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Development, Characterisation, and Consumer Acceptance of an Innovative Vegan Burger with Seaweed

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Abstract: What consumers choose when purchasing food is of most importance to promote sustainability. The consumption of more sustainable foods should be stimulated, for example, by using more sustainable ingredients and by consumer education. Therefore, an innovative and highly nutritious vegan burger with seaweed (VBS) was developed using sustainable ingredients, such as pulses—grass pea (*Lathyrus sativus* L.) and chickpea (*Cicer arietinum* L.)—and the seaweed Dulse (*Palmaria palmata* L.) from aquaculture. VBS was analysed for its physico-chemical and nutritional characteristics, including antioxidant activity (DPPH, TPC) and fatty acid and mineral element profiles. Shelf life and consumer acceptability were determined. The VBS was shown to be a source of protein (8.01 ± 0.14% fresh weight (FW)), fibre (5.75% FW), and mineral elements, such as P, Fe, rich in Mg, Mn, and Cu, while having low sodium content. Moreover, it presents a low sugar content. Furthermore, no antioxidant activity was detected. The pasteurised and vacuum-packed product had a shelf life of 90 days and was well accepted by consumers (64.0% acceptance). It may be concluded that an innovative VBS, nutritionally rich and with a shelf life of 90 days, was developed and well accepted by consumers, which is a good addition to a rich and diverse diet.

Keywords: sustainability; innovation; *Palmaria palmata*; *Lathyrus sativus* L.; macroalgae; low-value fish; highly nutritious; *Cicer arietinum* L.

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1. Introduction

The decisions made by consumers when purchasing and consuming food play a crucial role in promoting a more sustainable food production and sourcing. To encourage a more sustainable food consumption, various strategies can be employed, such as the development of food products that incorporate sustainable, locally sourced, and seasonal ingredients. Additionally, consumer awareness can be enhanced through marketing techniques and environmental education programs [1,2]. Sustainability should not only be seen from an availability perspective, but also from a social, economic, and especially environmental perspective [3].

Global warming is caused by high quantities of greenhouse gases in the atmosphere produced by human activities, such as burning of fossil fuels and deforestation, presenting serious consequences, including soil degradation, loss of productivity of farming lands, reduced sources of fresh water, and desertification [4–6]. Reducing the quantities of CO_2, a greenhouse gas, by carbon offsetting is a way of minimizing the effects of global warming by reducing, avoiding, or sequestering carbon. For this purpose, several researches suggest the use of seaweed farming as a safer and more sustainable method of carbon offsetting than the common method used nowadays—forest plantation [7–9]. Thereafter, seaweed can be used as a sustainable food and feed source with high nutritional value [8,10–12]. *Palmaria palmata* L., for example, has a high protein content and is rich in mineral elements, such as I, Fe, Ca, and K, as well as vitamins A and C, while being low in sodium [13–15]. New forms of agricultural practices must also be implemented to prevent loss of productivity, such as the usage of pulses as green fertiliser crops [16,17].

Since antiquity, pulses, such as the common pea (*Pisum sativum* L.), chickpea (*Cicer arietinum* L.), and grass pea (*Lathyrus sativus* L.) have been used to maintain productivity in Mediterranean agricultural systems by providing biologically fixed nitrogen [18,19]; therefore, this type of nitrogen is a sustainable way of improving agricultural productivity, avoiding the usage of synthetic fertilisers [16,17].

Grass pea, also known as Indian pea, white pea, chícharo, almorta, among other names, is used as a source of food and feed [20,21]. Cultivated since the Neolithic, it has spread over three continents and is considered a staple food in several developing countries, such as Ethiopia and India due to its drought and flood tolerance, high yield, and insect resistance, which is cultivated in Australia, Europe, and several other African and Asian countries [20–23]. The grass pea has been nutritionally analysed in several studies, standing out for its high protein content (31.6%) and low fat content (2.7%) [24]. Portuguese raw dry grass pea also has high protein content (31.7%) and low fat content (5.4%) with carbohydrates being the main nutrient (64.2%) [25]. Both represent a higher protein content than common peas and broad beans, making it a good source of protein for human consumption, as well as a good source of PUFA (58% of total fatty acids) [23].

Therefore, the application of these foods as ingredients in new products is of great importance for human nutrition, and has a high economic, social, and environmental impact. They are products that can minimise the environmental mistakes made and simultaneously revitalise the agri-food market. In addition, the new products developed should focus on new food trends—such as vegetarianism and veganism which are often a strategy to minimise the production of greenhouse gases by encouraging reduced livestock production [26].

The aim of the present work is to develop and characterise a new burger suitable for vegan and vegetarian diets using grass pea and seaweed as main ingredients, as well as inquire about consumer acceptance of this new product. The burger developed intends to help in increasing the consumption of more sustainable, tasty, and nutritionally rich foods by creating new ways of eating them.

2. Materials and Methods

2.1. Selection of Ingredients and Formulations

The ingredients selection was made on the basis of assumptions, such as the importance of using little or unexploited marine resources, the incorporation of local food resources, and the relevant added nutritional value, in order to achieve a more sustainable and simultaneously nutritious product. After choosing the key ingredients (described in Section 2.2), the formulation design was made following the intended nutritional value (high content of protein and minerals, low sodium content, and low saturated fats, while using seaweeds and pulses), by analysing formulations' theoretical chemical composition using a spreadsheet. After theoretical formulation selection, they would pass through several steps, as described in the flowchart in Figure 1.

2.2. Production of Vegan Burger

The vegan burger with seaweed (VBS) was produced using boiled grass pea and chickpea in a 50:50 ratio, powdered *Palmaria palmata* and rehydrated diced *Palmaria palmata*, grated raw carrot, diced onion, oatmeal flakes, lupin flour, apple cider vinegar, olive oil, and a mixture of herbs and spices. Dried *Palmaria palmata* was purchased from the aquaculture company "AlgaPlus". Grass pea was supplied by a producer from the Peniche area, Portugal. All other raw materials were purchased from the local supermarket.

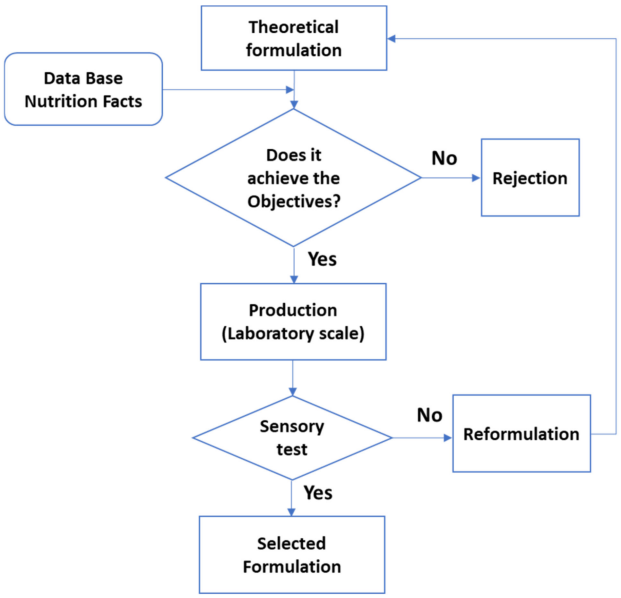

Figure 1. Formulation selection flowchart.

The production of VBS started by soaking the pulses in fresh tap water for 20 h and the soaked water was changed every 5 h. Thereafter, the pulses were cooked in an autoclave (Raypa, AES-28, Barcelona, Spain) for 15 min at 119 °C. After cooking, the pulses were removed from the water and left to cool down in the refrigerator (Liebherr, LKv, Biberach an der Riß, Germany). The cooled cooked pulses were then grated in a food processor (Vorwerk Elektrowerke GmbH & Co., Thermomix TM6, Wuppertal, Germany) for 2 s at speed 10. Diced onion was cooked in olive oil until transparent, and then the carrot, seaweed, and herbs and spices were added and cooked for 4 min. The processed pulses and cooked vegetables and herbs and spices were mixed by hand, along with the oatmeal flakes, lupin flour, and apple cider vinegar. The resulting mixture was separated in 100 g portions (ADAM, PGL 3002, UK) and moulded into patties, using a moulding tool.

The moulded burgers were vacuum sealed at 95% vacuum (Henkelman, Boxer 42, Hertogenbosch, The Netherlands) and thermal treated at 95 °C for 60 min in a water bath (Thermo Scientific, SWB 15, Newington, CT, USA). The finished product was stored at 4 °C in the refrigerator (Liebherr, LKv, Biberach an der Riß, Germany) until needed.

2.3. Chemical/Nutritional Composition

2.3.1. Sample Preparation

Fresh samples were prepared by homogenisation in a food processor (Moulinex, 1,2,3 XXL, Paris, France). Dried samples were prepared by freeze drying at −56 °C for 60 h (Scanvac, Coolsafe, Lynge, Dynamark). Thereafter, the VBS samples were freeze-dried at −80 °C (Thermo Electron Corp., Forma-86C Ult Freezer, USA) for 18 h, which were then ground with a coffee grinder (Kunft, KCG4380, Senhora da Hora, Portugal).

2.3.2. pH

The pH of fresh samples of VBS was measured by differential potentiometry, using a potentiometer (InoLab pH Level 2, WTW, Weilheim, Germany) equipped with a penetration probe for solid foods (SenTix®Sp-T 900, WTW, Weilheim, Germany).

2.3.3. Water Activity (aW)

The water activity of VBS samples was determined by hygrometry (ROTRONIC, HYGROPALM—HP23-AW, Bassersdorf, Switzerland), in accordance with the directions of the equipment manufacturer.

2.3.4. Crude Protein Content

Aliquots of 0.7 g of fresh samples of VBS (designated by m) were digested using 15 mL of H_2SO_4 and 2 Kjeldahl tabs (K_2SO_4 with Se) in a digestor (Foss, Digestor 2006, Hilleroed, Denmark) for 30 min at 220 °C, followed by 90 min at 400 °C. After cooling down, 70 mL of distilled water and 100 mL of aqueous solution of NaOH (40% m/V) were added to the digested sample and steam-distilled (Foss, Kjeltec™ 2100, Denmark) to a beaker containing 30 mL of H_3BO_3 (4% m/V) and indicators. The distillate was then titrated with HCL 0,1 M until the colour changed to a greyish pink, which is the total volume of titrant designated by V_a. A blank sample was processed in the same way, which is the total volume of titrant used as designated by V_b. The crude protein content in g/100 g of fresh VBS (FW) was calculated using the following equation:

$$\frac{6.25 * 0.014 * (V_a - V_b)}{m} * 100 \quad (1)$$

2.3.5. Total Fat Content

Following the Folch method [27] adapted from Duarte et al. (2020) [28], test tubes containing 1 g aliquots of fresh samples of VBS were added to 5 mL of Folch reagent and 0.8 mL of distilled water and homogenised for 1 min. Then, another 5 mL of Folch reagent was added and homogenised for 5 min, followed by the addition of 1.2 mL of $NaCl_{(aq)}$ 0.8% (m/V) and homogenisation for 2 min. This mixture was centrifuged at 7000× *g* for 10 min, after which the lower part was removed and made to pass through a water removing filter into a pear-shaped glass flask. To the other part, 5 mL of chloroform was added, homogenised, and centrifuged, in which the lower part was removed again and made to pass through the same filter into the same flask. The organic solvent was removed by low pressure evaporation (Heidolph, Laborota 4000, Schwabach, Germany) and left in the oven at 105 °C for 4 h, in which the fat content was measured after cooling down in a desiccator and expressed in % FW.

2.3.6. Water and Ash Content

VBS's water content, in % FW, was calculated by comparing fresh and dry weight. Briefly, 1 g of aliquots was dried in the oven (Memmert, UF110, Schwabach, Germany) at 105 °C for 24 h and weighed after cooling down in a desiccator.

Ash content, in % FW, was calculated by comparing fresh and burnt content, where previously dried samples were incinerated in a kiln (Nabertherm, B170, Lilienthal, Germany) at 525 °C for 5 h. After cooling down in a desiccator, they were weighed.

2.3.7. Dietary Fibre Content

The dietary fibre content, in % FW, was estimated considering the dietary fibre content of each one of the raw materials (designated by *tFi*) and their amount in the final product (*ti*), using an equation built for this purpose.

$$\sum tFi * ti \quad (2)$$

2.3.8. Sugar Content

Using the colorimetric phenol-sulphuric assay [29], an aqueous solution at 2.5 mg/mL of freeze-dried sample was used to determine the sugar content, in % FW. A glucose standard was also prepared.

To a test tube containing 1000 µL of sample solution (or glucose standard or distilled water for the blank), 500 µL of $Phenol_{(aq)}$ 4% (m/V) were added and mixed. Then, 2500 µL of H_2SO_4 were added to all tubes and mixed again. The tubes were left to cool down in a water bath at 25 °C for 10 min. The absorbance of the solutions was measured (Thermo Scientific, Evolution 201, Shanghai, China) at 490 nm using optical glass cells. Using the values from the glucose standard, an equation was created to calculate the sugar content.

2.3.9. Carbohydrate Content

The carbohydrate content was calculated using the method suggested by FAO [30] and expressed in % FW.

2.3.10. Energy

Energy, in kilojoule (kJ) and kilocalorie (kcal) per 100 g FW, was estimated as described in Regulation (EU) No. 1169/2011 of the European Parliament and of the Council of 25 October 2011 [31].

2.3.11. Mineral Elements Profile

A mineral elements profile (Na, K, Ca, Mg, P, S, Cu, Mn, Fe, Zn, Pb, Cd, As) was conducted using inductively coupled plasma optical emission spectrometry (ICP-OES) and freeze-dried samples [32].

Briefly, 7.5 mL HNO_3 65% (v/v) and 2.5 mL HCl 37% (v/v) were added to 0.5 g of freeze-dried VBS sample. The sample digestion occurred at 60 °C for 30 min, followed by 30 min at 90 °C and 60 min at 105 °C. Standards and blanks were also subjected to the same treatment. After cooling down, samples were diluted to a final volume of 25 mL, filtered, and analysed (Thermo Fisher Scientific, Inc., iCap 7000 series). All results were determined in mg/100 g FW.

2.3.12. Fatty Acid Profile

Gas chromatographer (Thermo Scientific, TRACE GC Ultra) equipped with a flame ionisation detector (GC-FID) was used to study the fatty acid profile of the vegan burger with seaweed.

Briefly, 50 mg aliquots of freeze-dried VBS sample were acid-methylated using 2.0 mL of a methanolic solution of H_2SO_4 2% (v/v) and left to react in a closed test tube in a water bath at 80 °C for 2 h. After cooling down, 1000 µL of Milli-Q water (Merk, Milli-Q® Advantage A10, Darmstadt, Germany) and 2000 µL of n-hexane were added to the contents of the test tubes and mixed. After centrifugation at 1500× g for 5 min, 1000 µL of supernatant was extracted and placed in a vial.

For the analysis, a Trace TR-FAME 60 m × 0.5 mm × 0.25 µm column was used, with helium at 1.5 mL/min. The temperature of the oven was: 75 °C for 1 min, 5 °C/min until 170 °C, 170 °C for 10 min, 5 °C/min until 190 °C, 190 °C for 10 min, 2 °C/min until 240 °C, 240 °C for 10 min. Injector temperature was 250 °C and detector temperature was 280 °C. The detector was supplied by 350 mL/min of air and 35 mL/min of hydrogen.

As a standard, Supelco 37 Component FAME Mix was used.

The fatty acid content in the VBS was then estimated using an equation [33,34] and conversion factors were supplied by USDA [35].

2.3.13. Total Phenolic Content (TPC)

The *Folin–Ciocalteu* colorimetric assay [36,37] was used, adapted for microplate, and gallic acid was used as a standard.

Briefly, 2 µL of ethanolic solution of fresh VBS sample at 250 mg/mL (or standard or ethanol for blank) was added to microplate wells, followed by 10 µL of *Folin–Ciocalteu* reagent which was left to react for 2 min at room temperature and protected from light. Then, 30 µL of $Na_2CO_{3\,(aq)}$ 20% (m/V) was added, which was left to react for 60 min at room temperature and protected from light. Absorbance was measured at 760 nm (Biotek, Epoch2, Winooski, VT, USA). For the determination, an equation based on the standard was used, and the results were expressed in mg of gallic acid equivalents per g of fresh VBS (mg GAE/g).

2.3.14. DPPH Assay

The DPPH colorimetric method was used, adapted for microplate [38,39]. Moreover, TROLOX was used as standard and ethanol as solvent.

Briefly, 2 µL of ethanolic solution of fresh sample at 250 mg/mL (or standard or ethanol for control) and 198 µL of ethanolic solution of DPPH 0.1 mM were added to microplate wells, which were left to react for 30 min at room temperature and protected from light. Ethanol was used as blank. After the reaction, absorbance was measured at 517 nm (Biotek, Epoch2, Winooski, VT, USA). The TROLOX equivalent antioxidant activity (TEAC) in mg per g of fresh VBS was determined using an equation based on the standard.

2.4. Shelf-Life Determination

Samples of VBS were stored in individual packs at refrigeration conditions at 4 °C (Liebherr, LKv, Germany) until analysis. Samples were tested at 0, 30, 90, and 180 days. The pH of VBS samples was measured as described in Section 2.3.2.

2.4.1. Thiobarbituric Acid Reactive Substances (TBARS) Assay

For TBARS assay, the NP 3356:2009 Portuguese Standard [40] was used. An extraction of desired compounds from 15.0 g of fresh VBS (exact mass designated by m) was made using 30.0 mL of a solution of trichloroacetic acid 7.5% (m/V) with EDTA disodium salt 0.1% (m/V) and propyl gallate 0.1% (m/V), which was mixed for 2 min and centrifuged (Eppendorf, 5810R) at 4000× *g* for 10 min.

Extract solutions at 10% (*v*/*v*), 20% (*v*/*v*), and 60% (*v*/*v*) were prepared in test the tubes, with a final volume of 5.0 mL (volume added to complete 5.0 mL is designated by V1). Moreover, standard solutions of 1,1,3,3-tetraethoxypropane (TEP) and blanks were prepared. Thereafter, 5.0 mL of thiobarbituric acid 0.02 M was added and mixed, and the test tubes were thoroughly closed. Then, they were left to react in a boiling water bath (Thermo Scientific, SWB 15) for 40 min. After cooling down, their absorbance was measured (Thermo Scientific, Evolution 201) at 530 nm using optical glass cells. Malondialdehyde (MDA) content (designated by nMDA) was determined using an equation based on the standard and the TBARS index was calculated using the following equation:

$$\frac{72.0636 * nMDA}{m * V1} * \left(30 + \frac{m * rH}{100}\right) \quad (3)$$

where rH is the water content of the samples analysed.

2.4.2. Total Volatile Basic Nitrogen (TVB-N) Assay

TVB-N was determined using the Conway microdiffusion method, according to the NP 2930:2009 Portuguese Standard [41]. An extraction of the desired compounds from 12.5 g of fresh VBS (exact mass designated by m) was made using 25.0 mL of a solution of trichloroacetic acid 5% (m/V), which was mixed and centrifuged (Eppendorf, 5810R, Hamburg, Germany) at 6000× *g* for 10 min at 4 °C.

To the centre part of a Conway cell, 1.0 mL of boric acid 1% (m/V) containing 1% colour indicator (methyl red 66 mg/L and bromocresol green 66 mg/L), and 1.0 mL of the sample extract (or distilled water for the blanks or $(NH_4)_2SO_4$ 0.1% m/V to the diffusion control), 0.5 mL of distilled water, and 1.0 mL of saturated solution of K_2CO_3 were added to the exterior ring, while immediately closing the cell. After mixing thoroughly, the cells were placed in the oven at 40 °C for 90 min. After cooling down, the content of the central part was titrated with HCl 0.02 M until the colour changed to pink, where V0 is the volume of titrant used on the blanks, in mL; V1 is the volume of titrant used on the diffusion control; V2 is the volume of titrant used on the sample; and rH is the water content of the sample. The TVB-N content, in mg/100 g FW, was determined by the following equation:

$$\frac{21 * (V2 - V0)}{(V1 - V0) * m} * (100 * rH) \quad (4)$$

2.4.3. Microbiological Study

Samples of VBS were analysed for enumeration of total aerobic microorganisms at 30 °C for 72 h and psychrophiles at 6.5 °C for 10 days by the incorporation technique using the PCA medium, as well as enumeration of moulds and yeasts at 25 °C for 5 days by the spreading technique using the DG-18 medium supplemented with glycerol. Suspension in buffered peptone water was prepared, followed by decimal dilutions.

2.4.4. Sensory Evaluation

The sensory characteristics of the VBS samples were evaluated by a semi-trained panel (n = 10) for their visual appearance, smell, texture, and taste, while searching for the unwanted characteristics. When the unwanted characteristics were found, the samples were considered improper for consumption.

2.5. Consumer Acceptance

Consumer acceptance of the VBS was evaluated using freshly produced samples (no more than 2 days old). Tasters aged 16–64 years old from all backgrounds (n = 89) were given a sample to taste and asked to evaluate the aspect, smell, taste, global evaluation, and purchase intent on a scale from 1 to 7, where 1 is a poor evaluation and 7 is a very good evaluation.

2.6. Statistical Analysis

The mean and sample standard deviation of the physical and chemical experimental data were conducted by Microsoft Office Excel 365 software.

3. Results

3.1. Raw Material Selection

The selection of raw materials was carried out by considering the sustainability, sensory properties, nutritional value, and technological advantages of the production process. Grass pea was chosen due to its resilience, sustainability, and capability of growing in difficult conditions. Locally produced, grass pea was used to increase the sustainability. This pulse has high protein, magnesium, and calcium content [10–14]. Moreover, the choice of chickpea was made due to its high protein, fibre and iron content, low price, and high availability [11,12]. It also helps in reducing the bitter taste of grass pea. *Palmaria palmata* was the selected seaweed due to its potential to substitute table salt, high potassium, iodine, and vitamin C content, as well as flavour [32,33]. All other raw materials were selected to improve flavour and texture.

3.2. Physical and Chemical/Nutritional Composition

The physical and chemical properties, as well as the nutritional composition of VBS were analysed and presented in relation to VBS's FW (Table 1). The pH and aW results show that VBS has low acidity and is a high aW food, which makes it more difficult to preserve for long periods of time. Therefore, this justifies the need for thermal processing and refrigerated storage before consumption [34,42].

The protein content obtained for VBS is lower than that found for other vegan burgers in the UK market [43]. Nevertheless, according to Regulation (EC) No. 1924/2006 of the European Parliament and of the Council of 20 December 2006 [44], the VBS can be classified as "source of protein" as protein represents 17% of its total energy. VBS contains plant-based protein with the potential of providing several essential amino acids, mainly from grass pea. Moreover, the consumption of this type of protein is associated with the reduction in the ingestion of saturated fats and cholesterol [45]. Following the same regulation, VBS is also classified as "low sugar content" and "source of fibre", when considering its total sugar and fibre content. The protein and fibre content is largely dependent on the addition of grass pea and seaweeds to the formulation, with these ingredients being rich in these two macronutrients [36,46,47]. VBS fibre content is higher than that found for other vegan

burgers but similar to that of soy burger [43]. Due to its fibre content, VBS has high potential in regulating the organism's sugar absorption and helping in the functioning of the correct excretory system [48].

Table 1. Physical and chemical/nutritional characteristics of the VBS (n = 3).

pH	5.15 ± 0.01
aW	0.955 ± 0.004
Water content	60.7 ± 0.6% FW
Energy	798.15 kJ (190.05 kcal)
Total fats	5.35 ± 0.11% FW
Saturated fats	0.87 ± 0.01% FW
Carbohydrates	24.59% FW *
Sugars	1.71 ± 0.03% FW
Fibre	5.75% FW *
Total protein	8.01 ± 0.14% FW
TPC	0.29 ± 0.01 mg GAE/g FW
DPPH	below the detection limit

* Theoretical values, determined by calculation.

Regarding the total fat, VBS presents lower fat content than reported for other vegan burgers [43]. As for the fatty acid profile of the VBS (Table 2), unsaturated fatty acids (UFA) represent the major part, with ca. 80% of the total fatty acids (TFA) divided into 58.5% of monounsaturated fatty acids (MUFA) and 21.4% of polyunsaturated fatty acids (PUFA). The other 20% of TFA are saturated fatty acids (SFA). From the PUFA present in the VBS, the most abundant are linoleic acid (C18:2n6c) and α-linolenic acid (C18:3n3). The most relevant MUFA are oleic acid (C18:1n9c) and palmitoleic acid (C16:1). Palmitic acid (C16:0) and stearic acid (C18:0) are the most abundant SFA.

Table 2. Fatty acid profile (% of total fat; n = 3).

C12	1.28 ± 0.05
C14	0.22 ± 0.01
C15	0.22 ± 0.01
C16	11.64 ± 0.14
C16:1	0.37 ± 0.02
C17	0.37 ± 0.01
C18	5.89 ± 0.20
C18:1	21.01 ± 0.64
C18:2	53.65 ± 1.75
C18:3	4.82 ± 1.17
C20	0.52 ± 0.14
SFA	20.15 ± 0.31
MUFA	21.38 ± 0.65
PUFA	58.47 ± 0.73

The high content in UFA as well as this specific fatty acid profile are mainly justified by the presence of olive oil as raw material. This type of fatty acid has been pointed out as a benefit to cardiovascular health [49].

The VBS presents a total ash content of 1.35 ± 0.01% FW. The mineral elements profile was analysed and expressed in mg per kg FW (Figure 2).

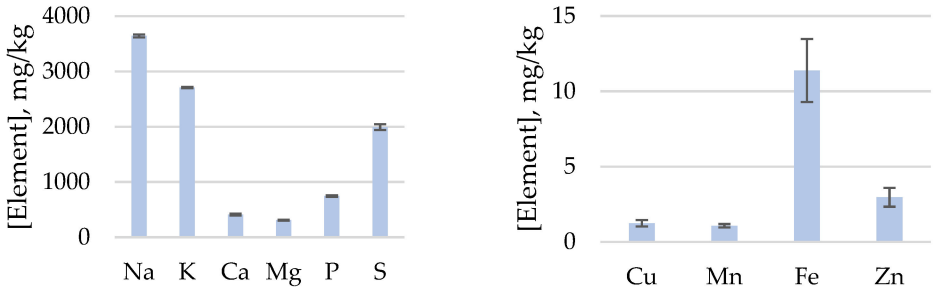

Figure 2. Mineral elements content of the vegan burger with seaweed (n = 3).

Following the previously mentioned regulation, as well as annex 3 of Regulation (EU) No. 1169/2011 of the European Parliament and of the Council of 25 October 2011 [31], and considering its mineral elements content, the VBS is compliant with the allegations "source of phosphorus, and iron" and has a "high content of magnesium, copper and manganese", while presenting "low sodium content". These claims were achieved by a combination of ingredients, highlighting once again the addition of seaweed but also grass pea [45,47]. When compared to other vegan burgers, VBS presents similar Ca and Fe contents but lower Cu, Mn, Zn, and Mg contents [50]. Additionally, it presents lower sodium content (0.36%) when compared to other vegan burgers in the UK market (1.0–1.9%) [43].

All these mineral elements are fundamental for the correct functioning of the human organism and its consumption in a suitable diet (by introducing this type of product) can stand up to the intake of food supplements. Phosphorus aids in the regulation of blood's pH, acts as an activator for some enzymes, and is a key element for bones and cellular membranes [51]. Iron is an essential constituent of hemoglobin and myoglobin, which is necessary for the physical and neurological development. Moreover, it plays a part in the synthesis of some hormones [52] and is given as a supplement during pregnancy [53]. Magnesium is fundamental for the correct functioning of more than 300 enzymes which is indispensable for the correct muscular functioning [54]. Copper is a co-factor in several enzymes responsible for energy production and nutrient metabolism [55]. Manganese is a co-factor in several enzymes with a role in amino acid metabolism, cholesterol, and carbohydrates [56]. Although sodium is essential for some metabolic processes, if ingested in excess according to the usual method in the diets of the majority of the developed countries, it is linked to hypertension and several cardiovascular diseases [57]. Therefore, according to the results obtained in the present study, the developed VBS has mineral elements content that can contribute to a healthy diet.

No antioxidant activity was identified on the VBS by the method used. Regarding total phenolic compounds content, this was measured as 0.29 ± 0.01 mg GAE/g FW. These low values seem to be justified by several thermal processes that the VBS and its raw material were subjected to during production [58–62].

In summary, VBS was shown to be a source of protein, fibre, and mineral elements, such as P, Fe, with high content of Mg, Mn, and Cu, low sodium, and sugar content. Therefore, the consumption of the VBS will perfectly fit in with a balanced diet rich in nutrients and low in sodium, which is suitable for all ages.

3.3. Shelf-Life

The shelf life of VBS in refrigeration at 4 °C was studied. Microbiological growth during the period of the study was analysed (Table 3), as well as chemical degradation of lipids (Figure 3a) and proteins (Figure 3b).

Table 3. Microbiological results for 1, 30, 90, and 180 days (n = 4).

	Colony Forming Units (CFU/g)		
	Total Aerobic Microorganisms at 30 °C	**Psychrophiles**	**Moulds and Yeasts**
Day 1	<10	<10	<10
Day 30	<10	<10	<10
Day 90	<10	<10	<10
Day 180	<10	<10	<10

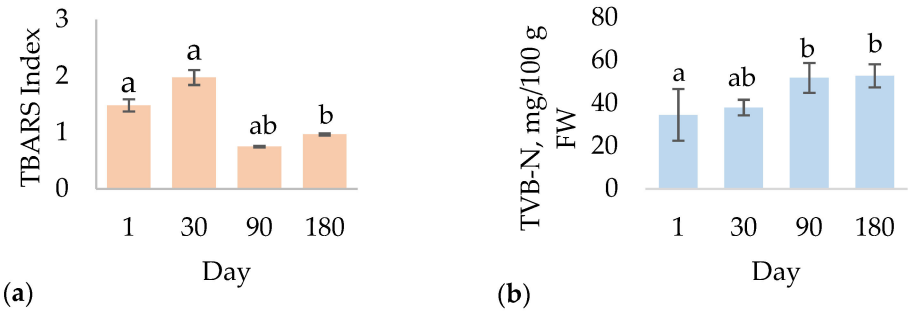

Figure 3. Thiobarbituric Acid Reactive Substances and TVB-N measured during the shelf-life determination study (n = 3). (**a**) TBARS index (**b**) TVB-N in mg/100 g FW. Different letters indicate statistically significant differences ($p < 0.5$).

No microbiological growth was observed during the entire duration of this study (180 days). The values registered are consistent with a "satisfactory" classification, according to the Portuguese National Institute of Health Doctor Ricardo Jorge's (INSA) criteria to "hygiene and spoilage indicator microorganisms in ready-to-eat foods" [63]. This classification indicates that the foods presenting these results are safe for human consumption.

TBARS index and TVB-N were used as degradation indicators, even if not existing in the regulated maximum values for this specific type of food. No tendency was observed on these parameters during the period of the study. This suggests that no relevant degradation phenomena related to these parameters occurred. Small variations were observed and could be justified by small variations on reagent's concentrations, on reaction times, and possible evaporation phenomena during reaction.

Moreover, pH was measured, remaining at 5.14 ± 0.03 during the 180 days of the study. The inexistence of significative variations on this parameter along the study's duration is consistent with the results observed for the microbiological growth and chemical degradation indicators. This is due to the fact that several of the products resulting from microbiological and chemical degradation present acid characteristics which are not observed here [64].

Sensory analysis was performed by a panel of 10 semi-trained tasters, instructed to evaluate the VBS for its general sensory characteristics and search for noticeable changes. No changes were identified on days 30 and 90, when compared with day 1. Changes in texture were identified by all tasters at day 180, when compared with day 1, namely, tasters considered the 180-day-old VBS as dry and brittle. These characteristics were considered unwanted, and thus the study was terminated.

Shelf-life criteria were summarised in Table 4.

Table 4. Criteria for the definition of the vegan burger with seaweed's shelf life.

	Criteria		
	Microbiological	**Physical and Chemical**	**Sensory**
Estimated shelf life	>180 days	>180 days	90 days

As a result, VBS's shelf life was estimated as being 90 days, when stored in its closed original vacuum-seal package and maintained in refrigeration at 4 °C, which is higher than other similar products stored at the same conditions [65,66].

3.4. Consumer Acceptance

To inquire about consumer acceptance of the VBS, 89 people of both genres, ages between 16 and 64 and all diets were invited to taste this innovative food product and provide an answer. They were asked about how pleasant it was (Figure 4a) as well as their purchase intention (Figure 4b).

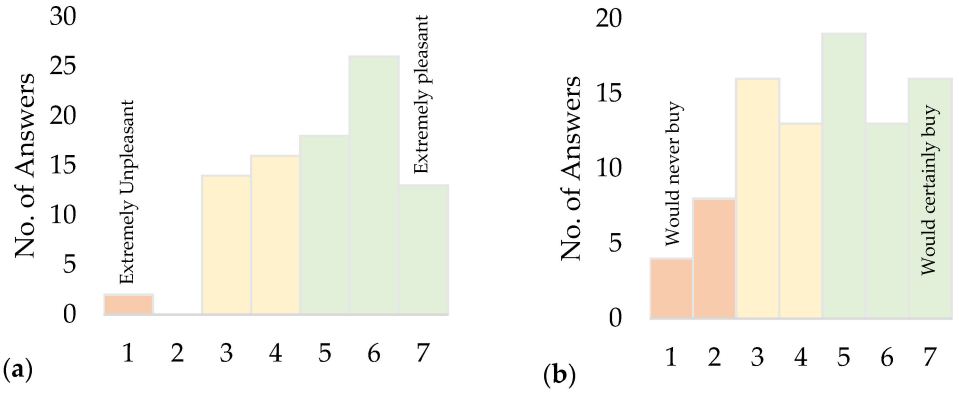

Figure 4. (**a**) Overall acceptance and (**b**) purchase intention of the vegan burger with seaweed.

Moreover, 64.0% of the inquired sample considered the VBS to be at least pleasant (5/7 to 7/7), of which 53.9% considered buying it.

These results show that the population is open to the introduction of new kinds of products on the market.

4. Conclusions

In this study, an innovative vegan burger with seaweed using pulses was developed with environmental and economical sustainability in mind and analysed for physical, chemical, and nutritional characteristics, including mineral element and fatty acid profiles, as well as its shelf life and consumer acceptance.

Results obtained allowed us to understand that this innovative food product presents a "source of protein", "low sugar content", "source of fibre", "source of phosphorus and iron", and "rich in magnesium, copper, and manganese", while presenting a "low sodium content". Its composition in fats is mainly characterised by unsaturated fatty acids and does not present any quantifiable antioxidant activity. The shelf-life analysis allowed us to confirm that the vegan burger with seaweed is safe for consumption for at least 90 days, if stored in its original package and in refrigeration. When the consumers were inquired, a high degree of acceptance and purchase intention were shown. These overall results show that this sustainable and nutritionally rich product with a good shelf life presents a good acceptance by the consumer, showing that there is a market for this innovative type of product.

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