
- c). Press lightly to remove excess seawater.
- d). Dry in Drying Oven to 12% at 90°C.
- M17.** a). Dry 500g x 10 in Drying Oven to 12% at 40°C.
- M18.** a). Dry 500g x 10 in Drying Oven to 12% at 90°C.

Analytical Methodology

Analysis of all processed samples was carried out at Advanced Laboratory Testing (ALT), a specialist food testing facility which is fully accredited. The details of the methods used presented hereunder.

Enumeration of micro-organisms: aerobic colony count

Reference Standard: I.S. EN ISO 4833:2013

Marks & Spencer Manual of Microbiological Methods, Method 1.1 March 2007

Method Summary: 30°C Pour plate using PCA, 48-hour incubation

EC Compliance: Yes, INAB Accredited: Yes

Determination of total fat and moisture in foods

Reference Standard: The method was derived from SMART Trac II CEM Operation Manual, 600153 Rev 0. AOAC Official method of analysis 19th edition, 2012 (39.1.39, Chapter 39 p. 27).

Method Summary: This test utilises the CEM SMART Trac II™ Rapid Fat and Moisture/Solids Analyser. This instrument accurately measures the fat content of virtually any type of food product. Smart Trac produces moisture results by removing water (evaporation) via microwave energy and measuring the weight loss on drying. The dried sample is transferred into a plastic sleeve using the Compression Station and inserted into the NMR instrument where it then receives a pulse of radio-frequency energy from the NMR for analysis of fat content. The moisture and fat results are then displayed and reported by the SMART Trac as a percentage (g/100g).

INAB Accredited: Yes

Determination of ash in foods

Reference Standard: CEM Application note for ashing using Microwave Furnace SOP 025. AOAC Official method of analysis 19th edition, 2012 923.03 (32.105), Chapter 32, p.2 Phoenix Operating Manual 600134 Rev. 12.

Method Summary: Ash in food is the inorganic residue remaining after the organic matter has been burnt away. A temperature controlled Phoenix Microwave Furnace (CEM) is used to determine the Total Ash in different food samples. The Phoenix is a special purpose microwave system. This instrument's primary purpose is the rapid oxidation of samples for the gravimetric determination of ash content. The weight of residue is expressed as a percentage of the sample weight taken.

INAB Accredited: Yes

Determination of nitrogen (protein) in food

Reference Standard: (I) Nitrogen Determinator Instruction Manual FP628. (II) Pearson's Composition and Analysis of Foods, pg.20. (III) AOAC Official Method 992.15, - Section 39.1.16, Chapter 39, pg.6

Method Summary: Nitrogen in a food sample is determined by complete combustion in the presence of oxygen using the LECO Nitrogen Determinator (FP628). The resulting gases pass through various filters to remove interfering gases/ particles and nitrous oxide gases are reduced to nitrogen by means of a heated catalyst. An aliquot of the filtered gases is analysed using a thermal conductivity cell, with helium being used as the reference and carrier gas. The output voltage that results is processed by the internal computer and converted to give the nitrogen content of the sample. This is then converted into a protein value by calculation using an appropriate conversion factor. In this study, the Protein Conversion Factor employed was 6.25.

INAB Accredited: Yes

Total carbohydrate (by difference)

Method Summary: Total Carbohydrate (CHO) is calculated as follows:

$\% \text{ Carbohydrate} = 100 - (\% \text{ Moisture} + \% \text{ Fat} + \% \text{ Protein} + \% \text{ Ash})$

INAB Accredited: Yes

Energy calculations for foods

Reference Standard: Regulation (EU) No 1169/2011 on nutrition labelling for foodstuffs. McCance and Widdowson. The composition of foods Seventh Summary Edition. Food Safety Authority Ireland, Accuracy of Nutrition Labelling of Pre-Packaged Food in Ireland, 2010.

Method Summary: This procedure describes the equations used to determine energy and other values from analytical results previously determined. Energy is calculated in kcals and in kj/ 100g. The calculations performed are as those described in the reference method.

INAB Accredited: Yes

Results

The following tables show the results of each processing method at 12% Moisture and also calculated on a DMB. Using Method 17 as the Control Alaria, i.e. seaweed that was dried to 12%, the bar charts following each table demonstrate the changes found using each processing method. It should be noted that Method 1 – 18 are referred to as M1 – M18 in the following tables and the bar charts are labelled as figures after each table.

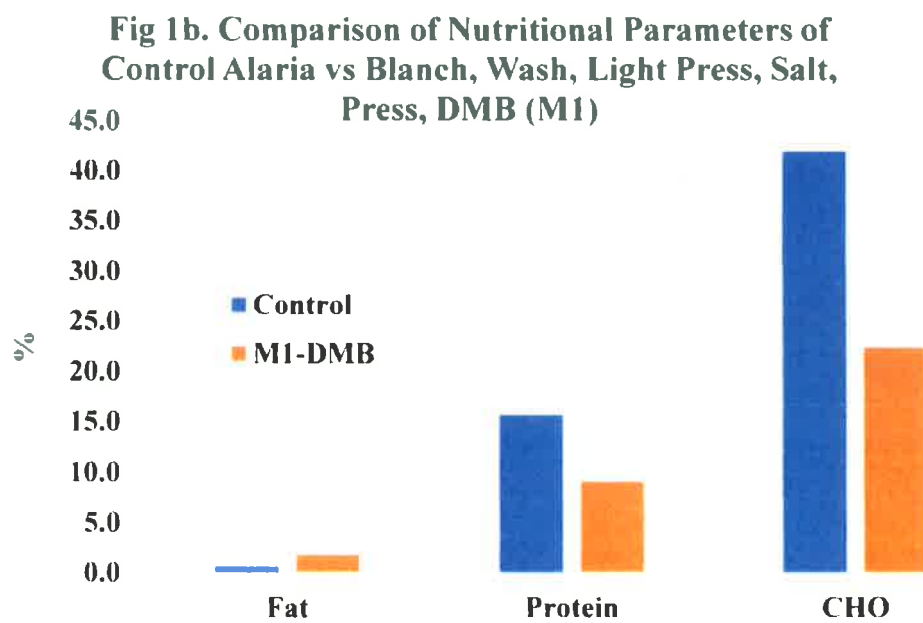
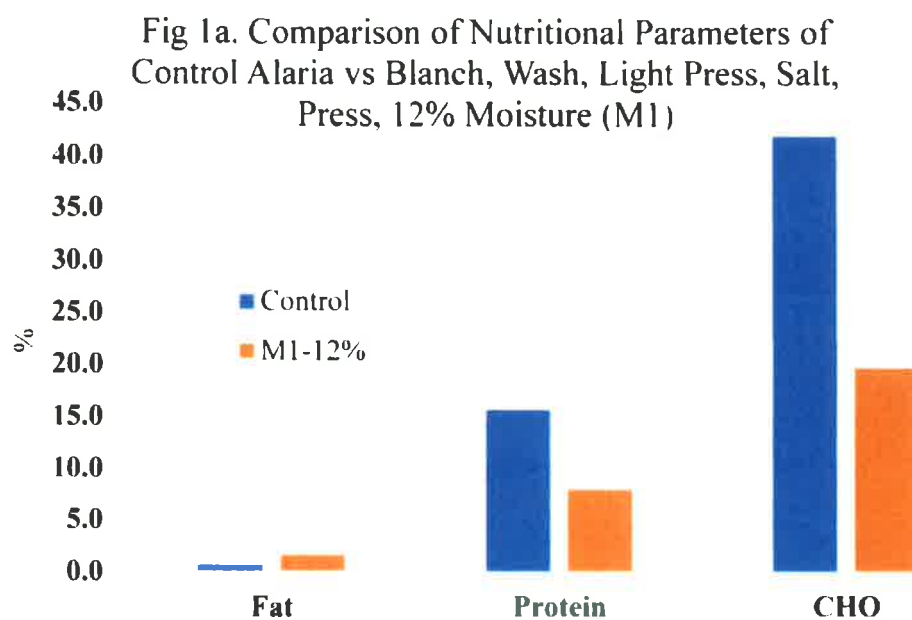
Method 1. (M1)

The 500g batch of seaweed was blanched for 1 minute, washed in seawater bath at ambient temperature for 2 minutes, pressed lightly to remove excess seawater, salted with 150g table salt (30 parts salt/100 parts seaweed) for 24 hours and pressed with 25kg for 24 hours.

Table 1. Nutritional Parameters of Alaria by M1 - Blanch, Wash, Light Press, Salt, Press. Results at 12% Moisture and DMB.

	12% Moisture	DMB	Refence Level
Fat %	1.6	1.8	1 to 2 (13)
Ash %	58.7	66.8	14 to 32 (13)
Protein %	8	9.1	9 to 18 (13)
E (kcal)	124.2	141.2	
E (kJ)	528	600	
CHO %	19.7	22.4	46 to 51 (14)

TVC cfu/ml 730



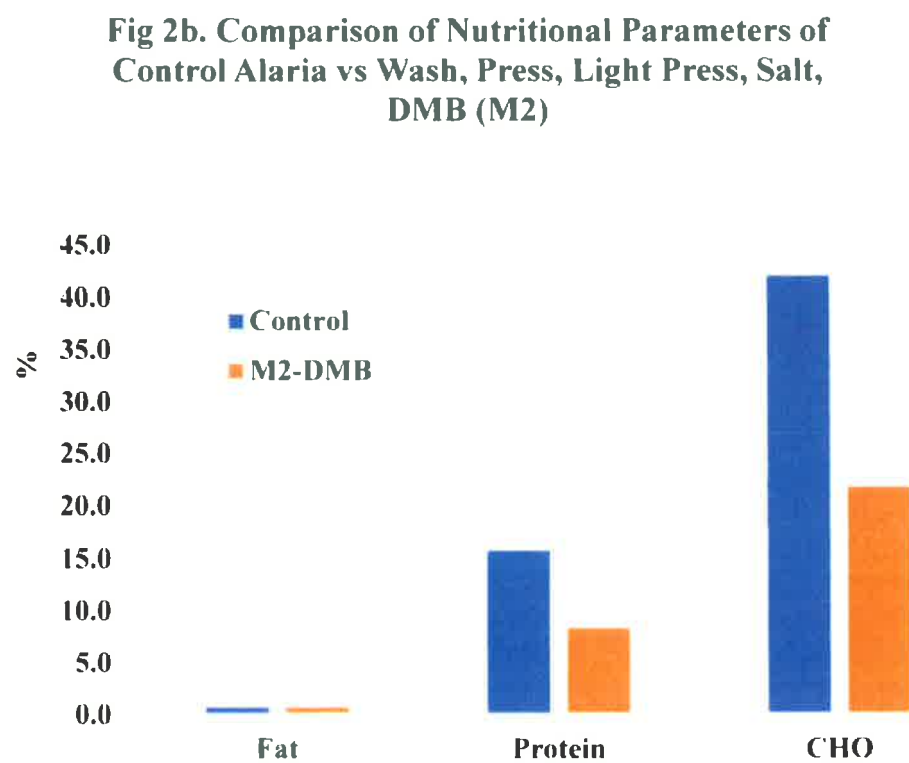
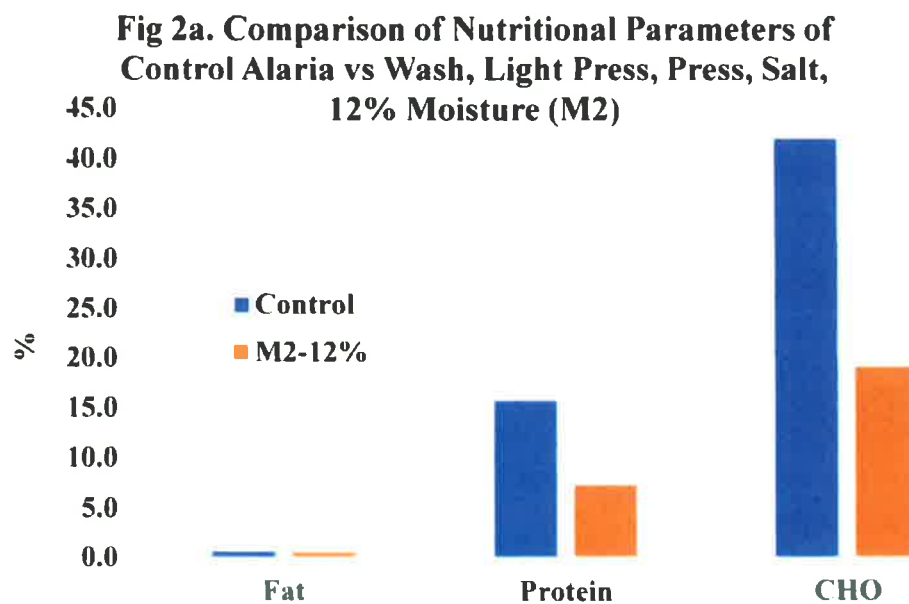
Method 2. (M2)

The 500g batch of seaweed was washed in seawater bath at ambient temperature for 2 minutes, pressed lightly to remove excess seawater and salted with 150g table salt (30 parts salt/100 parts seaweed) for 24 hours.

Table 2. Nutritional Parameters of Alaria by M2 - Wash, Light Press, Press, Salt. Results at 12% Moisture and DMB.

	12% Moisture	DMB	Reference Value
Fat %	0.5	0.6	1 to 2 (13)
Ash %	61.4	69.7	14 to 32(13)
Protein %	7.3	8.3	9 to 18 (13)
E (kcal)	107.6	122.3	
E (kJ)	462.9	526.0	
CHO %	19.1	21.7	46 to 51 (14)

TVC cfu/ml <10



Method 3. (M3)

The 5kg batch of seaweed was blanched for 1 minute, washed in seawater bath at ambient temperature for 2 minutes, pressed lightly to remove excess seawater, salted with 150g table salt (30 parts salt/100 parts seaweed) for 24 hours and pressed for 24 hours with 25kg, desalted in seawater for 2 minutes, chopped and dried at 40°C.

Table 3. Nutritional Parameters of Alaria by M3 – Blanch, Wash, Light Press, Salt, Press, Desalt, Chop, Dry at 40°C. Results at 12% Moisture and DMB.

	12% Moisture	DMB	Reference Value
Fat %	0.3	0.3	1 to 2 (13)
Ash %	63.8	72.5	14 to 32 (13)
Protein %	8.1	9.2	9 to 18 (13)
E (kcal)	98.8	112.3	
E (kJ)	419.1	476.3	
CHO %	16.0	18.2	46 to 51 (14)
TVC cfu/ml	3,600		

Fig 3a. Comparison of Nutritional Parameters of Control Alaria vs Blanch, Wash, Light Press, Salt, Press, Desalt, Chop, Dry 40°C, 12% Moisture (M3)

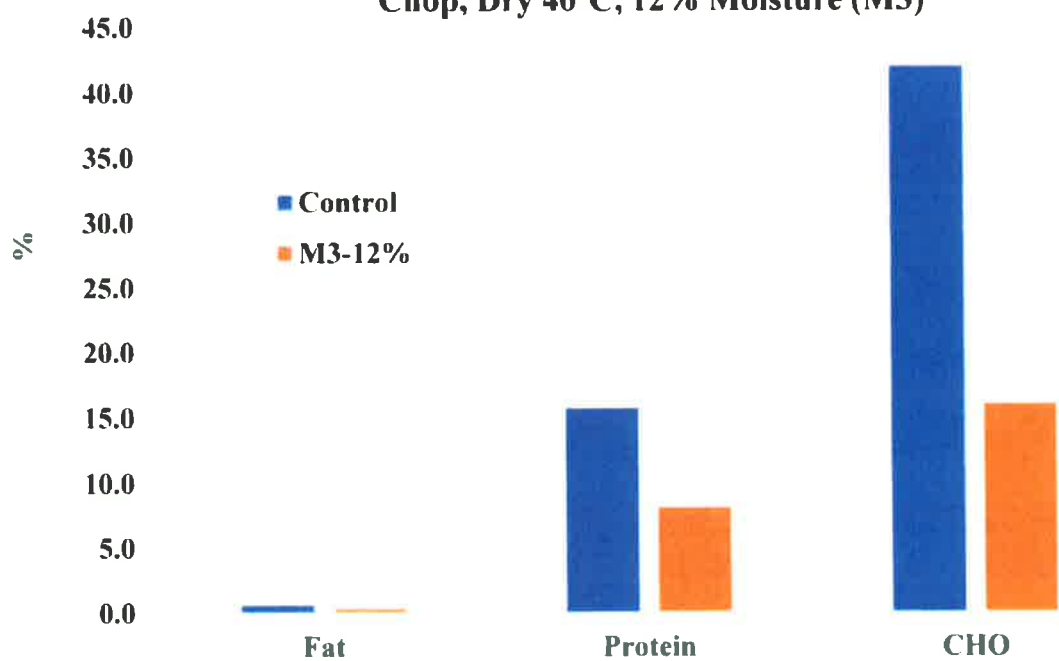
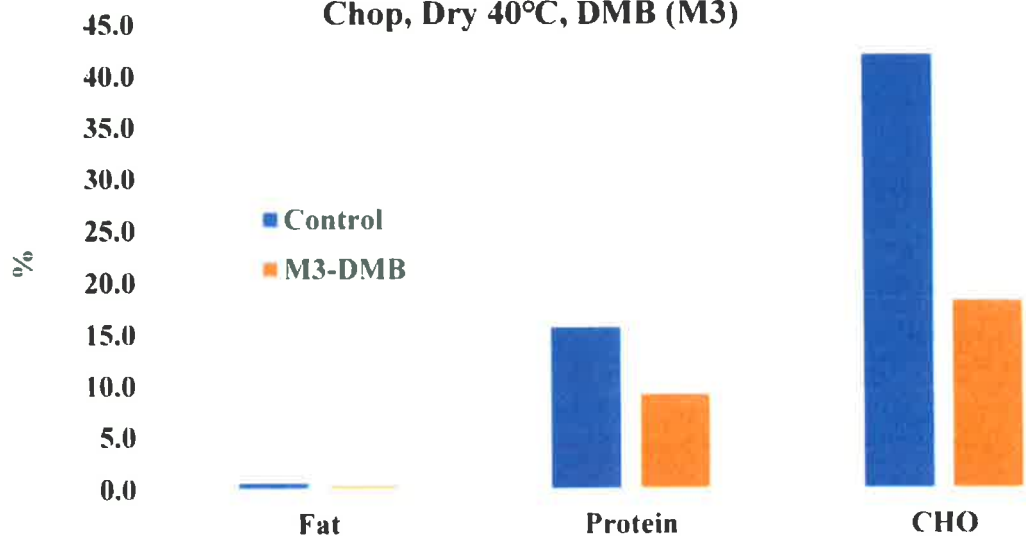


Fig 3b. Comparison of Nutritional Parameters of Control Alaria vs Blanch, Wash, Light Press, Salt, Press, Desalt, Chop, Dry 40°C, DMB (M3)



Method 4. (M4)

The 5kg batch of seaweed was blanched for 1 minute, washed in seawater bath at ambient temperature for 2 minutes, pressed lightly to remove excess seawater, salted with 150g table salt (30 parts salt/100 parts seaweed) for 24 hours and pressed for 24 hours with 25kg, desalted in seawater for 2 minutes, chopped and dried at 70°C.

Table 4. Nutritional Parameters of Alaria by M4 – Blanch, Wash, Light Press, Salt, Press, Desalt, Chop, Dry at 70°C. Results at 12% Moisture and DMB.

	12% Moisture	DMB	Reference Value
Fat %	0.6	0.6	1 to 2 (13)
Ash %	51.9	59.0	14 to 32 (13)
Protein%	10.3	11.8	9 to 18 (13)
E (kcal)	144.1	163.8	
E (kJ)	614.9	698.8	
CHO %	25.3	28.8	46 to 51 (14)

TVC cfu/ml 90

Fig 4a. Comparison of Nutritional Parameters of Control Alaria vs Blanch, Wash, Light Press, Salt, Press, Desalt, Chop, Dry 70°C, 12% Moisture (M4)

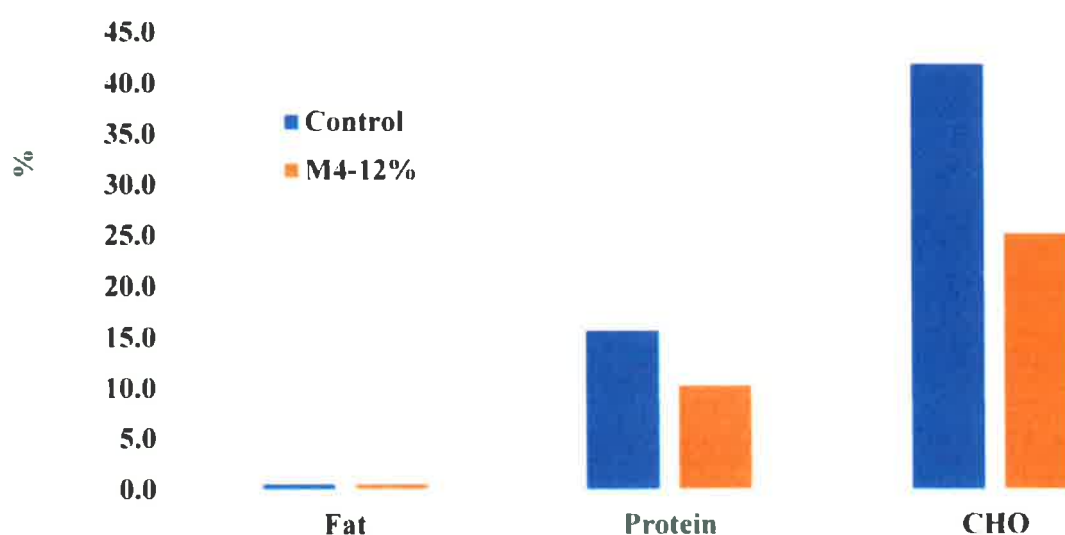
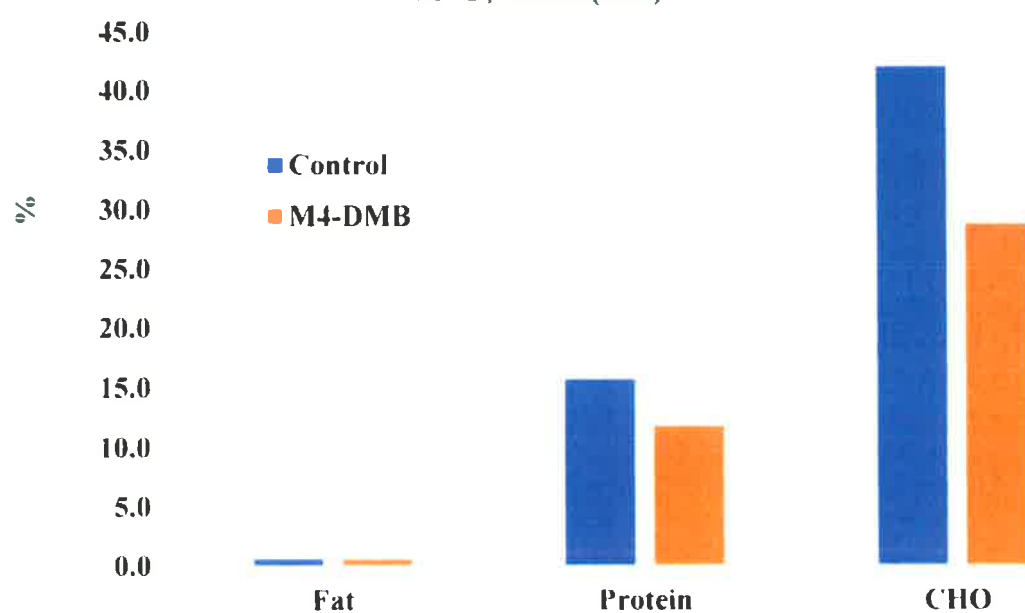


Fig 4b. Comparison of Nutritional Parameters of Control Alaria vs Blanch, Wash, Salt, Press, Desalt, Chop, Dry 70°C, DMB (M4)



Method 5. (M5)

The 5kg batch of seaweed was blanched for 1 minute, washed in seawater bath at ambient temperature for 2 minutes, pressed lightly to remove excess seawater, salted with 150g table salt (30 parts salt/100 parts seaweed) for 24 hours and pressed for 24 hours, desalted in seawater for 2m minutes, chopped and dried at 90°C.

Table 5. Nutritional Parameters of Alaria by M5 – Blanch, Wash, Light Press, Salt, Press, Desalt, Chop, Dry at 90°C. Results at 12% Moisture and DMB.

	12% Moisture	DMB	Reference Value
Fat %	0.7	0.8	1 to 2 (13)
Ash %	47.9	54.4	14 to 32 (13)
Protein %	10.5	11.9	9 to 18 (13)
E (kcal)	163.3	185.5	
E (kJ)	696.2	790.7	
CHO %	28.9	32.9	46 to 51 (14)

TVC cfu/ml 210

Fig 5a. Comparison of Nutritional Parameters of Control Alaria vs Blanch, Wash, Light Press, Salt, Press, Desalt, Chop, Dry 90°C, 12% Moisture (M5)

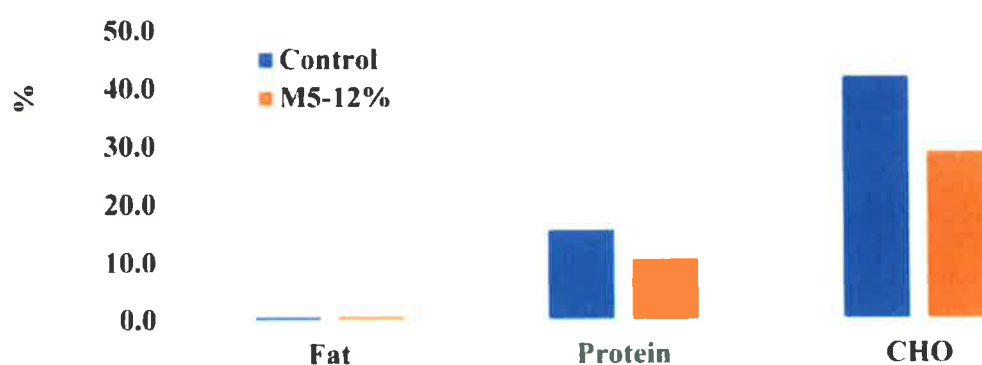
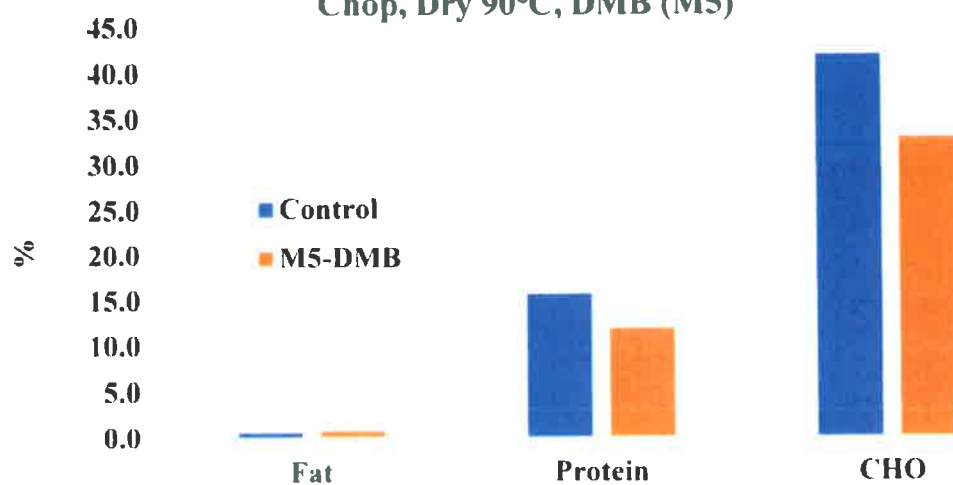


Fig 5b. Comparison of Nutritional Parameters of Control Alaria vs Blanch, Wash, Light Press, Salt, Press, Desalt, Chop, Dry 90°C, DMB (M5)



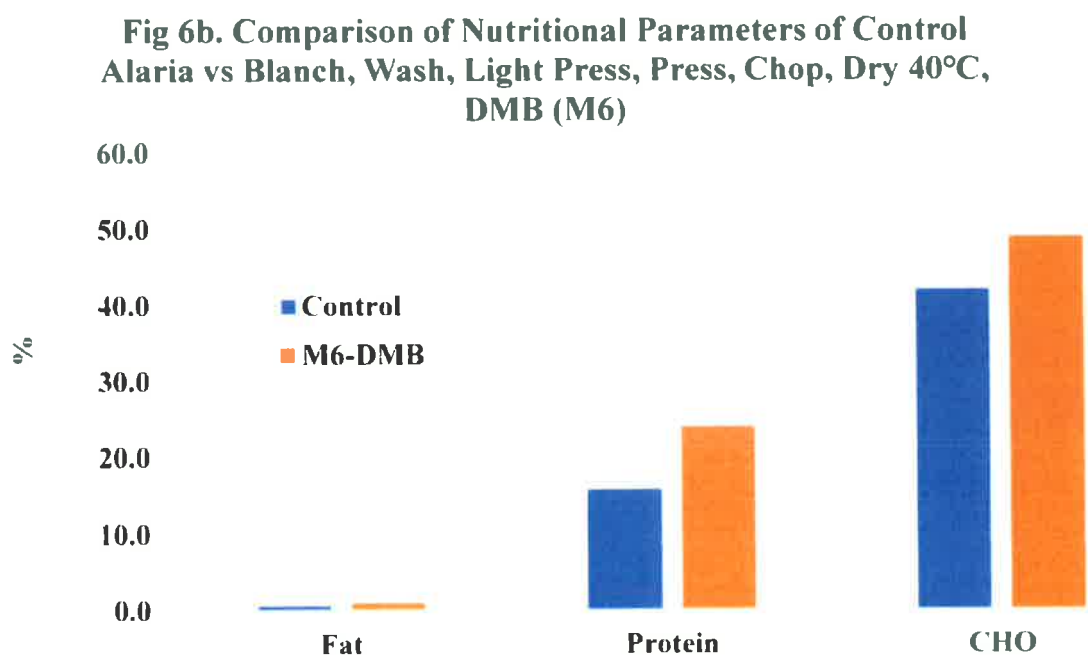
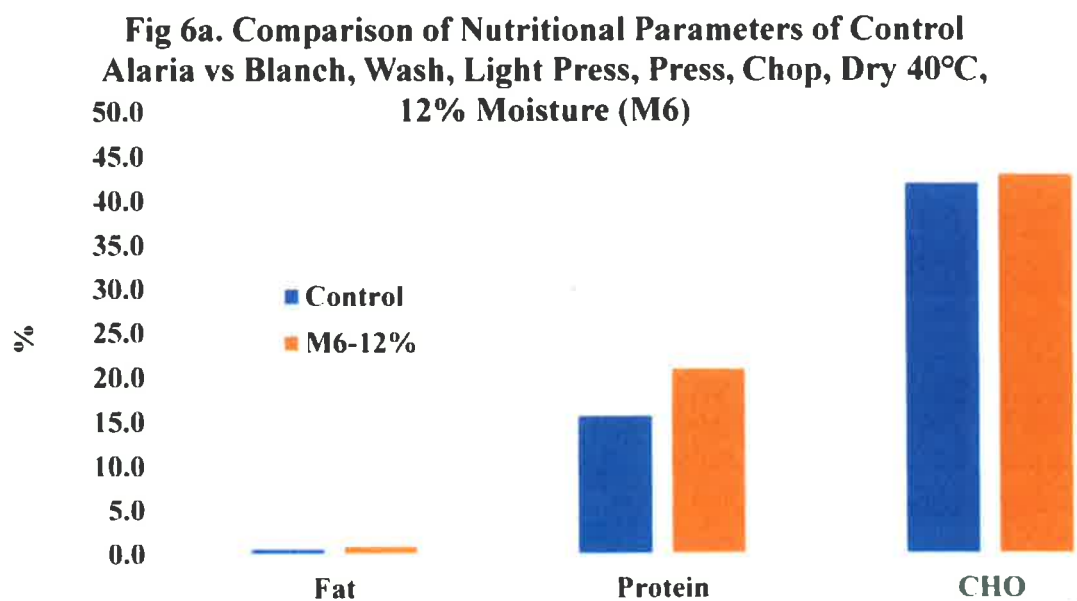
Method 6. (M6)

The 5kg batch of seaweed was blanched for 1 minute, washed in seawater bath at ambient temperature for 2 minutes, pressed lightly to remove excess seawater, pressed for 24 hours with 25kg, chopped and dried at 40°C.

Table 6. Nutritional Parameters of Alaria by M6 – Blanch, Wash, Press Lightly, Press, Chop and Dry at 40°C. Results at 12% Moisture and DMB.

	12% Moisture	DMB	Reference Value
Fat %	0.9	1.0	1 to 2 (13)
Ash %	23.2	26.4	14 to 32 (3)
Protein %	21.0	23.9	9 to 18(13)
E (kcal)	263.7	299.7	
E (kJ)	1121.4	1274.4	
CHO %	43.0	48.9	46 to 51 (14)

TVC cfu/ml >300,000



Method 7. (M7)

The 5kg batch of seaweed was blanched for 1 minute, washed in seawater bath at ambient temperature for 2 minutes, pressed lightly to remove excess seawater, pressed for 24 hours with 25kg, chopped and dried at 70°C.

Table 7. Nutritional Parameters of Alaria by M7 – Blanch, Wash, Press Lightly, Press, Chop and Dry at 70°C. Results at 12% Moisture and DMB.

	12% Moisture	DMB	Reference Value
Fat %	0.9	1.1	1 to 2 (13)
Ash %	23.3	26.4	14 to 32 (13)
Protein %	20.8	23.6	9 to 18 (13)
E (kcal)	263.9	299.8	
E (kJ)	1119.1	1271.5	
CHO %	43.0	48.8	46 to 51 (14)

TVC cfu/ml <10

Fig 7a. Comparison of Nutritional Parameters of Control Alaria vs Blanch, Wash, Light Press, Press, Desalt, Chop, Dry 70°C, 12% Moisture (M7)

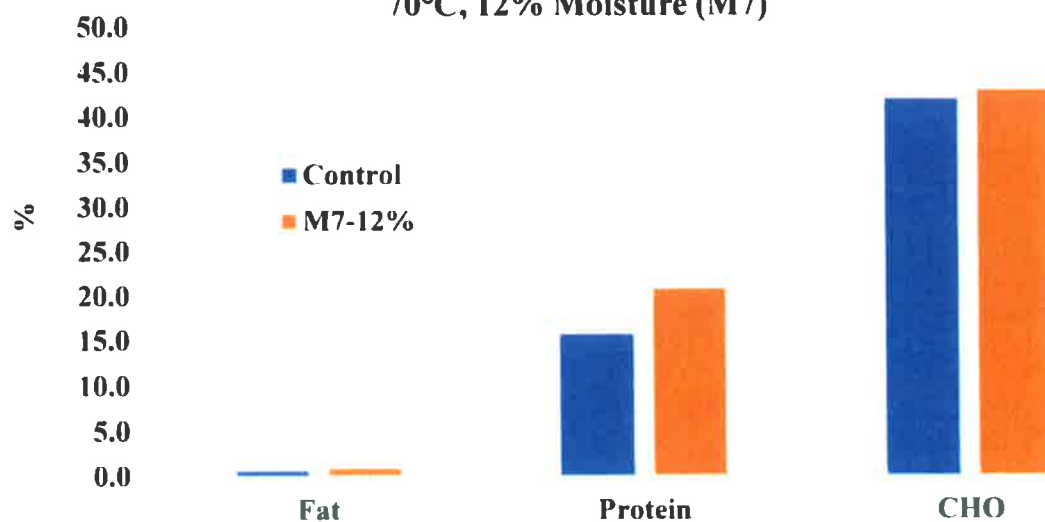
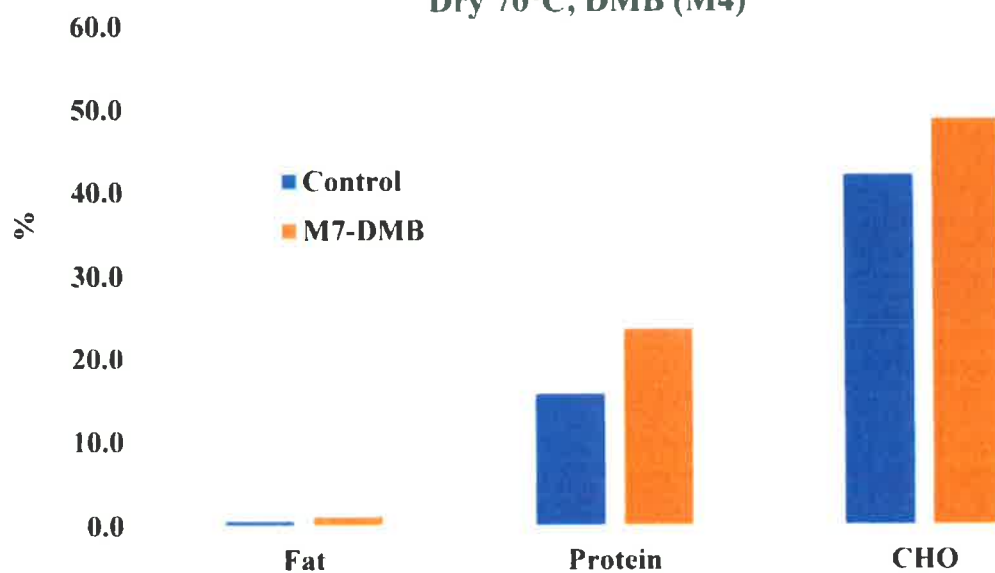


Fig 7b. Comparison of Nutritional Parameters of Control Alaria vs Blanch, Wash, Light Press, Salt, Press, Desalt, Chop, Dry 70°C, DMB (M4)



Method 8. (M8)

The 5kg batch of seaweed was blanched for 1 minute, washed in seawater bath at ambient temperature for 2 minutes, pressed lightly to remove excess seawater, pressed for 24 hours with 25kg, chopped and dried at 90°C.

Table 8. Nutritional Parameters of Alaria by M8 – Blanch, Wash, Press Lightly, Press, Chop and Dry at 90°C. Results at 12% Moisture and DMB.

	12% Moisture	DMB	Reference Value
Fat %	1.0	1.1	1 to 2 (13)
Ash %	27.6	31.3	14 to 32 (13)
Protein %	18.3	20.8	9 to 18 (13)
E (kcal)	246.8	280.4	
E (kJ)	1047.5	1190.2	
CHO %	41.3	46.9	46 to 51 (14)

TVC cfu/ml <10

Fig 8a. Comparison of Nutritional Parameters of Control Alaria vs Blanch, Wash, Light Press, Press, Chop, Dry 90°C, 12% Moisture (M8)

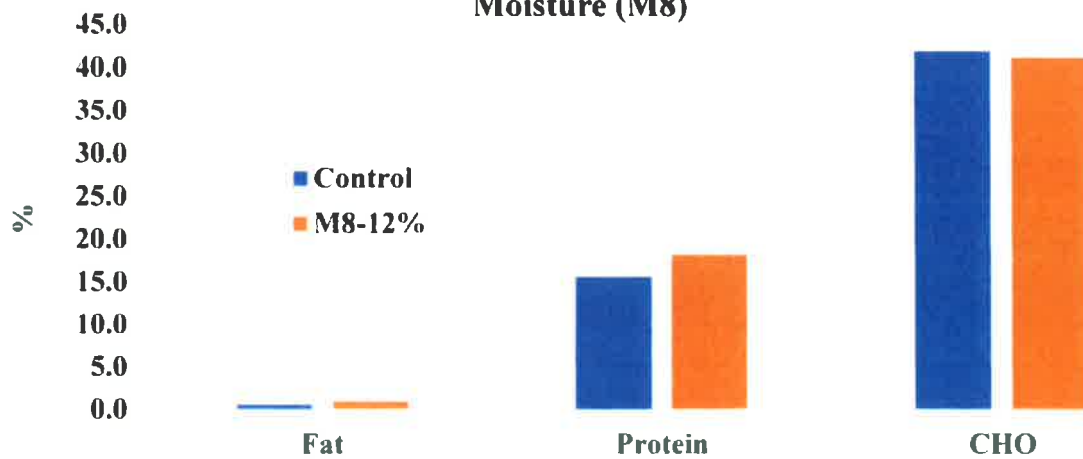
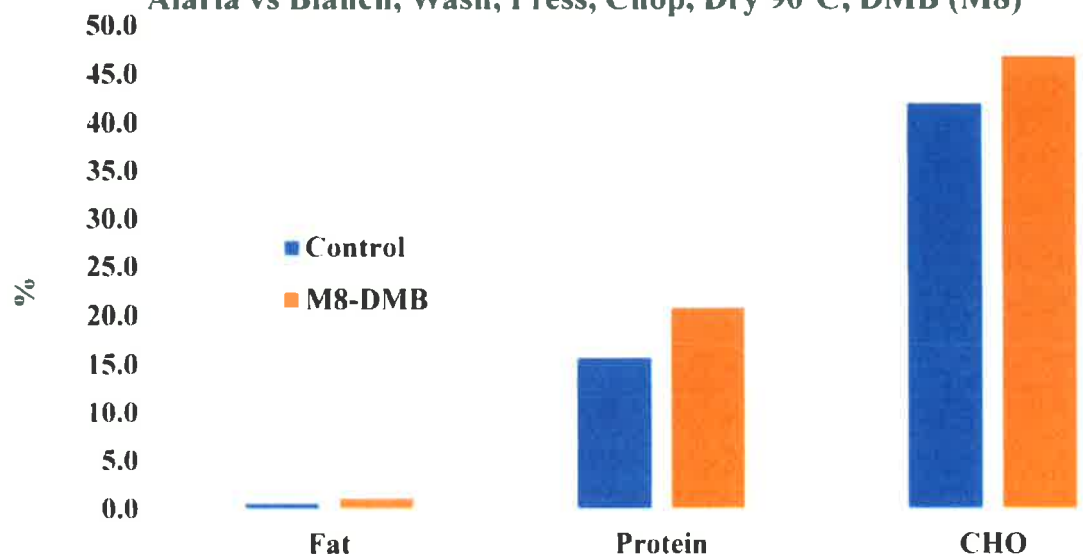


Fig 8b. Comparison of Nutritional Parameters of Control Alaria vs Blanch, Wash, Press, Chop, Dry 90°C, DMB (M8)



Method 9. (M9)

The 5kg batch of seaweed was washed in seawater bath at ambient temperature for 2 minutes, pressed lightly to remove excess seawater, pressed for 24 hours with 25kg, chopped and dried at 40°C.

Table 9. Nutritional Parameters of Alaria by M9 - Wash, Press Lightly, Press, Chop and Dry at 40°C. Results at 12% Moisture and DMB.

	12% Moisture	DMB	Reference Value
Fat %	0.6	0.7	1 to 2 (13)
Ash %	50.6	57.5	14 to 32 (13)
Protein %	10.6	12.0	9 to 18 (13)
E (kcal)	150.9	171.5	
E (kJ)	641.8	729.2	
CHO %	26.0	29.5	46 to 51 (14)
TVC cfu/ml	80		

Fig 9a. Comparison of Nutritional Parameters of Control Alaria vs Wash, Salt, Press, Desalt, Chop, Dry 40°C, 12% Moisture (M9)

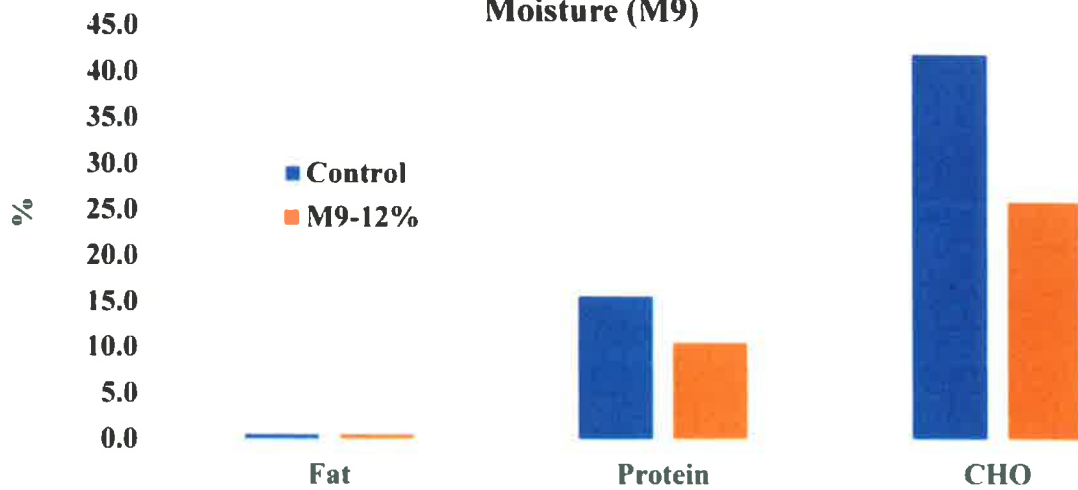
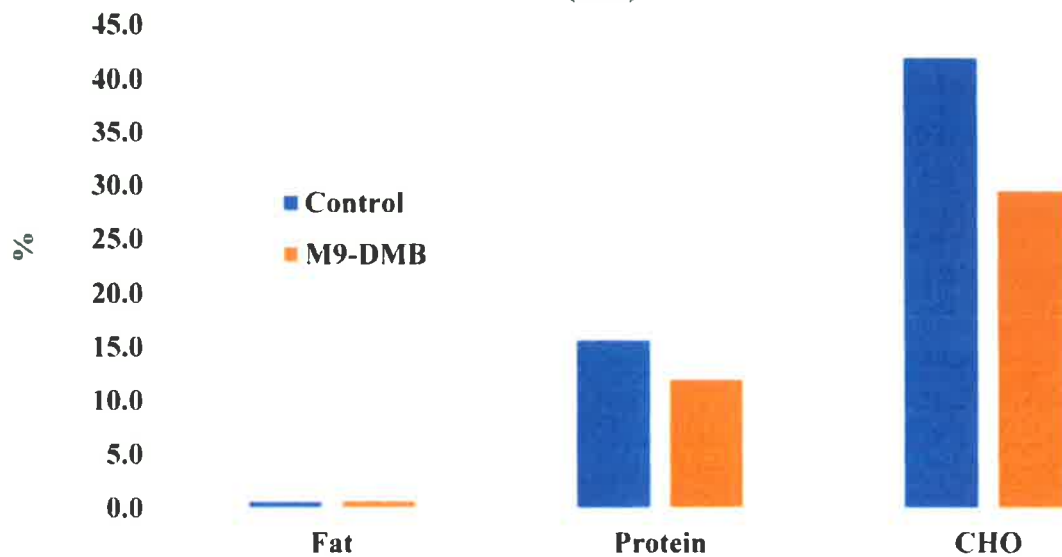


Fig 9b. Comparison of Nutritional Parameters of Control Alaria vs Wash, Salt, Press, Desalt, Chop, Dry 40°C, DMB (M9)



Method 10. (M10)

The 5kg batch of seaweed was washed in seawater bath at ambient temperature for 2 minutes, pressed lightly to remove excess seawater, pressed for 24 hours with 25kg, chopped and dried at 70°C.

Table 10. Nutritional Parameters of Alaria by M10 - Wash, Press Lightly, Press, Chop and Dry at 70°C. Results at 12% Moisture and DMB.

	12% Moisture	DMB	Reference Value
Fat %	0.6	0.7	1 to 2 (13)
Ash %	50.4	57.3	14 to 32 (13)
Protein %	11.5	13.1	9 to 18 (13)
E (kcal)	154.2	175.2	
E (kJ)	652.4	741.2	
CHO %	25.4	28.9	46 to 51 (14)
TVC cfu/ml	50		

Fig 10a. Comparison of Nutritional Parameters of Control Alaria vs Wash, Salt, Press, Desalt, Chop, Dry 70°C, 12% Moisture (M10)

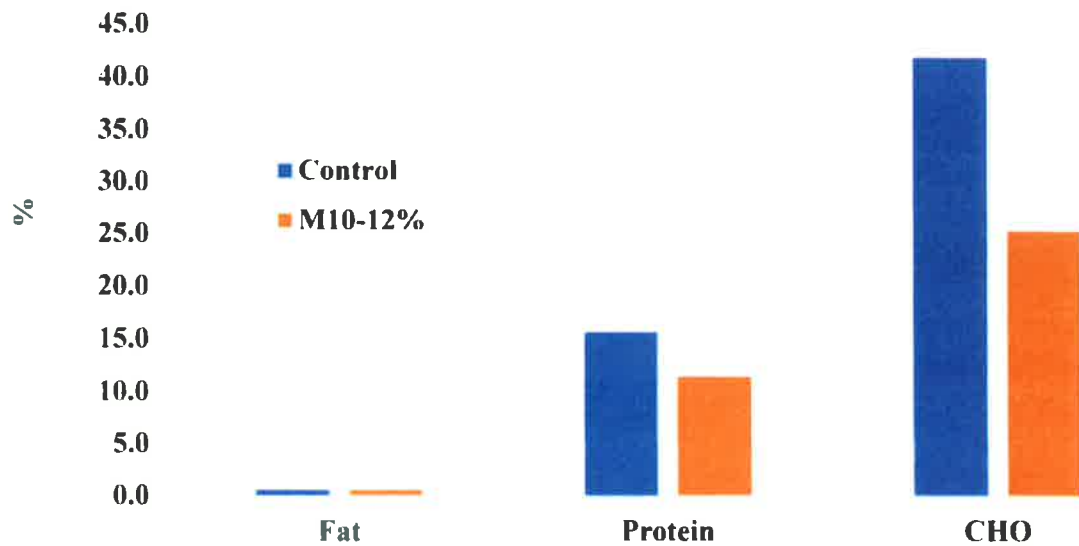
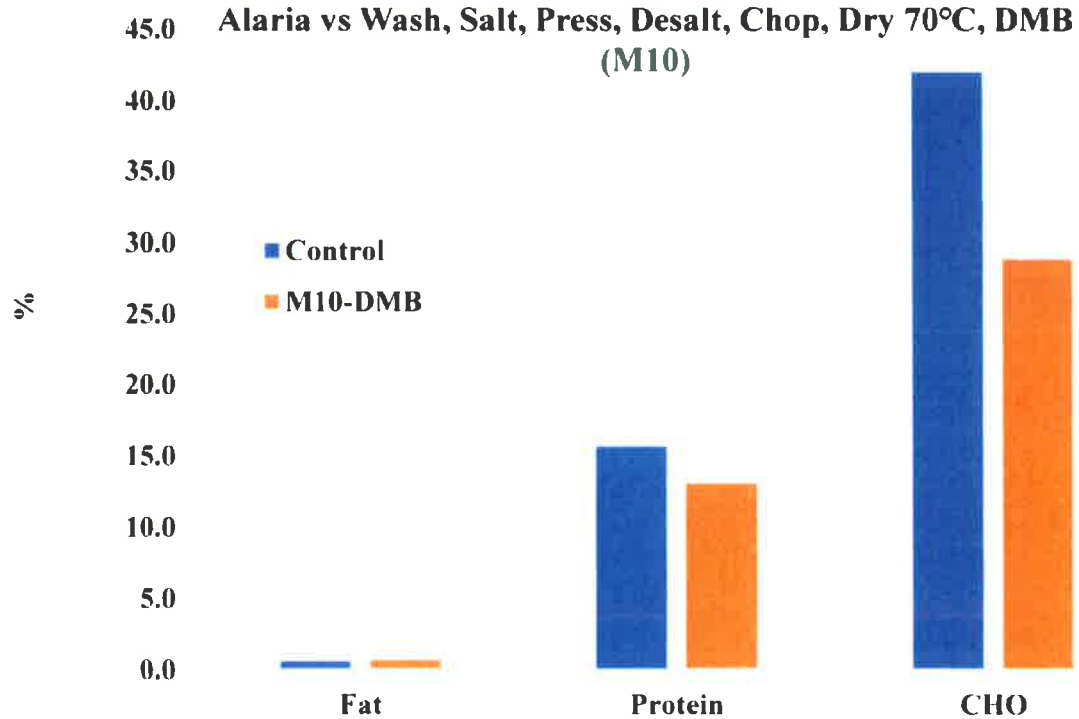


Fig 10b. Comparison of Nutritional Parameters of Control Alaria vs Wash, Salt, Press, Desalt, Chop, Dry 70°C, DMB (M10)



Method 11. (M11)

The 5kg batch of seaweed was washed in seawater bath at ambient temperature for 2 minutes, pressed lightly to remove excess seawater, pressed for 24 hours with 25kg, chopped and dried at 90°C.

Table 11. Nutritional Parameters of Alaria by M11 - Wash, Press Lightly, Press, Chop and Dry at 90°C. Results at 12% Moisture and DMB.

	12% Moisture	DMB	Reference Value
Fat %	0.8	0.9	1 to 2 (13)
Ash %	40.0	45.5	14 to 32 (13)
Protein %	12.7	14.4	9 to 18 (13)
E (kcal)	196.2	223.0	
E (kJ)	830.7	944.0	
CHO %	34.4	39.1	46 to 51 (14)

TVC cfu/ml 130

Fig 11a. Comparison of Nutritional Parameters of Control Alaria vs Wash, Salt, Press, Desalt, Chop, Dry 90°C, 12% Moisture (M11)

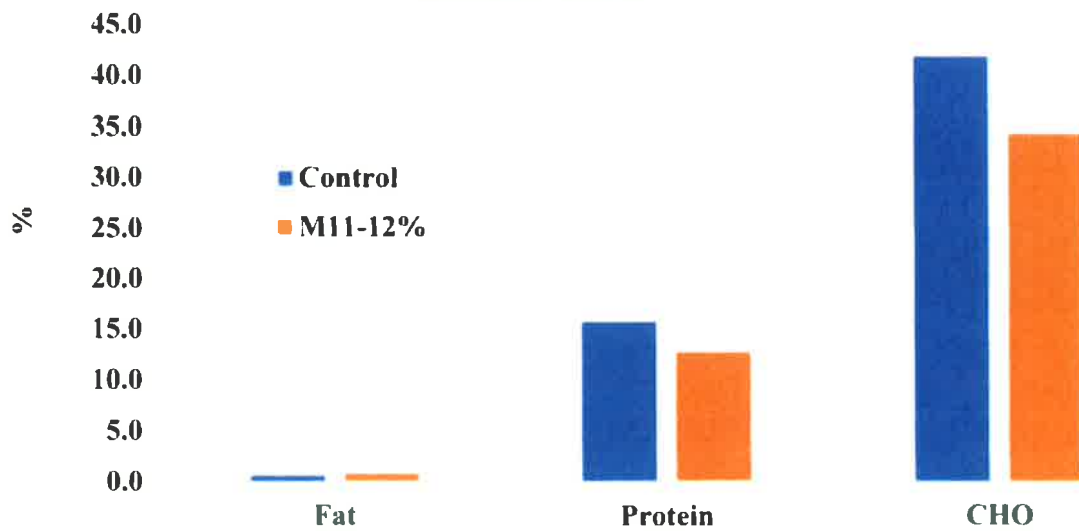
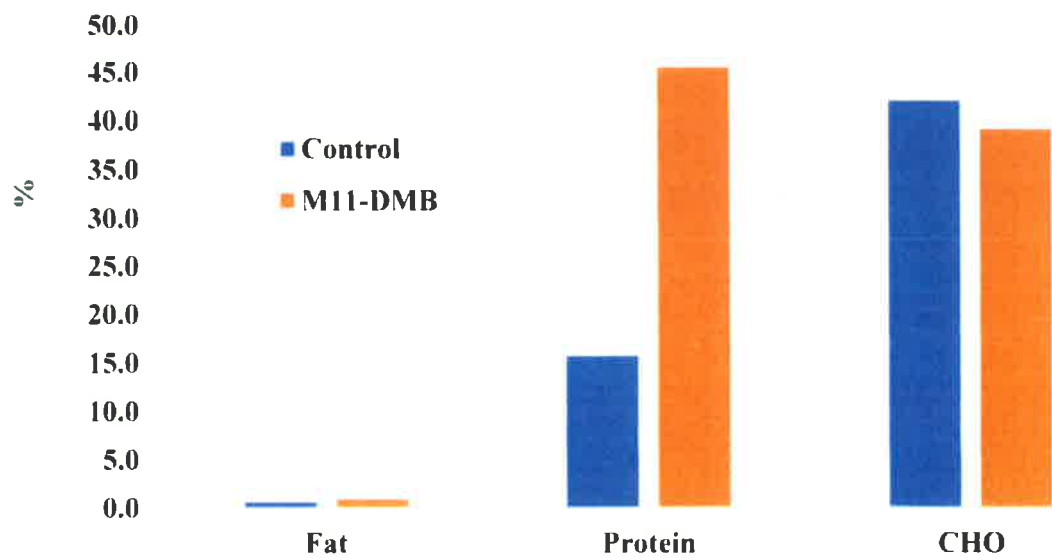


Fig 11b. Comparison of Nutritional Parameters of Control Alaria vs Wash, Salt, Press, Desalt, Chop, Dry 90°C, DMB (M11)



Method 12. (M12)

The 500g batch of seaweed was blanched for 1 minute, washed in seawater bath at ambient temperature for 2 minutes, pressed lightly to remove excess seawater, salted with 150g table salt (30 parts salt/100 parts seaweed) for 24 hours, pressed with 25kg for 24 hours and Frozen at -20°C.

Table 12. Nutritional Parameters of Alaria by M12 - Blanch, Wash, Light Press, Salt, Press, Freeze. Results at 12% Moisture and DMB.

	12% Moisture	DMB	Reference Value
Fat %	0.6	0.7	1 to 2 (13)
Ash %	50.7	57.7	14 to 32 (13)
Protein %	12.3	14.0	9 to 18 (13)
E (kcal)	152.5	173.3	
E (kJ)	642.3	729.9	
CHO %	24.3	27.7	46 to 51 (14)

TVC cfu/ml 5,100

Fig 12a. Comparison of Nutritional Parameters of Control Alaria vs Blanch, Wash, Salt, Press, Freeze, 12% Moisture (M12)

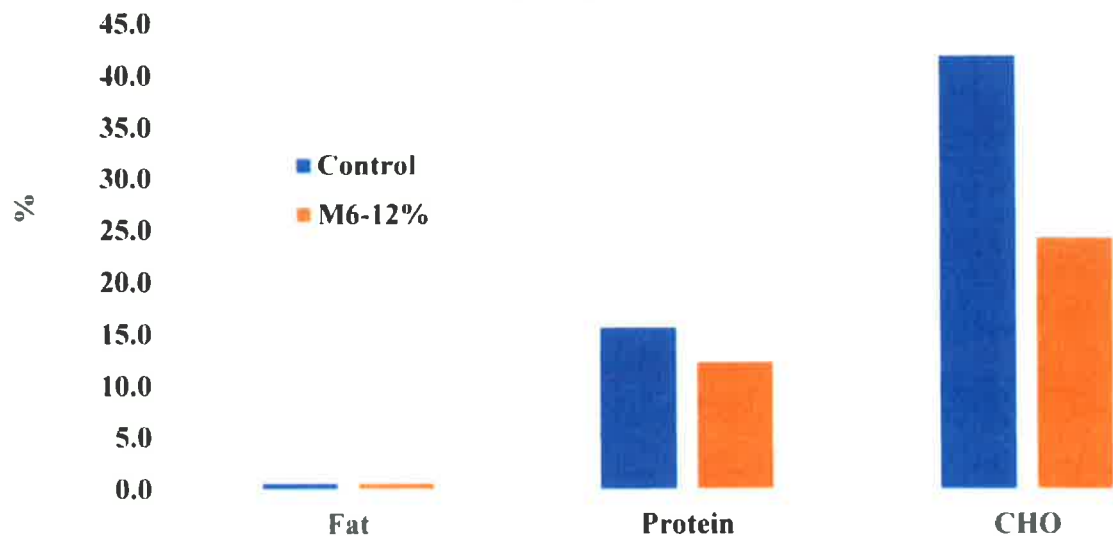
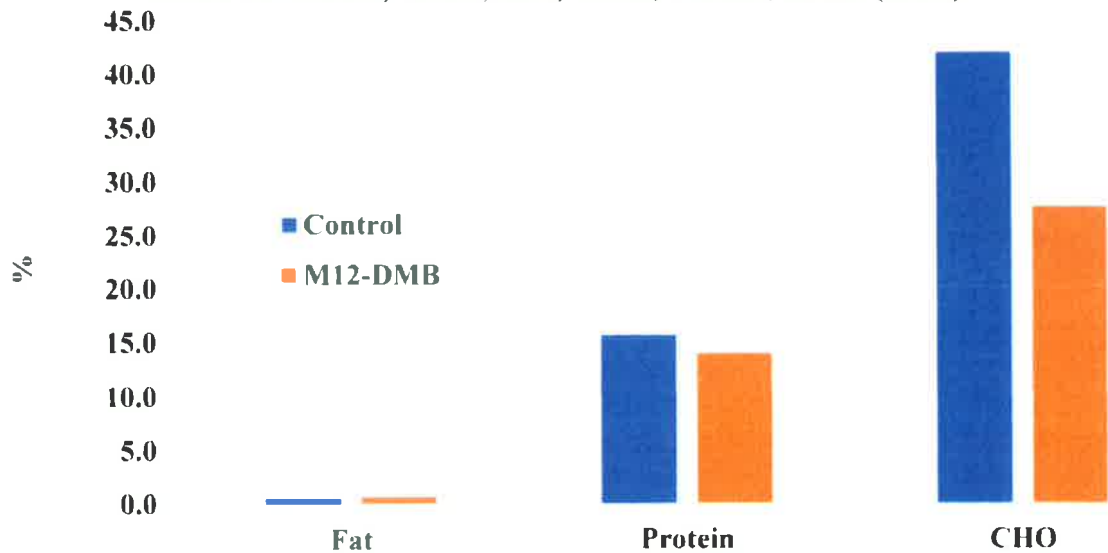


Fig 12b. Comparison of Nutritional Parameters of Control Alaria vs Blanch, Wash, Salt, Press, Freeze, DMB (M12)



Method 13. (M13)

The 500g batch of seaweed was blanched for 1 minute, washed in seawater bath at ambient temperature for 2 minutes, pressed lightly to remove excess seawater, pressed with 25kg for 24 hours and Frozen at -20°C.

Table 13. Nutritional Parameters of Alaria by M13 - Blanch, Wash, Light Press, Press, Freeze. Results at 12% Moisture and DMB.

	12% Moisture	DMB	Reference Value
Fat %	0.6	0.6	1 to 2 (13)
Ash %	23.4	26.6	14 to 3 (13)
Protein %	24.6	27.9	9 to 18 (13)
E (kcal)	102.9	116.9	
E (kJ)	434.3	493.5	
CHO %	40.0	45.5	46 to 51 (14)
TVC			
cfu/ml	120		

Fig 13a. Comparison of Nutritional Parameters of Control Alaria vs Blanch, Wash, Press, Freeze, 12% Moisture (M13)

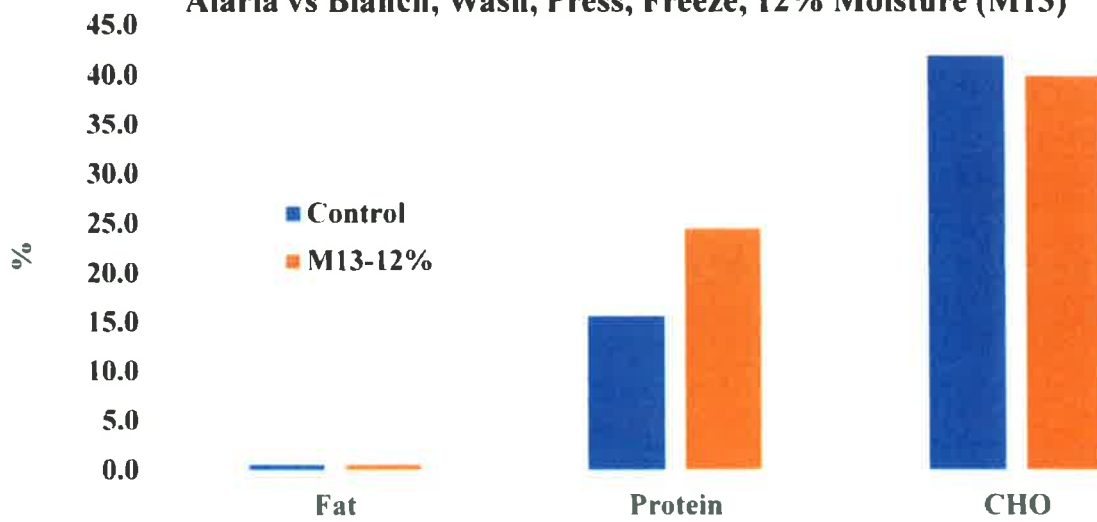
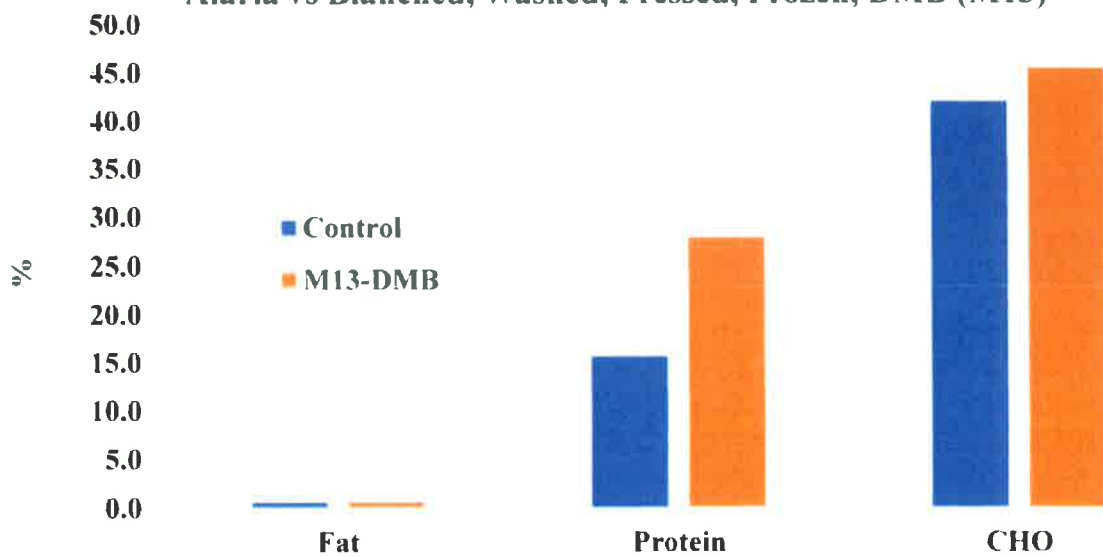


Fig 13b. Comparison of Nutritional Parameters of Control Alaria vs Blanched, Washed, Pressed, Frozen, DMB (M13)



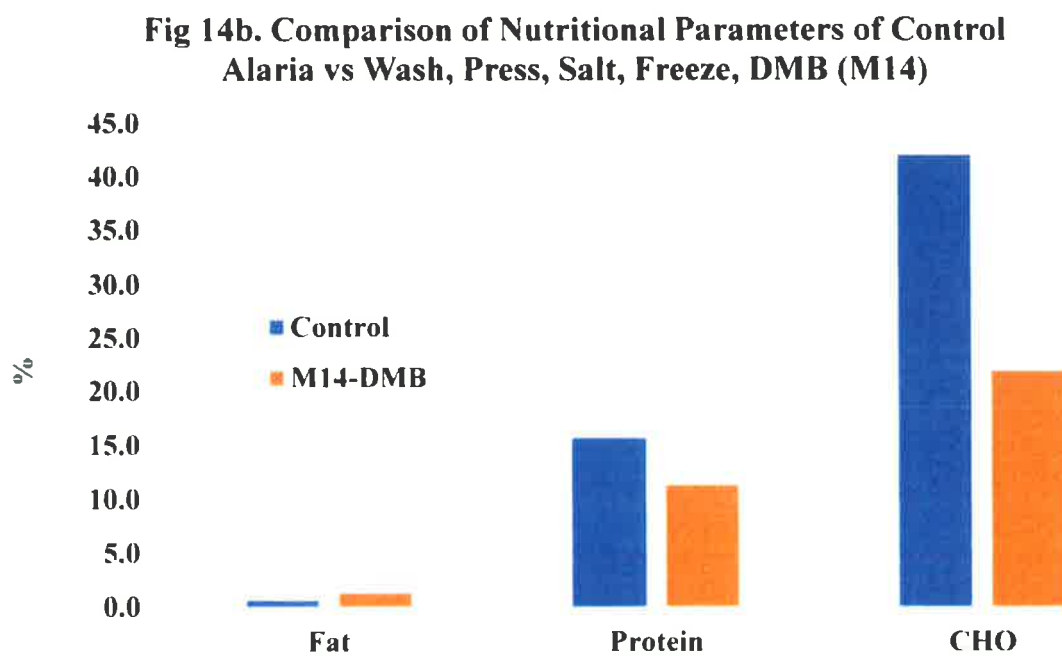
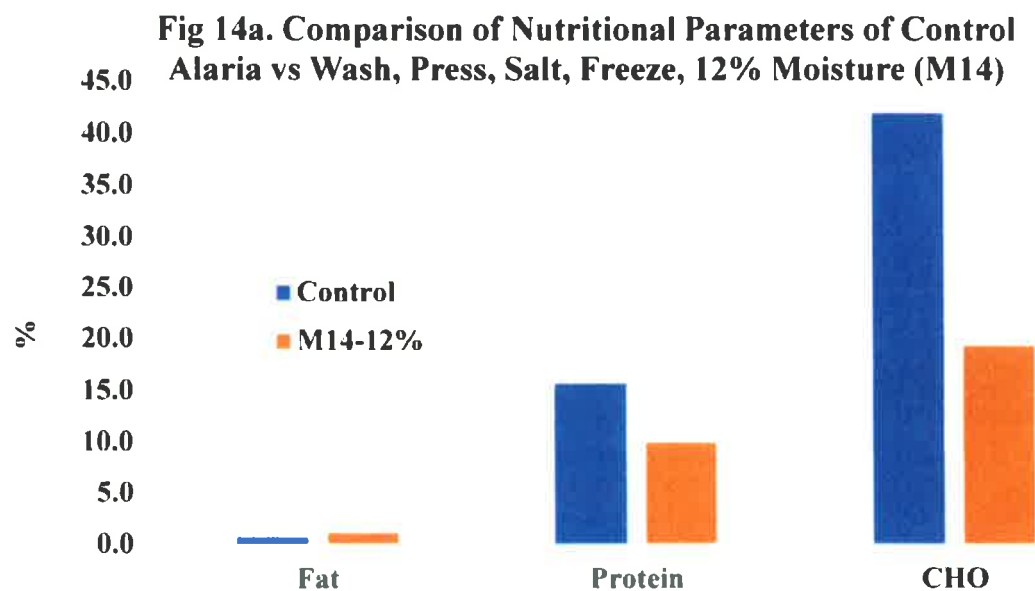
Method 14. (M14)

The 500g batch of seaweed was washed in seawater bath at ambient temperature for 2 minutes, pressed lightly to remove excess seawater, pressed with 25kg for 24 hours, salted with 150g table salt (30 parts salt/100 parts seaweed) for 24 hours and frozen at -20°C.

Table 14. Nutritional Parameters of Alaria by M14 - Wash, Light Press, Press, Salt, Freeze. Results at 12% Moisture and DMB.

	12% Moisture	DMB	Reference Value
Fat %	1.1	1.3	1 to 2 (13)
Ash %	57.6	65.5	14 to 32 (13)
Protein %	9.9	11.3	9 to 18 (13)
E (kcal)	124.9	141.9	
E (kJ)	533.7	606.5	
CHO %	19.3	21.9	46 to 51 (14)

TVC cfu/ml 67,000



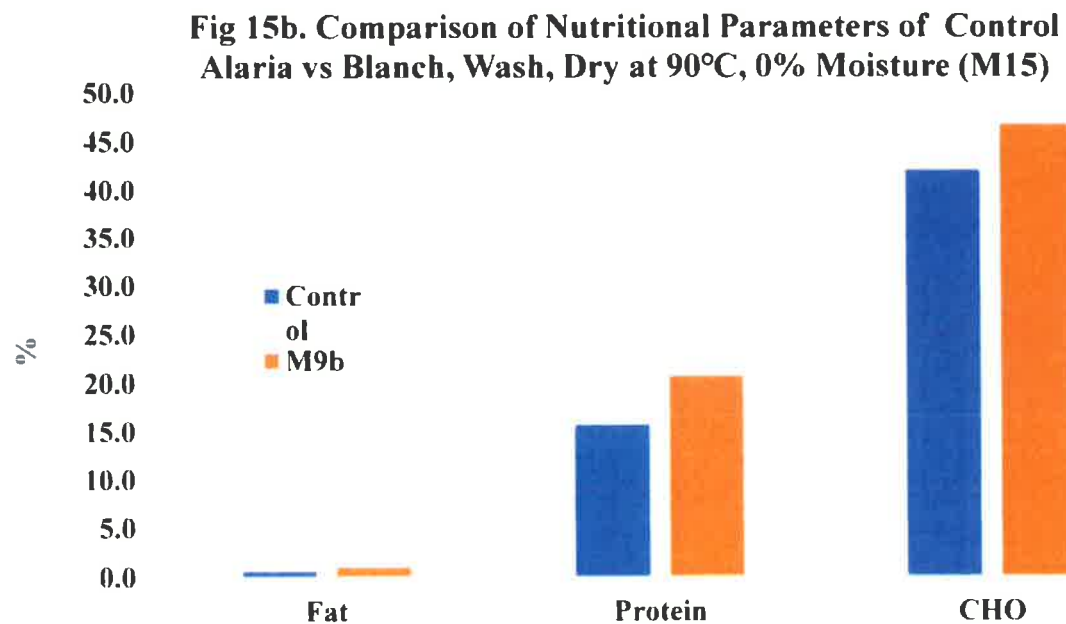
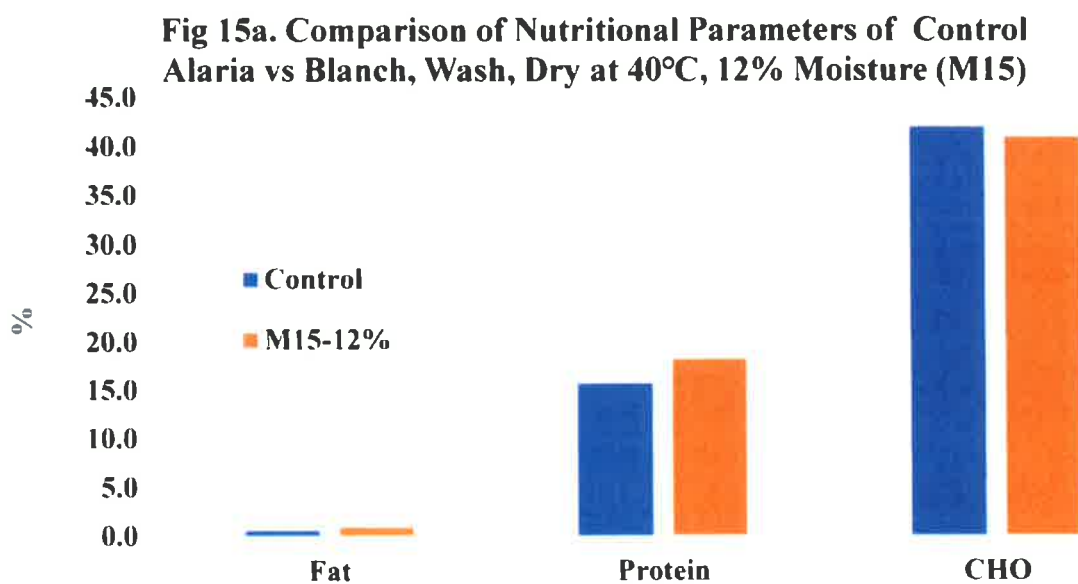
Method 15. (M15)

The 5kg batch of seaweed was blanched for 1 minute, washed in seawater bath at ambient temperature for 2 minutes, pressed lightly to remove excess seawater and dried at 40°C.

Table 15. Nutritional Parameters of Alaria by M15 – Blanch, Wash, Light Press, Dry at 40°C. Results at 12% Moisture and DMB.

	12% Moisture	DMB	Reference Value
Fat %	0.9	1.0	1 to 2 (13)
Ash %	28.0	31.8	14 to 32 (13)
Protein %	18.2	20.7	9 to 18 (13)
E (kcal)	244.1	277.4	
E (kJ)	1037.0	1179.0	
CHO %	41.0	46.6	46 to 51 (14)

TVC cfu/ml 7,800



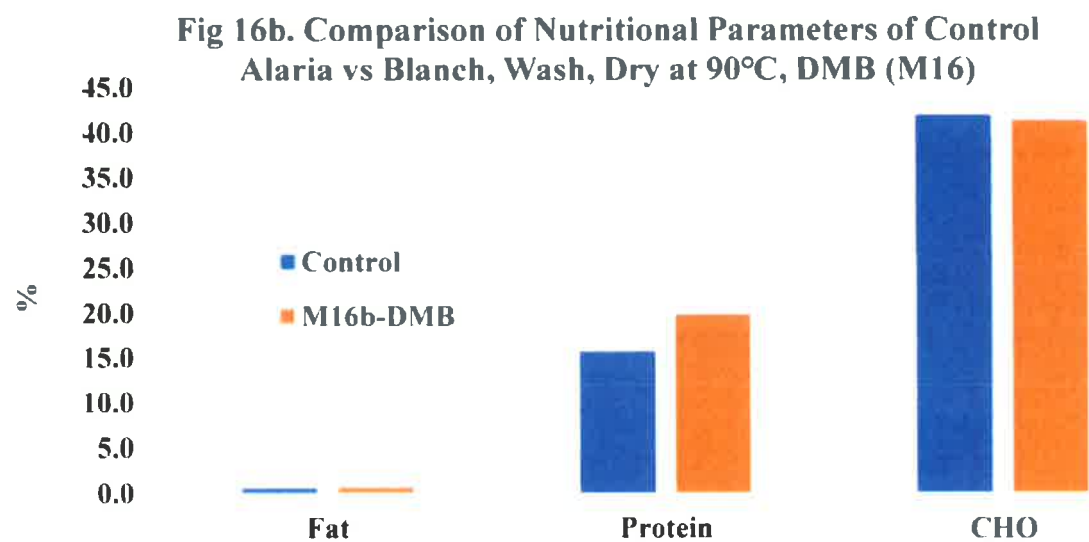
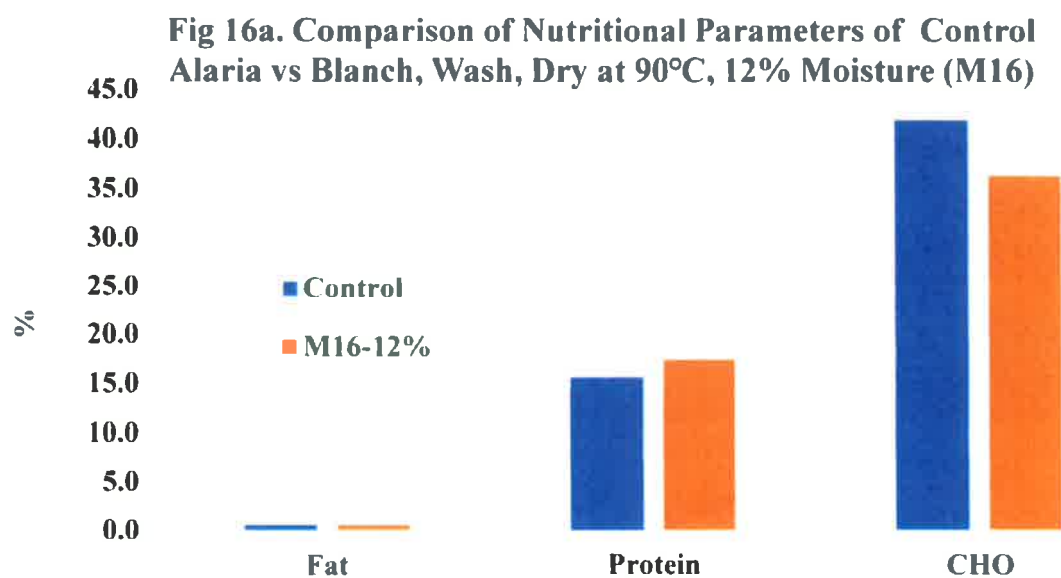
Method 16. (M16)

The 5kg batch of seaweed was blanched for 1 minute, washed in seawater bath at ambient temperature for 2 minutes, pressed lightly to remove excess seawater and dried at 90°C.

Table 16. Nutritional Parameters of Alaria by M16 – Blanch, Wash, Light Press, Dry at 90°C. Results at 12% Moisture and DMB.

	12% Moisture	DMB	Reference Value
Fat %	0.6	0.7	1 to 2 (13)
Ash %	33.5	38.1	14 to 32 (13)
Protein %	17.5	19.9	9 to 18 (13)
E (kcal)	237.4	269.7	
E (kJ)	1007.8	1145.0	
CHO %	36.4	41.4	46 to 51 (14)

TVC cfu/ml <10

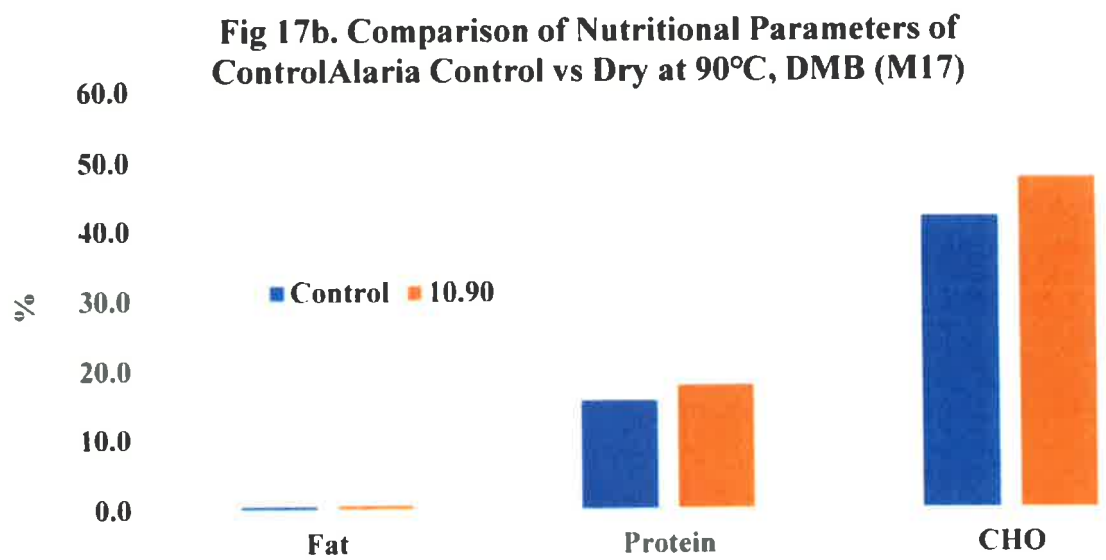


Method 17. (M17)

The 5kg batch of seaweed dried at 40°C and the 12% Moisture sample was employed as the Control sample for this study. There is no comparative bar chart for the Control (Fig 17a) but there is one for Control vs DMB.

Table 17. Nutritional Parameters of Alaria by M17 – Dry at 40°C. Results at 12% Moisture and DMB.

	12% Moisture	DMB	Reference Value
Fat %	0.6	0.7	1 to 2 (13)
Ash %	29.6	33.6	14 to 32 (13)
Protein %	15.7	17.9	9 to 18 (13)
E (kcal)	236.9	269.1	
E (kJ)	1004.7	1141.4	
CHO %	42.0	47.7	46 to 51 (14)
TVC cfu/ml	230,000		



Method 18. (M18)

The 5kg batch of seaweed dried at 90°C. and the 12% Moisture sample was employed as the Control sample for this study.

Table 18. Nutritional Parameters of Alaria by M18 – Dry at 90°C. Results at 12% Moisture and DMB.

	12% Moisture	DMB	Reference Value
Fat %	0.7	0.7	1 to 2 (13)
Ash %	28.4	32.3	14 to 32 (13)
Protein %	18.5	21.0	9 to 18 (13)
E (kcal)	241.2	274.3	
E (kJ)	1025.7	1166.1	
CHO %	40.4	45.9	46 to 51 (14)

TVC cfu/ml 110

Fig 18a. Comparison of Nutritional Parameters of ControlAlaria Control vs Dry at 90°C, 12% Moisture (M18)

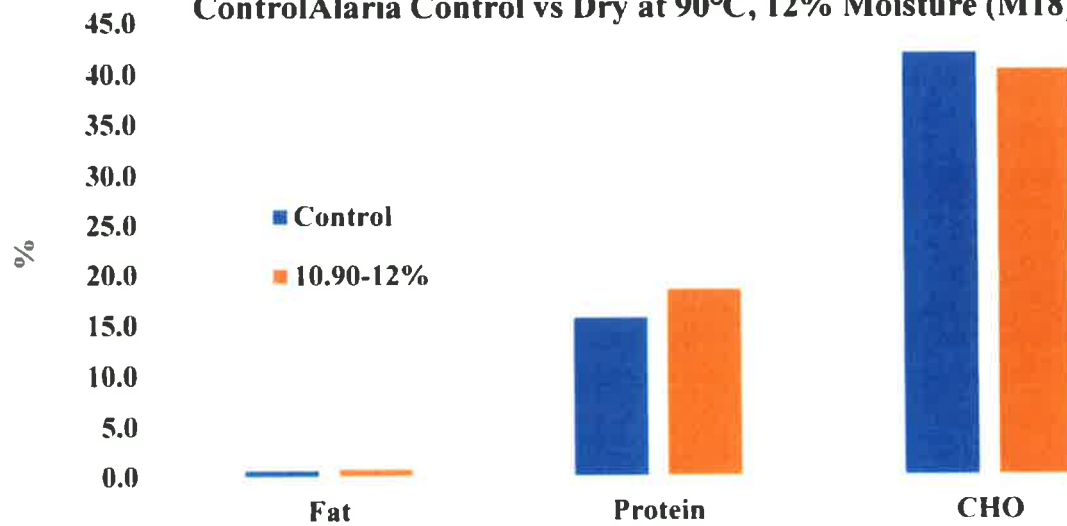
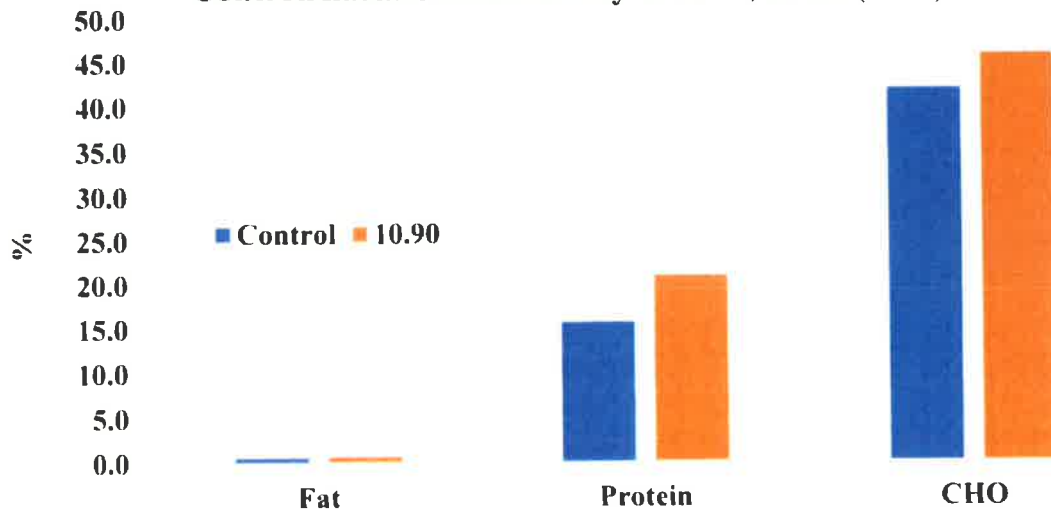


Fig 18b. Comparison of Nutritional Parameters of ControlAlaria Control vs Dry at 90°C, DMB (M18)



Discussion

Table 1 showed normal Fat and Protein levels, but low CHO compared to reported reference values. It should be noted that the reference values cited were on a on a dry matter basis. Although a blanching step was employed the TVC of 730cfu/ml was low.

Fig 1a and Fig 1b showed a slightly raised Fat level compared to the Control, but Protein and CHO were significantly lower that the Control levels.

Table 2 showed low Fat, Protein levels and CHO compared to reported reference values. Although a blanching step was not employed, the TVC of <10cfu/ml was unexpected and would not be considered a hazard to human health.

Fig 2a and Fig 2b showed similar Fat level compared to the Control, but Protein and CHO were significantly lower that the Control levels.

Table 3 showed lowest Fat, low Protein levels and CHO compared to reported reference values. Although a blanching step was employed the TVC of 3,600cfu/ml may have been related to the drying step at 40°C which would have allowed bacterial growth. However, this value was low.

Fig 2a and Fig 2b showed low Fat level compared to the Control, and Protein and CHO were significantly lower that the Control levels.

Table 4 showed low Fat and CHO levels compared to reported reference values. Although a blanching step was employed the reduced TVC compared to M3 of 90cfu/ml may have been related to the drying step at 70°C which would have inhibited bacterial growth. However, this value was low and would not be considered a hazard to human health.

Fig 4a and Fig 4b showed similar Fat level compared to the Control, but Protein and CHO were significantly lower that the Control levels.

Table 5 showed low Fat and slightly higher CHO levels compared to reported reference values. Although a blanching step was employed the reduced TVC compared to M3 of 210cfu/ml may have been related to the drying step at 90°C which would have inhibited bacterial growth.

Fig 5a and Fig 5b showed similar Fat level compared to the Control, with Protein and CHO were slightly higher than the batch dried at 70°C.

Table 6 showed Fat within the guide level and Protein above the reference levels. Higher CHO levels compared to previous results were also noted, being also above the Control level. Although a blanching step was employed the TVC of >300,000cfu/ml may have been related to the drying step at 40°C which would have allowed bacterial growth. However, this value was very high and may be considered a hazard to human health.

Fig 6a and Fig 6b showed similar Fat level compared to the Control, with Protein and CHO higher than previous batches and above the Control levels.

Table 7 showed similar results to Table 6, with Fat within the guide level and Protein above the reference levels. Higher CHO levels compared to previous results were also noted, being also above the Control level and close to the Reference level. Although a blanching step was employed the TVC <10cfu/ml may have been related to the drying step at 70°C which would have inhibited bacterial growth.

Fig 7a and Fig 7b showed similar Fat level compared to the Control, with Protein and CHO higher than previous batches and above the Control levels.

Table 8 showed similar results to Table 6, with Fat within the guide level and Protein above the Reference levels. Higher CHO levels compared to previous results were also noted, being also above the Control level on a DMB basis and close to the Reference level. Although a blanching step was employed the TVC <10cfu/ml may have been related to the drying step at 90°C which would have inhibited bacterial growth.

Fig 8a and Fig 8b showed similar Fat level compared to the Control, with Protein and CHO higher than previous batches and above the Control levels.

Table 9 showed Fat below the guide level and Protein within the reference levels. Lower Protein and CHO levels compared to previous results were also noted, with the CHO being also below the Reference levels. Although no blanching step was employed the TVC 80cfu/ml was low considering the drying step at 40°C which should have allowed bacterial growth.

Fig 9a and Fig 9b showed similar Fat. Protein and CHO lower than previous batches and below the Control levels in both batches.

Table 10 showed Fat below the guide level and Protein within the reference levels. Lower Protein and CHO levels compared to previous results were also noted, with the CHO being also below the Reference levels. Although a blanching step was not employed the TVC 50cfu/ml was and may have been related to the drying step at 70°C which would have inhibited bacterial growth.

Fig 10a and Fig 10b showed similar Fat, Protein and CHO lower than previous batches and below the Control levels in both batches.

Table 11 showed Fat below the guide level and Protein within the reference levels. Lower Protein and CHO levels compared to previous results were also noted, with the CHO being higher than Table 10, but below the Reference levels. Although a blanching step was not employed the TVC 130cfu/ml was low and may have been related to the drying step at 90°C which would have inhibited bacterial growth.

Fig 11a and Fig 11b showed similar Fat, Protein and CHO to Fig 10a and Fig 10b, and below the Control levels in both batches.

Table 12 showed Fat below the Reference levels and Protein within the Reference levels. Similar Protein but lower CHO levels compared to Table 11, were also noted, but below the Reference levels. Although a blanching step was employed, the TVC 5,100cfu/ml may have been related to the drying step not being employed.

Fig 12a and Fig 12b showed similar Fat, Protein and CHO to Table 10. With CHO below the Control levels in both batches.

Table 13 showed Fat below the Reference levels and Protein higher than the Reference levels. Protein levels were within the Reference levels with high CHO levels compared to Table 12. A blanching step was employed and the TVC 120cfu/ml was low.

Fig 13a and Fig 13b showed similar Fat, higher Protein and CHO compared to Fig 12a and Fig 12b.

Table 14 showed Fat within the Reference levels and Protein just within the Reference levels, with low CHO levels compared to Table 12. No blanching step was employed the TVC 67,000cfu/ml was high which may have allowed bacterial growth.

Fig 14a and Fig 14b showed similar Fat, lower Protein and CHO compared to Fig 13a and Fig 13b.

Table 15 showed Fat within the Reference levels and Protein just above the Reference levels, with similar CHO levels compared to Table 12. A blanching step and a drying step at 40°C was employed and the TVC of 7,800cfu/ml was high which may have allowed bacterial growth.

Fig 15a and Fig 15b showed similar Fat, higher Protein and CHO compared to Control levels.

Table 16 showed Fat lower than the Reference levels and Protein at the higher end of the Reference levels, with CHO below the Reference levels. A blanching step and a drying step at 90°C was employed and the TVC of <10cfu/ml was demonstrated, which would have inhibited bacterial growth.

Fig 16a and Fig 16b showed similar Fat, slightly higher Protein and CHO just below the Control levels.

Table 17 showed Fat lower than the Reference levels and Protein at the higher end of the Reference levels, with CHO below the Reference levels for the 12% Moisture only. A drying step at 40°C was employed and the TVC of 230,000cfu/ml was high which may have allowed bacterial growth.

Fig 17b showed similar Fat, slightly higher Protein and CHO above the Control level.

Table 18 showed Fat lower than the Reference levels and Protein above the Reference levels, with CHO just below the Reference levels. A drying step at 90°C was employed and the TVC of 110cfu/ml was demonstrated, which would have inhibited bacterial growth.

Fig 18a and Fig 18b showed similar Fat, slightly higher Protein and CHO just below the Control level.

F. J. Bloomfield, Ph.D.

Scientific Director.

29th May 2017

Chemical Analysis Laboratories Limited. Directors: Dr. Jack Bloomfield, Philip Morgan. Company Registration No. 30465

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