

*Full Length Research Paper*

# **The nutritional values of two species of sea cucumbers (*Holothuria scabra* and *Holothuria lessoni*) from Madagascar**

**Valerie Lalao RAZAFINDRATOVO ANDRIAMANAMISATA<sup>1\*</sup> and Antonio  
Fidinirina TELESPHORE<sup>2\*</sup>**

Department of Food Science and Nutrition, Faculty of Science and Technology, University of Antananarivo,  
Madagascar.

Received 17 April, 2019; Accepted 29 October, 2019

Sea cucumbers are fishery products that are not used for consumption by the Malagasy people. In this study, nutritional values of two species of trepangs (air dried sea cucumber) from Madagascar *Holothuria scabra* and *Holothuria lessoni* were determined including moisture, ash, fat, protein and carbohydrate. The results showed that *H. scabra* and *H. lessoni* had respective water contents of 13 and 14%, the dry matter contents were around 86%. Results on crude protein were around 47% for *H. scabra* and 41% for *H. lessoni*, which constitute almost half of their total composition. Crude ash represented 32% of the dry matter for *H. scabra* and 39% for *H. lessoni*. Lipids and carbohydrates are low (between two and three percent for lipids and about eight percent for carbohydrates). The findings from this study have shown that there are significant differences (p-value <0.005) in nutrient composition in the two species. This low moisture content ensures a long preservation time of the food. These two species are very rich in proteins; this richness in proteins could be an asset in the fight against the malnutrition existing in Madagascar. The high quantity of ash is already sufficient to assume that there are a high quantity and quality of mineral elements. The objective of this study is to determine the nutritional value of the two species for possible presentation to consumers.

**Key words:** Sea cucumbers, Trepangs, nutritional analysis, *Holothuria scabra*, *Holothuria lessoni*, Madagascar.

## **INTRODUCTION**

For many centuries, sea cucumbers have been used for culinary and traditional therapeutic purposes in many Asian countries (Conand, 1994). They have been exploited in Madagascar since 1920, and they rank 6<sup>th</sup> in terms of economic value compared to other fishery products. The island has a wide variety of these species, but only more than thirty are exploited. Their exploitation is an excellent source of income for fishermen and

collectors (between 25000 Ariary and 90000 Ariary per kilogram for dried sea cucumbers, between 500 Ariary and 4000 Ariary per kilogram for fresh ones in 2007 (Eeckhaut et al., 2009) (Ariary is the basic monetary unit of Madagascar). The commercial importance is high, which reached 699 tons in 2012 (MRHP, 2014). All the productions are exported to Asian countries where trepangs (sea cucumber air-dried) are highly appreciated;

\*Corresponding author. E-mail: lalaovalerie@yahoo.fr; antonio.telesphore@gmail.com.

no document mentions its use in Malagasy meals.

Madagascar is a country that presents a wide range of varied pedoclimatic contexts and enjoys a remarkable richness in terms of both its specific biodiversity (flora and fauna) and eco-system, and its soil (MNHN, 2016). The country thus has assets that are a real economic potential. Despite the existence of food resources with high nutritional potentials in the country, more than half of the households in it suffer from chronic or temporary food insecurity (FAO and PAM, 2017). The Malagasy diet is characterized by a predominance of carbohydrate intake (77 to 79%) and a low intake or bioavailability of various important micronutrients and macronutrients, such as proteins (10%) and lipids (11 to 13%), essential fatty acids, iron, vitamins and calcium (Bader et al., 2005). For fighting this problem of nutrition, promoting untapped resources must be a priority in order to increase the availability and variety of foods, especially foods rich in proteins, in minerals and in vitamins. Since Madagascar is an island, coastal environments are likely to provide the population with fishery resources including sea cucumbers.

Sea cucumbers are considered to be excellent nutritional foods, rich in proteins, low in fat, high in amino acids and trace elements (Chen, 2004). Oedjoe (2017) described the nutrient content of fresh sea cucumbers which included protein (21% to 44.07%), fat (1.01% to 1.19%), carbohydrate (0.5% to 2.34%), ash (2.01 to 3.07%) and water (76.03 to 79.43%). Meanwhile Attaran et al. (2017) reported respectively the proximate composition of moisture, protein, fat and ash were 93, 4.4, 0.2 and 2% in *H. arenicola*. Hanna et al. (2017) reported that body wall of *H. scabra* (air dried) content around 12.13% of moisture, 55.18% of protein, 27.97% of ash and 1.02% of lipid. However, their nutritional importance remains unrecognized by the majority of the Malagasy population.

The introduction of this food could have some advantages for the Malagasy diet, and hence it seemed useful to study the theme: "the nutritional values of two species of sea cucumbers (*Holothuria scabra* and *Holothuria lessona*) from Madagascar". The main objective of this study was to determine the nutritional values of two sea cucumber species found in Madagascar, and to measure the acceptance and the appreciation of these products which are new in the Malagasy diet to promote their consumption for they may contribute to the improvement of the dietary diversity. The samples are collected from farmed sea cucumber (aquaculture/ holothuriculture) "which is a mode of sea cucumber farming in different types of ponds". The breeding process is divided into three stages during which the individuals develop at the larval, juvenile and adult stages.

## MATERIALS AND METHODS

### Sampling

Both species of sea cucumber came from aquaculture or

holothuriculture. Batches of processed specimens (dried, also called trepangs) were obtained, then crushed and reduced to flour by a MICROTRON® MB 800 mill in order to be homogenized before.

### Determination of moisture content

The moisture composition was determined by calculating the weight loss of the sample while drying in an oven. In a calibrated aluminum capsule;  $5 \pm 0.001$  g of the sample was weighed and dried at  $103^\circ\text{C}$  for 12 h in an oven. At the end of the drying process, the capsule containing the sample was cooled in a desiccator under vacuum for about 30 min and then weighed. Moisture content was expressed as a percentage of the weight loss from the original weight (Aubry, 2013).

$$M \% = \frac{p.e - w}{p.e} \times 100$$

Where:  $M \%$  = moisture percentage,  $w$  = weight after drying and  $p.e$  = weight of the test sample in g.

### Determination of ash content

The determination of the ash content consists of a gravimetric determination by high temperature calcination. Five grams of sample were weighed then was introduced into a muffle furnace at  $550^\circ\text{C}$  for three to five hours, until white, light gray or reddish ash was obtained, apparently free of carbonaceous particles. The capsule containing the ashes was then cooled in a desiccator and weighed. The ash content was expressed as a percentage ratio of the weight of the ash to the oven dry weight (AOAC, 2008).

$$A \% = \frac{(w - t) \times 100}{p.e}$$

Where:  $A \%$  = ash percentage,  $w$  = weight of ash after calcination in g,  $t$  = weight of the capsule, and  $p.e$  = weight of the test sample in g

### Determination of protein content

The percentage of protein was obtained after the determination of the total organic nitrogen by the Kjeldahl method (AFNOR, 2002). Exactly 0.25 g of sample prepared was weighed. Then 10 milliliter (ml) of concentrated  $\text{H}_2\text{SO}_4$  and a pinch of catalyst were added to a flask containing the sample. Then everything was placed in a heating system or mineralizer. The reaction was complete only in the absence of undigested material; the solution became clear after 3 h 15 min. The tubes were then allowed to cool at room temperature. Their content was quantitatively transferred to Kjeldahl distiller followed by addition of distilled water and 30% (w/v) sodium hydroxide. In a receiving flask, 10 ml of 4% boric acid solution and three drops of Tashiro's reagent color indicator were prepared. The titration was done using 0.1 N HCl directly after the distillation. The mixture of distillate and boric acid was assayed in the presence of Tashiro's reagent until its light green color turned into a persistent pink. Blank titration was carried out in the same way. The percentage of nitrogen content was then calculated by the following formula:

$$N \% = \frac{(V_1 - V_0) \times T \times 14 \times 100}{w \times 1000}$$

Where:  $w$  = weight of the test sample in g,  $T$  = titre of hydrochloric acid in normality,  $V_1$  = volume of acid used for the test portion in ml,

and  $V_0$  = volume of the acid used for the blank test in ml.

The protein content was calculated using the nitrogen conversion factor of 6.25 [(FAO/OMS, 1973) (Merrill and Watt, 1973)]. The total protein content was obtained by the following formula:

$$\text{Protein \%} = N\% \times 6.25$$

### Determination of lipid content

The extraction and dosage of lipids were carried out by extraction using a mixture of chloroform solvent, methanol and water. One gram of sample and 3 ml of distilled water was homogenized with a mixture of chloroform (10 ml) and methanol (5 ml). It was then stirred for one hour using a magnetic stirrer. The mixture was then vacuum filtered with a sintered glass and a vacuum pump. 4 ml of NaCl were added to the mixture and allowed to settle. NaCl and water separates the homogenate into two layers: The chloroform layer containing all the lipids and the methanol layer containing all the non-lipids. A purified lipid extract was obtained after isolation of the chloroform layer (Folch et al., 1957). If  $w$  was the mass of the residue and  $W$  the mass of the sample, the lipid level (expressed as a percentage) was given by the following formula:

$$L \% = \frac{w}{W} \times 10$$

Where: L % = lipid percentage,  $w$  = weight of lipid and  $W$  = weight of sample.

### Determination of carbohydrate content

The carbohydrate content was deduced from the difference between the total content of the elements and the sum of the protein, lipid, ash and moisture contents.

$$\text{Percentage carbohydrate} = 100 - (\% \text{ moisture} + \% \text{ ash} + \% \text{ protein} + \% \text{ lipid})$$

### Sensory analysis

For sensory analysis, the hedonic test was performed. The cooked sea cucumber sample was presented in front of the subjects who had to express their opinion regarding the pleasing characters on the rating scale from one to nine. The scale ranged from "extremely unpleasant" to "extremely pleasant", the median expresses that the character was neither pleasant nor unpleasant. A score above five qualifies the appreciation of the product. The test was conducted on 63 naïve consumers, selected from the students residing at the University Campus Ankatso II (36), University Campus Ambolokandrina (13) and 14 people living in the neighborhoods of Ambohipo Antananarivo: 28 women and 35 men, 15 young people who were under 18 years of age (adolescent), 34 young people aged between 18 and 30 years and 14 adults over 30 years.

The scales of quotation proposed by AFNOR (2000) are:

1. This product is extremely unpleasant (bad)
2. This product is very unpleasant (bad)
3. This product is unpleasant (bad)
4. This product is rather (quite) unpleasant (bad)
5. This product is neither unpleasant nor pleasant (neither good nor bad, pleasant or unpleasant)
6. This product is rather (quite) pleasant
7. This product is pleasant (good)
8. This product is very pleasant (good)

9. This product is extremely pleasant (good)

### Statistical analysis

All data were processed statistically by the RStudio-1.0.153 software: using the T test and ANOVA.

The T. test was used to find out the difference between the nutrient contents of the two sea cucumber species. This test is a test used to know the difference between two variables by referring to the probability obtained.

The principle is to put a null hypothesis  $H_0$  at the beginning which asserts that there is no difference between individuals. After doing the difference test, the probability or  $p$  = value is obtained. If it is less than 0.05,  $H_0$  is rejected, hence the individuals are different, and otherwise, the samples are similar.

To determine the difference between the means obtained after hedonic test, ANOVA or Analysis of Variance was used. The ANOVA principle follows Fisher's law. The Fisher test consists of comparing the variance between the groups and the variance within the group, the ratio gives the calculated value: F-value which will be compared with the F theoretical obtained by the statistical table (F distribution table) as a function of the DF or degree of freedom and of risk  $\alpha$  = 5%.

1. If F-value  $\geq$  F theoretical ((t-1) and (N-t));  $\alpha$   $H_0$  is rejected, at least one of the means is different
2. If Fvalue = F theoretical ((t-1) and (N-t));  $\alpha$   $H_0$  is correct the means are equal

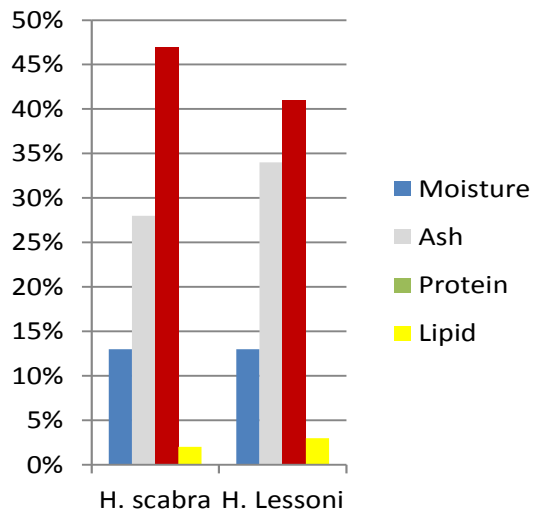
The probability obtained also makes it possible to assert the difference between the means:

1. If p-value < 5%,  $H_0$  is rejected, at least one of the means is different
2. If p-value > 5%,  $H_0$  is correct the means are equal

## RESULTS AND DISCUSSION

The results were divided in two parts: The results of physico-chemical analysis and that of sensorial analysis. Figure 1 and Table 1 show the nutrient levels of the two dried sea cucumbers, *H. scabra* and *H. lessoni*, while Table 2 shows the differences between the nutrient levels of these two species. Tables 3 to 5 present the results of the hedonic test performed on 63 naïve subjects and the analysis of variance of the notes.

Both species of sandfish had low water content: 13.80  $\pm$  1.45 g per 100 g for the *H. scabra* sample and 13.47  $\pm$  0.89 g per 100 g for *H. lessoni* sample. It is due to the practice of smoking and drying processes that remove a significant amount of water during their transformation from fresh to trepangs. As a result, the rate of dry matter was high, about 86%. The moisture content of fresh sea cucumber is approximately 76.3 to 79.43 g per 100 g of edible material (Oedjoe, 2017), which means that approximately 62.83 g of water is removed during treatment. The difference between the moisture content of these two species was not significant ( $p$ -value > 0.05). This low water content corresponds to the standard requirement for their quality and conservation, 30% is the maximum tolerable level (Baird, 1974). It proves the good



**Figure 1.** Physicochemical compositions of two species of dried sea cucumbers: *H. scabra* and *H. lessoni*.

**Table 1.** Means and standard deviation (SE) of the physicochemical compositions of two dry sea cucumber species: *H. scabra* and *H. lessoni* (in g per 100 g of sample).

Species	Parameter	Means $\pm$ SE
<i>H. scabra</i>	Moisture	13.80 $\pm$ 1.45
	Ash	28.18 $\pm$ 1.35
	Protein	47.84 $\pm$ 2.11
	Lipid	2.070 $\pm$ 0.26
	Carbohydrates	8.11
	Dry matter	86.2
<i>H. lessoni</i>	Moisture	13.47 $\pm$ 0.89
	Ash	34.51 $\pm$ 0.59
	Protein	41.18 $\pm$ 2.11
	Lipid	3.020 $\pm$ 0.12
	Carbohydrates	7.86
	Dry matter	86.57

**Table 2.** The difference test between physicochemical compositions of *H. scabra* and *H. lessoni*.

Parameter	P-value
Moisture	> 0.05
Ash	< 0.01
Protein	< 0.01
Lipid	< 0.01

quality of these samples, and helps to promote the exportation of trepangs and increase their market value.

In fact, the lower their water content, the less the trepangs are perishable, and the longer the preservation is.

**Table 3.** Mean and standard deviation of sea cucumber ratings

Preferred character	Hedonic value (Mean ± SD)
Odour	5.73 ± 1.49
Taste	5.86 ± 1.71
Global	5.86 ± 1.68

**Table 4.** Difference of assessment between sex groups: Man and woman (ANOVA Table).

	Df	Sum of square	Mean square	F value	Pr(>F)
Sex	1	28.35	28.350	11.73	0.0011 **
Residual	61	147.36	2.416		

Significant codes: 0 '\*\*\*\*' 0.001 '\*\*\*' 0.01 '\*\*' 0.05 '.' 0.1 ' ' 1; With Df = 1 and 61,  $\alpha = 5\%$ ,  $F_{5\%;1,61} = 3.99$ ; 4.

**Table 5.** Difference in appreciation between age groups: under 18, between 18 and 30 and over 30 (ANOVA Table).

	Df	Sum square	Mean square	F value	Pr(>F)
Age	2	8.43	4.217	1.513	0.229
Residual	60	167.28	2.788		

With DF = 2 and 60;  $\alpha = 5\%$ ,  $F_{5\%;2,60} = 4$ .

The raw ash content provides a rough estimate of the total inorganic matter in the sample. The results obtained in this study showed a high ash content for these two species, about 34 g per 100 g for the *H. lessoni* sample; this was higher than that of *H. scabra*, which was about 28%. The difference was significant for both species, p-value equaled 0.006735 (less than 0.01). The high ash content can reflect a high concentration of minerals, which means that this food is an interesting solution in case of deficiency in these minerals, since it is one of the problems of Malagasy diets. Proteins make up the bulk of the flesh of sea cucumbers or trepangs. In this study, a high protein value was found, and it was almost half the content of the samples,  $47.84 \pm 2.109$  g per 100 g for *H. scabra* sample and  $41.18 \pm 2.109$  g per 100 g for the *H. lessoni* sample. The difference between the protein contents of these two species was significant, p-value equaled 0.01814 (less than 0.05). The results are consistent with data provided by many scientific papers with respect to protein ratios regarding the same or other species. As an example: Ali et al. (2015) found a ratio of about 60% for *H. scabra* and around 53% for other specimens, Hanna et al (2017) found a ratio of 55.18% for body wall of *H. scabra*. The difference in values can be attributed to the influences of several factors, such as the different types of sea cucumber species and the substrates they use during their development. Bouriga et al. (2013) found that fresh sea cucumbers contain between 8 and 14% of protein, and Oedjoe (2017) found between 21 and 44, 07%, which means that the

treatments applied to the fresh sea cucumbers during their transformation into trepangs improves protein availability. Compared to protein composition of other foods of fish origin, such as fish and crustaceans whose protein content range between 4 and 26 g per 100 g of sample (Santé Canada, 2008), dried sea cucumbers are assumed to be more nutritious. The high total protein content is a characteristic of sea cucumber species. As a result, it is a food adapted to a diet in regions where the protein-energy deficiency exists, as is the case with Madagascar. They can replace meat and beans as protein rich dishes.

These sandfish species had very low lipid content, of around two percent for *H. scabra* and three percent for *H. lessoni*. Statistically, the composition is different for the two species with p-value equal to 0.01306 (less than 0.05). Sea cucumbers have never been known as a source of lipid, which has been proven by many authors: 100 g of fresh sea cucumber contains around 1.12 g of lipids (Oedjoe, 2017); according to Hanna et al. (2017), dried *H. scabra* contains only about 1.55% of lipids, which complies with a fat free diet. The difference between these two species was significant.

Like any animal flesh, sea cucumbers contain only a tiny portion of carbohydrates. In this study, only eight percent of the flesh consisted of carbohydrates for both species with a difference that was minimal between the two species. These results are comparable to those reported by Berger and Carbonneau (2014) and by Hanna et al. (2017). Some other authors have found no

trace of carbohydrates in their samples (Ali et al., 2015). During their use as food, a supplementation with a carbohydrate source food is necessary, which means that they are adapted to the Malagasy rice diet. The combination of these two foods is a perfect nutritional supplement.

The means of the hedonic values obtained after the hedonic test were greater than five, which means that this product is appreciated and accepted by the juries even if it is a moderate acceptance. However, for both sex groups (males and females), the assessment is not the same, men value more than women. According to the analysis of variance, F-value = 11.73, which is much higher compared to  $F_{5\%;1,61}$  ( $11.73 > 3.99; 4$ ), and the probability p-value is lower than 0.01 ( $0.0011 < 0.01$ ), confirming this which states that the means of the grades are different.

In relation to the age of the consumers, the difference of appreciation is not observed as F-value was less than  $F_{5\%;2,60}$  ( $1.51 < 4$ ) and the probability p-value was greater than 0.05 ( $0.23 > 0.05$ ).

## Conclusion

A significant difference was noted for some nutrients between the two species. Sea cucumbers contain a very high protein content of between 40 and 55% and a high ash content of 28 to 34%. On the other hand the lipid content ranges between 2 and 3% and the carbohydrate content between 7 and 8% which are very low. The product was accepted and appreciated by the juries even if it was a moderate appreciation. However, the research remains unfinished. It would be interesting to determine the composition of amino acids and mineral elements. In addition, research on protein valorization would be an advantage for the population.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## ACKNOWLEDGEMENTS

This work was part of the priority research axis of LABASAN (Laboratory of Biochemistry Applied to Food Sciences and Nutrition (University of Antananarivo)), which is "The valorization of local food resources and the identification of foods of high nutritional value for a better use. LABASAN is gratefully acknowledged for its laboratory facility provision.

## REFERENCES

Ali S, Elamin M, Gideiri B, Ibrahim M (2015). The proximate composition and the nutritional value of some sea cucumber species inhabiting

- the sudanese red sea. Food Science and Quality Management 41:12-14.
- Association Française de Normalisation (AFNOR) (2000). Directives générales pour la réalisation d'épreuves hédoniques en laboratoire d'évaluation sensorielle ou en salle en conditions contrôlées impliquant des consommateurs. AFNOR, recueil de normes, Analyse sensorielle 6th edition.
- Association Française de Normalisation (AFNOR) (2002). Détermination de la teneur en azote totale et calcul de la teneur en protéines, Méthode de Kjeldal. CSIC Library of standards pp. 1-13.
- Association of Official Analytical Chemists (AOAC) (2008). Official methods of analysis of the Association of Official Analytical Chemists. 15th edition, Washington DC.
- Attaran G, Barzkar N, Taheri A (2017). Proximate composition and mineral contents in the body wall of two species of sea cucumber from the Oman Sea. Environmental Science and Pollution Research 24(23):1-5.
- Aubry M (2013). Détermination de la teneur en humidité dans les aliments pour les animaux et les pains. Agence Fédérale Pour La Sécurité De La Chaîne Alimentaire pp.1-5.
- Bader E, Claude M, Deligia C, Ralison C (2005). Profils nutritionnel de Madagascar. Division de l'Alimentation et de la Nutrition pp.1-49.
- Baird H (1974). Bêche de mer dans les îles du Pacific Sud, Manuel à l'usage des pêcheurs. South Pacific Commission Library pp.1-29.
- Berger K, Carbonneau M (2014). Valeur nutritionnelle du concombre de mer (*Cucumaria frondosa*). Merinov, Rapport De Recherche-Developpement 14-07:1-7.
- Bouriga N, Chalhaf M, Ghouaie S, Missaoui H, Skandrani Y (2013). Etude de la composition biochimique de *Holothuria polii* de Tunisie. Rapp. The Mediterranean Science Commission 40:895.
- Chen J (2004). Present status and prospects of sea cucumber industry in China. Advances in Sea Cucumber Aquaculture and Management pp. 25-38.
- Conand C (1994). Les holothuries, ressource halieutique des lagons. Rapports scientifiques et techniques science de la mer 65: 1-79.
- Eeckhaut I, Jangoux M, Lavitra T, Rachelle D, Rasolofonirina R (2009). Traitement et commercialisation des holothuries dans la région de Toliara au sud-ouest de Madagascar. La Bêche-De-Mer- Bulletin de la CPS 28:24-33.
- FAO, PAM (2017). Mission de la FAO/PAM d'évaluation des récoltes et de la sécurité alimentaire à Madagascar.
- FAO, WHO (1973). Energy and protein requirements. Report of a Joint FAO/WHO Ad Hoc Expert Committee, FAO Nutrition Meetings 52:105-106.
- Folch J, Lees M, Sloane-stanley G (1957). A simple method for isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry 266:497-509.
- Hanna P, Jattujan P, Poomtong T, Siangcham T, Sobhon P, Sroyraya M, Tinikul R (2017). Nutritional components of the sea cucumber *Holothuria scabra*. Functional Foods in Health and Disease 7(3):168-181.
- Merrill A, Watt B (1973). Energy value of foods, basis and derivation. Human nutrition research Branch. Agriculture Research Service, United States Department of Agriculture. Agriculture Handbook No 74.
- Ministère des Ressources Halieutiques et de la pêche (MRHP) (2014). Lettre de politique bleue. Ministère des Ressources Halieutiques et de la Pêche de Madagascar pp. 1-23
- Muséum national d'histoire naturelle (MNHN) (2016). Le Muséum à Madagascar. Muséum National D'histoire Naturelle.
- Oedjoe MR (2017). Composition of Nutritional Content of Sea Cucumbers (Holothuroidea) in Mania Waters, Sabu Raijua Regency, East Nusa Tenggara. Aquaculture Research and Development 8(7):1-3.
- Santé Canada (2008). Valeur nutritive de quelques aliments usuels. www.santecanada.gc.ca/fcen