METHODOLOGICAL SCOPE OF STANDARD NCH0765 FOR THE DETERMINATION OF TOTAL IMPURITIES BY WASHING IN ALGAE OF THE GENUS *Gracilaria*

MICHAEL ARAYA [1] AND FADIA TALA [1,2,3]*

[1]*Centro de Investigación y Desarrollo Tecnológico en Algas y otros Recursos Biológicos (CIDTA), Facultad de Ciencias del Mar, Universidad Católica del Norte, Coquimbo (Chile).*
[2]*Departamento de Biología Marina, Facultad de Ciencias del Mar, Universidad Católica del Norte, Coquimbo (Chile).*
[3]*Instituto Milenio en Socioecología Costera (SECOS), Chile.*

ABSTRACT

Due to contradictory experimental results obtained in the determination of impurities by washing in algae of the genus *Gracilaria*, compared to sieving and manual separation, the methodological basis described by the Instituto Nacional de Normalización (INN) to the normative NCh0765 was analyzed. Our results show that for the same sample, 2.4 ± 0.9% of impurities were determined by sieving and manual separation analysis, while 31.9 ± 2.9% was detected by the washing methodology. This difference would be mainly due to the exudation of organic compounds, principally the phycocolloid Agar, during the washing procedure which loss can range from 10 - 20%. The differences in impurities according to the methodology could have consequences for the export restrictions of algal resources due to current Chilean regulations and buyers' demands.

Keywords: *Gracilaria chilensis, Impurities, phycocolloids, exports*.

INTRODUCTION

To date, Chile is the main producer of algae in America, averaging 403,608 ± 75,344 tons between 2012 and 2020[1,2]. These volumes of algae harvest are even higher than those recorded in 2019 for Europe (287,033 tons), Africa (144,909 tons), and Oceania (16,572 tons), while productions from Asia (34,826,750 tons) largely leads the total worldwide, mainly from aquaculture activities[3]. The algae industry in Chile is mainly based on harvesting from natural populations and then exporting as raw materials, without added value and at low prices for the extraction of phycocolloids[4]. *Gracilaria chilensis* (a.k.a. pelillo) is the only species cultivated in Chile, whose biomass is intended for agar extraction at the national level and a fraction is exported as raw material. In 2022, 6,509 tons were allocated to produce 80 tons of agar, while 3,401 tons were exported [2] .

To export algae for human consumption from Chile, certain requirements must be met, including: a) the establishment must have a "Valid Sanitary Resolution"; b) be enabled under the framework of the National Fisheries and Aquaculture Office (SERNAPESCA), at least in category D, according to Chapter IV of the Safety and Certification Manual; and c) the raw material must meet physical, chemical, microbiological, and organoleptic criteria (details at www.sernapesca.cl). Among the chemical parameters, the requirement of <20% moisture and <10% impurities are critical to export authorization, both human consumption and industrial use[5]. Nowadays, the methodologies to perform these analyses are described in the Official Chilean Normative NCh0765 Of2002[6] issued by the Instituto Nacional de Normalización (INN). In this specification, the determination of moisture is performed independently of the algal genus; on the contrary for total impurity content es there are different methods according to the algal species. Only for the genus *Gracilaria*, the methodology described includes washing procedure, while for other species it includes visual inspection and separation, without a rationale that differentiates the methodologies. When reviewing international standards for algae certification commercialization, it was not possible to find the use of similar washing metodology[7,8,9]. The objective of this study was to evaluate the implications of methodological selection in the determination of impurities of a type of species (*Gracilaria chilensis*) and theirs effect on stock availability for exportation.

EXPERIMENTAL SECTION

Reagents and Equipment

The red algae *Gracilaria chilensis* (Rhodophyta) was provided by the company COMERCIALIZADORA MARIA GRACIELA ORTIZ SALINAS E.I.R.L. in January 2024 from aquaculture farm in Ba. Coquimbo (30°S). For the analyses, ultrapure water obtained by a Heal Force Smart series purifier was used. A ZFD-5140 oven was used for algae drying. A Phoenix vertical autoclave was used to obtain agar yield. Weights were recorded using a BOECO BAS 31 plus analytical balance.

METHODOLOGY

Moisture Determination

The moisture (%) was determined according to point 7.1 of normative NCh0765. 100 g of algae were dried in an oven at 103°C for 4 hours, until constant weight.

Impurity Determination

For the determination of impurities by sieving and manual separation, the protocol described in the point 7.3 of normative NCh0765 was followed. 100g of algae were sieved on a 500 µm mesh to remove sand and small impurities. The algae were manually inspected, and impurities (other algae, shells, stones, etc.) were removed. The percentage of impurities was established relative to the initial weight of the sample (algae + impurities).

For the determination of impurity by washing, the protocol of point 7.2 in the normative NCh0765 was followed. All sample from the moisture determination were washed in 10L of ultrapure water for 10 minutes. The washed algae were separated and placed on absorbent paper. The liquid and smaller algae residues were sieved through a 300 µm mesh and reincorporated into the rest of the washed algae. Then, it was dried in an oven according to point 3.1, and the impurity (%) was estimated.

The mathematical formula used for calculating the percentage of impurities is defined as follows:

$$\%\text{ impurity MB}=\frac{\text{impurity weigth}}{\text{sample weigth}}\cdot 100\cdot\frac{(100\text{-}\%\text{M})}{100} \qquad \text{Eq. 1}$$

Where the weights are expressed in grams, impurity weight corresponds to the difference between the dried sample before cleaning and the weight of the dried sample after washing; sample weight corresponds to the weight of the sample before cleaning, and the moisture percentage as %M, (100-%M) / 100 is considered a moisture factor, which is used to convert from dry to wet basis.

Total Agar Yield Determination.

For the agar yield determination, the methodology described by Craigie et al. (1978)[10] was followed. 10.0g of dried *Gracilaria* cut between 3-5cm long were introduced into a 1L Erlenmeyer flask and rehydrated in 375mL of ultrapure water for 3 hours. They were subsequently autoclaved at 120°C at a pressure of 1kg/cm^2 for one hour. It was cooled to room temperature, and the supernatant was centrifuged at 3500rpm. The viscous liquid obtained was lyophilized at -80°C and 5mTorr pressure for 72 hours using an IlShin TFD 5503 lyophilizer. The residue obtained was weighed, and the agar yield percentage on a dry basis was calculated.

**Corresponding author email: mmaraya@ucn.cl*

RESULTS AND DISCUSSION

Moisture and Impurity

The average moisture percentage was 12.06 ± 1.01, obtaining a moisture factor between 0.89 – 0.86 for the lowest and highest moisture values, respectively.

The analytical determination of moisture can be affected by the sampling, transportation, and storage of the sample, in addition to the analysis conditions such as how the sample is placed on the weighing pan. An attempt should be made to evenly distribute the sample on the pan, avoiding thicker areas with algae to ensure homogeneous drying. Storage in vacuum-sealed aluminum bags could help control these factors. For sampling, the Chilean normative NCh43 Of61 on random sample collected considered for algae does not specify transportation and storage protocols according to algal type. Therefore, an increase or loss of moisture in the sample will have implications for impurity content.

Table 1. *Moisture and impurity content by both methods in Gracilaria chilensis.*

Sample	Moisture (%)	Impurity Sieving (% Imp_{wb})	Impurity Washing (% Imp_{wb})
1	11.13	2.05	33.50
2	11.19	2.55	33.66
3	13.64	1.07	35.08
4	11.51	2.39	30.33
5	12.85	3.78	26.97
Average ± SD	12.06 ± 1.01	2.37 ± 0.87	31.91 ± 2.92

The impurity content in wet bases (wb) determined by sieving and manual separation was 2.4 ± 0.9%, while this increases to 31.9 ± 2.9% with washing method for the same sample. The difference between these methods is contradictory observing the algae with a clean appearance, with minimal sand, some pieces of other algae, and animal remains. The selection of impurities removed by sieving and manual separation could be considered subjective, as an error associated with the method, as it depends on the analyst's criteria.

In the washing methodology, conditions such as washing time, water temperature, type of water, pressure exerted on the algae, could be affecting the analytical results, and there are not established in the normative. However, the weight loss in the sample after the washing process was clearly observed.

Agar yield.

During the washing process of the algae, a yellowish-green coloration of the supernatant water was observed within the first 90 seconds, suggesting that organic constituents, including agar, were being released into the aqueous medium. For this reason, the agar content between the initial algae and after washing was compared. The results showed a decrease in agar yield of between 10-20% for washed samples. Therefore, impurity contents over 30% according to the washing method would be overestimated due to weight loss in the algae. Release of natural organic constituents from the tissue does not correspond to the standard definition of impurities, which defines it as: "all those substances foreign to the algae and quantifiable by this standard."

Table 2. *Comparison of Agar content in Gracilaria chilensis samples before and after the washing process.*

Sample	Agar yield (%) Initial sample	Agar yield (%) Post-Washing sample
1	56,6	40,2
2	56,9	41,9
3	54,4	41,3
4	56,6	41,9
5	57,3	46,7
Average ± DS	56.4 ± 1.0	42.4 ± 2.2

We assume that the washing methodology has been introduced with the purpose of measuring the weight loss due to water-soluble external salts; however, it is not possible for 30% of the matter to come from salts. As previously mentioned, apparent cleanliness from visual inspection is not concordant with impurities content. The rehydration process after drying could be causing the loss by deteriorating the algae cell walls, coupled with the osmotic shock of washing with ultrapure water.

CONCLUSIONS

The determination of moisture and impurities are critical points to validate the export permits of algae in Chile by authorized entities. The washing method should be justified in its application to algae of the genus *Gracilaria chilensis*. The loss of agar, the main bioproduct of this specie, during the washing process generates high values of impurities, which is not consistent with the definition. The methodological details found here request precise research to establish a more robust and accurate standard method for the determination of impurities in algae of the genus *Gracilaria*.

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