Algal Research 95 (2026) 104610



Contents lists available at ScienceDirect

Algal Research

journal homepage: www.elsevier.com/locate/algal





Use of *Palmaria palmata* and *Fucus vesiculosus* hydrolysates for *Yarrowia lipolytica* biomass and erythritol production

Bruna Dias [a], Marlene Lopes [a,b], Isabel Belo [a,b,*]

[a] CEB – Centre of Biological Engineering, University of Minho, Campus de Gualtar, Braga, Portugal
[b] LABBELS – Associate Laboratory, Braga/Guimarães, Portugal

ARTICLE INFO

Keywords:
Yarrowia lipolytica
Seaweed hydrolysates
Erythritol
Protein-rich biomass
Microbial lipids

ABSTRACT

The relatively low protein content of *Fucus vesiculosus* and *Palmaria palmata* compared to other seaweeds, combined with their high carbohydrate content, makes these seaweeds particularly suitable as biorefinery feedstocks. This study explored the two algae *F. vesiculosus* and *P. palmata* to obtain hydrolysates and use them as substrates for *Yarrowia lipolytica* NCYC 2904 cultivation in stirred-tank bioreactors. Hydrolysates from *F. vesiculosus* (FH), non-washed *P. palmata* (PH), and washed *P. palmata* (WPH) were obtained after hydrothermal pretreatment (121 °C, 30 min) followed by enzymatic hydrolysis (Cellic CTec3). *Y. lipolytica* efficiently assimilated different carbon sources from PH and WPH (glucose, xylose, and formic acid), and from FH (fucose, mannitol, glucuronic acid, and acetic acid). To date, the consumption of fucose and glucuronic acid by *Y. lipolytica* has not been described. The yeast consumed high amounts of xylose (60 $g{\cdot}L^{-1}$) and produced erythritol from PH and WPH, a feature not previously reported. The highest erythritol concentration (40 $g{\cdot}L^{-1}$) and protein content in yeast biomass (29%, w/w, dry basis) were obtained in PH cultures, while the highest cellular lipids accumulation (15%, w/w, dry basis) was attained using WPH. Lipids produced under all conditions were enriched in mono- (71%–78%) and polyunsaturated (20%–22%) fatty acids, while the yeast biomass from PH, WPH, and FH exhibited a well-balanced amino acid profile, including essential amino acids. These findings have significant implications for the development of sustainable blue biorefineries based on *Y. lipolytica* as a microbial platform for valorizing seaweed biomass into bioproducts, such as protein-rich yeast biomass (approved for human consumption), lipids, and erythritol, supporting sustainable food and feed applications.

1. Introduction

The global exploitation of macroalgae has rapidly expanded over the last few years. The global market for seaweed farming is expected to reach USD 25.4 billion by 2025 and is projected to grow at a compound annual growth rate (CAGR) of 12.9% until 2029 [1]. This expansion is driven by the diverse applications of seaweeds and their biomolecules across multiple industries, including their use as nutrient-rich foods, dietary supplements, feed additives, agricultural biofertilizers, and soil conditioners [2,3]. Due to their carbohydrate and/or lipid content, macroalgae are also considered promising feedstocks for biofuels production via microbial fermentation or physicochemical conversion processes [4,5]. Furthermore, macroalgae cultivation plays a crucial role in sustainable aquaculture by assimilating carbon dioxide and metabolizing nitrogen and phosphorus excretions within integrated multi-trophic aquaculture systems [6]. Several advantages, such as high biomass yield, fast growth rates, minimal environmental requirements, positive carbon balance, and the absence of competition for freshwater and arable land with terrestrial plants, support the economical and sustainable use of seaweeds [7]. However, the rapid expansion of seaweed cultivation and accidental releases has led to the spread of invasive species, which threaten coastal ecosystems. The dual role of seaweeds, as a renewable source of bioactive compounds and as an ecological management challenge, underscores the need for strategies that both exploit and manage seaweed biomass [8]. Beyond their current applications, seaweeds (including invasive species) or by-products from bioactive compounds extraction can be valorized as feedstocks for microbial bioprocesses within an integrated biorefinery framework. This approach promotes blue biorefineries within a circular economy concept, aiming to achieve zero waste and aligns with the objectives of the European Green Deal [9].

The absence of lignin in seaweeds enables the use of milder

* Corresponding author at: CEB – Centre of Biological Engineering, University of Minho, Campus de Gualtar, Braga, Portugal.
E-mail address: ibelo@deb.uminho.pt (I. Belo).

https://doi.org/10.1016/j.algal.2026.104610
Received 25 August 2025; Received in revised form 21 November 2025; Accepted 23 February 2026
Available online 25 February 2026
2211-9264/© 2026 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

pretreatment conditions compared to lignocellulosic biomass, thereby enhancing polysaccharides accessibility for enzymatic hydrolysis [10]. Seaweed hydrolysates obtained through pretreatment followed by enzymatic hydrolysis have been extensively studied as substrates for bacterial fermentation to produce bioethanol, biohydrogen, butyric acid, propionic acid, acetone/isopropanol, butanol, and lactic acid [4,11]. However, the use of seaweed hydrolysates in yeast-based bioprocesses remains relatively underexplored and has been primarily limited to bioethanol production using *S. cerevisiae* [11–13], *Brettanomyces custersii* [14], and *Wickerhamomyces anomalus* [15]. The use of seaweed hydrolysates for yeast biomass and microbial protein synthesis was reported by Sharma et al. [16], who cultivated *Candida utilis* in *Saccharina latissima* hydrolysates. Furthermore, the production of yeast biomass and microbial lipids was only reported by Dobrowolski et al. [17], who cultivated the wild-type *Yarrowia lipolytica* A101 in *Fucus vesiculosus* and *S. latissima* hydrolysates. Yeast-based bioprocesses can transform seaweed hydrolysates into valuable compounds but also produce protein- and lipid-rich yeast biomass, thereby enhancing the nutritional value compared to raw macroalgae for food and feed applications [16–18].

The non-conventional yeast *Y. lipolytica* has an exceptional ability to use a wide range of carbon sources, including compounds of lignocellulosic hydrolysates [19–21]. This yeast is also a rich source of lipids [19] and protein [18] and has been extensively studied to produce various biocompounds, such as organic acids [20,22], polyols [23,24], aroma compounds [25], among others. Furthermore, *Y. lipolytica* biomass and its metabolites have been granted Generally Recognized as Safe (GRAS) status by the Food and Drug Administration (FDA) in 2019, and the European Food Safety Authority (EFSA) has approved its biomass as an innovative novel food [18].

Palmaria palmata, commonly known as dulse or dillisk, is a red algae species belonging to the *Rhodophyta* group, typically found along the rocky coasts of the Northern Hemisphere [5]. This seaweed is composed primarily of xylan [26,27]. Protein content can vary significantly depending on factors such as geographic origin, season, and cultivation conditions [26]. Traditionally consumed due to its distinctive umami flavor, *P. palmata* is gaining renewed interest due to its content of vitamins, iron, essential minerals, dietary fibers, and phenolic compounds [5,26,27]. *Fucus vesiculosus*, traditionally known as bladder wrack, is one of the most prominent brown algae of the genus *Fucus*, commonly found in cold to temperate waters along the rocky shorelines of the Northern Hemisphere, typically in areas of high salinity [5]. Rich in macro- and micronutrients, *Fucus* species are consumed in various forms by populations across Asia, coastal Western Europe, and Alaska [5,28]. This seaweed contains several bioactive compounds, including fucoidans, phlorotannins, alginate, mannitol, laminarin, polyphenols, fucoxanthin, and essential minerals, with potential applications in the food, animal feed, cosmetic, biofertilizer, and pharmaceutical industries [28,29]. Despite their richness in bioactive compounds, both *P. palmata* and *F. vesiculosus* generally present relatively low protein content compared to other seaweeds [18], opening new opportunities for valorization by yeasts.

The use of *P. palmata* hydrolysates in yeast-based bioprocesses has only been explored by Mutripah et al. [30], and *F. vesiculosus* was only studied by Dobrowolski et al. [17]. Therefore, this work aimed to unravel the potential of *P. palmata* and *F. vesiculosus* hydrolysates (two distinct types of seaweeds with different biochemical compositions) as substrates for biocompounds production by *Y. lipolytica* NCYC 2904 in submerged fermentation. The seaweed hydrolysates, obtained through hydrothermal pretreatment followed by enzymatic hydrolysis, were used in STR bioreactors to explore the production of lipid- and protein-rich biomass and erythritol. The influence of washing *P. palmata* on hydrolysate composition and production of biomass and metabolites was also evaluated.

2. Materials and methods

2.1. Seaweeds characterization

The seaweeds were purchased at AlgaPlus (Ílhavo, Portugal), an IMTA-producer of seaweeds company. *Fucus vesiculosus*, supplied whole and dried, was milled to sizes smaller than 5 mm, and *Palmaria palmata* was supplied dried and milled (flakes <5 mm). Seaweeds were stored in hermetic plastic bags and kept in the dark at room temperature until use.

Moisture and ash contents were determined by oven-drying at 105 °C and calcination in a muffle at 575 °C, respectively, until constant weight [31]. Total carbon and nitrogen content was quantified using a UNICUBE® elemental analyzer (Elementar, Langenselbold, Germany). A conversion factor of 5 was applied to the nitrogen content to estimate total protein. Total lipids were quantified using the method described by Ferreira et al. [32], after lipids extraction with a chloroform and methanol mixture. The carbohydrate composition was assessed through a two-step quantitative acid hydrolysis following the NREL/TP-510-42618 protocol [33]: (i) 72% H_2SO_4 at 30 °C for 60 min, and (ii) 4% H_2SO_4 at 121 °C for 60 min. The concentration of monomeric sugars, and glucuronic and galacturonic acids released during quantitative acid hydrolysis was quantified by high-performance liquid chromatography (HPLC), and the corresponding polymeric fractions - glucan, xylan, fucoidan, and uronic acids - were calculated using the standardized stoichiometric correction factors (0.88 for xylan, 0.90 for glucan and fucoidan, and 0.92 for uronic acids). Mannitol released during hydrolysis was also quantified by HPLC. The insoluble phase obtained was oven-dried at 105 °C and expressed as acid-insoluble residue.

The mineral elements (sodium, potassium, magnesium, calcium, phosphorus, iron, manganese, lead, and zinc) were measured by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES, PerkinElmer, Waltham, MA, USA). Before analysis, seaweed samples were digested using a defined protocol for algae from the Berghof microwave digestion system (Speedwave four DAP-60+, Berghof Products + Instruments GmbH, Eningen, Germany). The digested samples were diluted with distilled water and analyzed under the following ICP-OES operating conditions: radio frequency power of 1350 W; 10 $L{\cdot}min^{-1}$ argon plasma flow; 0.2 $L{\cdot}min^{-1}$ auxiliary gas flow; and 0.6 $L{\cdot}min^{-1}$ nebulizer gas flow. The emission wavelengths (nm) used for each element were: Na, 589.59; K, 766.49; Mg, 285.21; Ca, 317.93; P, 213.62; Fe, 238.20; Mn, 257.61; Pb, 283.31; Zn, 213.86.

2.2. Microorganisms

Yarrowia lipolytica NCYC 2904, grown in YPD medium (20 $g{\cdot}L^{-1}$ glucose, 20 $g{\cdot}L^{-1}$ peptone, 10 $g{\cdot}L^{-1}$ yeast extract) for 2 days, was stored in 20% (v/v) glycerol at −80 °C until pre-inoculum preparation.

2.3. Hydrothermal pretreatment and enzymatic hydrolysis

Seaweeds were hydrothermally pretreated in an autoclave at 121 °C for 30 min using a 15% (w/w) dry solid loading in 50 mM citrate buffer (pH 4.8). The pretreatment was carried out in 1000-mL Erlenmeyer flasks with a working volume of 200 mL. The slurries obtained were subjected to enzymatic hydrolysis at 50 °C and 200 rpm of agitation using the commercial enzyme cocktail Cellic CTec3 (Novozymes, Bagsværd, Denmark) at a dosage of 15 filter paper units (FPU) per gram of dry solids. Hydrolysis was performed for 48 h for *P. palmata* and 96 h for *F. vesiculosus*. An additional condition was assessed for *P. palmata*, in which the seaweed was washed four times with distilled water and oven-dried at 60 °C for 16 h before pretreatment. The hydrolysates obtained were filtered, centrifuged, and analyzed by HPLC to quantify monomeric and oligomeric sugars.

2.4. Bioreactor experiments

A 2-L DASGIP Parallel Bioreactor System (Eppendorf, Hamburg, Germany), with a working volume of 400 mL, was used to evaluate the effect of different seaweed hydrolysates (PH- *P. palmata* hydrolysate, WPH- washed *P. palmata* hydrolysate, and FH- *F. vesiculosus* hydrolysate) as substrates for *Y. lipolytica* growth, intracellular compounds accumulation, and metabolites production. Culture media were supplemented with 2 $g{\cdot}L^{-1}$ corn steep liquor (CSL) and $(NH_4)_2SO_4$ for a C/N ratio of 20 (2.2 $g{\cdot}L^{-1}$ in PH, 2.8 $g{\cdot}L^{-1}$ in WPH, and 0.6 $g{\cdot}L^{-1}$ in FH). *Yarrowia lipolytica* NCYC 2904 cells, grown overnight in YPD, were centrifuged and resuspended to an initial concentration of 0.5 $g{\cdot}L^{-1}$ in *P. palmata* or *F. vesiculosus* hydrolysates medium.

Batch cultures were conducted at 27 °C, an agitation rate of 800 rpm, and an aeration rate of 3 vvm. The pH was automatically maintained at 5.5 ± 0.5 using 4 M HCl or 4 M NaOH.

2.5. Analytical methods

Yarrowia lipolytica biomass concentration was quantified by cell counting with a Neubauer counting chamber in a binocular bright-field microscope and converted to cell dry weight ($g{\cdot}L^{-1}$) by a conversion factor of 1.94×10^{10} $cells{\cdot}g^{-1}$.

The concentration of glucose, xylose, fucose, glucuronic acid, galacturonic acid, formic acid, mannitol, and erythritol was quantified by high-performance liquid chromatography (LC 2060C, Shimadzu, Japan) using an Aminex HPX-87H column (300 mm × 7.8 mm, 8 μm particle size), equipped with refractive index (RI) and UV (λ = 210 nm) detectors. The column temperature was maintained at 60 °C, and 5 mM sulfuric acid was used as the mobile phase at a flow rate of 0.7 $mL{\cdot}min^{-1}$.

Cuvette test kit LCK 338 (Hach-Lange GmbH, Germany) was used for the spectrophotometric determination of total nitrogen in hydrolysates.

Intracellular lipids were quantified in lyophilized cells using the phospho-vanillin colorimetric method, and lipids composition was determined after extraction with chloroform and methylation with a sulfuric acid–methanol mixture (85:15, v/v), following the protocols of Lopes et al. [34].

The total protein of lyophilized yeast cells was estimated by applying a conversion factor of 6.25 to the nitrogen content, determined using a UNICUBE® elemental analyzer (Elementar, Langenselbold, Germany).

The amino acid composition of biomass was determined after acid hydrolysis of cells and analysis by ultra-high performance liquid chromatography (UHPLC, SCL-40, Shimadzu, Japan) equipped with an automated pre-column derivatization module (RF-20AXS, Shimadzu). Lyophilized cells were hydrolyzed with 2 mL of 6 N HCl at 100 °C for 24 h. The hydrolysate was diluted in ultrapure water (1:100, v/v) and mixed (1:1, v/v) with an internal standard solution of homoserine (5 $mg{\cdot}L^{-1}$). Pre-column derivatization was performed automatically using the autosampler's integrated pretreatment function with a mixture of mercaptopropionic acid/ortho-phthalaldehyde (MPA/OPA), 9-fluorenylmethyl chloroformate (FMOC), and aqueous phosphoric acid, following the protocol provided by Hakyakawa [35]. The amino acids were analyzed on a Shim-pack XR-ODS II maintained at 40 °C. The mobile phases consisted of 20 $mmol{\cdot}L^{-1}$ sodium acetate buffer (pH 6.0), water:acetonitrile (1:9, v/v), and 20 $mmol{\cdot}L^{-1}$ sodium acetate buffer (pH 5.0) containing 0.5 $mmol{\cdot}L^{-1}$ EDTA-2Na. The elution was carried out at a flow rate of 0.8 $mL{\cdot}min^{-1}$ using a gradient method. Fluorescence detection was employed, with excitation/emission wavelengths set for OPA and FMOC derivatives as per the manufacturer's protocol. Amino acids standards solutions at several concentrations in HCl 0.1 M were prepared and submitted to the same derivatization and analysis procedures as the samples, to establish the calibration curves. Amino acids were identified by comparing retention times with those of standard amino acid mixtures.

2.6. Statistical analysis

One-way analysis of variance (ANOVA) and Tukey's test were performed using GraphPad Prism 7 software (Dotmatics, California, USA) to identify significant differences among means ($p < 0.05$).

3. Results and discussion

3.1. Seaweeds composition

The ash content, an indicator of the mineral concentration of seaweed, was similar in *F. vesiculosus* and *P. palmata* (Table 1). The red seaweed *P. palmata* had a 1.9-fold higher protein content than *F. vesiculosus*. The reliability of protein quantification in seaweeds is substantially affected by the choice of nitrogen-to-protein conversion factor, introducing significant uncertainty into the interpretation of results. The commonly used factor of 6.25 assumes that protein is the only source of nitrogen, which may lead to overestimation, as seaweeds contain significant non-protein nitrogen sources such as free amino acids, chlorophyll, nucleic acids, and inorganic nitrogen [36]. Mæhreet al. [37] proposed an average factor of 4.92 for seaweeds, while Maribu et al. [27] reported values ranging from 3.61 to 5.13 specifically for *P. palmata* samples. Given the lack of consensus, this study adopted the factor of 5 recommended by Angell et al. [36] as a more reliable universal conversion factor for seaweeds. The protein content in *P. palmata* can reach up to 42% of dry mass [38]; however, it varies significantly depending on geographic origin, season, and cultivation conditions, and values can be substantially lower than this maximum, as observed in the present study. As expected, lipids content was low in both species, consistent with the typical biochemical profile of seaweeds [39]. Regarding carbohydrate profiles, the brown seaweed *F. vesiculosus* was composed of glucan (10%), fucoidan (10%), mannitol (8%), uronic acids (6%), and xylan (5%). In contrast, the red seaweed *P. palmata* was rich in xylan (47%) and had a low amount of glucan (5%). These differences reflect the distinct polysaccharide composition associated with seaweed taxonomy. Brown seaweeds typically contain laminarin, mannitol, alginate (a polymer of uronic acids), and fucoidans (complex fucose-rich polysaccharides that may also include galactose, mannose, xylose, glucose, and glucuronic acid) [40]. In contrast, most red seaweeds are composed primarily of floridean, cellulose, mannans, and agar (which consists of galactopyranose and galactose) [40]. However, an evolutionary innovation in the family *Palmariaceae* led to the production of xylans as the major structural polysaccharides in the cell wall of species such as *P. palmata* [41]. Despite the known variability in seaweed composition influenced by seasonality, environmental conditions, geographical origin, and other factors, the ash, protein, lipids, and carbohydrate values in this study align with the reported ranges for *F. vesiculosus* [29,37,40] and *P. palmata* [26,27,37]. Nova et al. [42]

Table 1
Composition of *Fucus vesiculosus* and *Palmaria palmata*. Data are the average ± standard deviation of three independent analyses. Values followed by the same letter in each line do not present statistically significant differences ($p \geq 0.05$).

Component (g/100 g dry seaweed)	*Fucus vesiculosus*	*Palmaria palmata*	Washed *Palmaria palmata*
Ash	25.3 ± 0.7[a]	21.0 ± 1.4[a]	3.6 ± 0.4[b]
N	1.5 ± 0.3[a]	2.8 ± 0.2[b]	3.4 ± 0.3[b]
C	32.2 ± 1.2[a]	28.0 ± 2.7[a]	37.7 ± 1.3[b]
Total protein	7.5 ± 1.3[a]	13.8 ± 0.9[b]	17.2 ± 0.3[c]
Total lipids	1.2 ± 0.2[a]	1.8 ± 0.3[a]	1.7 ± 0.4[a]
Glucans	10.1 ± 0.4[a]	4.6 ± 0.5[b]	6.0 ± 0.6[b]
Xylans	5.1 ± 0.2[a]	46.5 ± 1.3[b]	42.9 ± 1.5[b]
Fucanoids	10.1 ± 0.5	n.d.	n.d.
Mannitol	8.2 ± 0.3	n.d.	n.d.
Uronic acids	6.0 ± 0.2	n.d.	n.d.
Acid-insoluble residue	9.7 ± 0.5[a]	2.2 ± 0.5[b]	3.0 ± 0.7[b]

n.d.: not detected.

reported values of 27% ash, 42% protein, and 0.3% lipids for *P. palmata*, and 30%, 12%, and 3%, respectively, for *F. vesiculosus*. Values closer to those obtained in the present study were reported by Dussan et al. [26], who found protein contents of 10% –16% and ash contents of 13%–27% in *P. palmata*.

Both unwashed seaweeds were rich in key macro-elements, particularly sodium and potassium (Table 2). *Palmaria palmata* had higher sodium content, while *F. vesiculosus* was richer in potassium and calcium. Among the micro-elements, iron and manganese were more abundant in *F. vesiculosus*, while lead and zinc were found in comparable amounts in both species. Dussan et al. [26] identified potassium (49 $g{\cdot}kg^{-1}$) and sodium (33 $g{\cdot}kg^{-1}$) as the two most abundant macro-elements in *P. palmata*. Lafeuille et al. [43] reported variations ranging from 1.6-fold to 12.6-fold in the mineral composition (I, K, Na, Ca, Mg, and Fe) of *P. palmata*, depending on the harvest period, but potassium (34–92 $g{\cdot}kg^{-1}$) and sodium (13–26 $g{\cdot}kg^{-1}$) remained the dominant elements. For *F. vesiculosus*, potassium and sodium contents were not quantified in studies by Obluchinskaya et al. [28] and Mæhre et al. [37]; however, comparable concentrations of calcium (9.8–18 $g{\cdot}kg^{-1}$) and magnesium (7.4–11.5 $g{\cdot}kg^{-1}$) were reported. According to Obluchinskaya et al. [28], elemental concentrations in *F. vesiculosus* exhibit significant variability depending on geographical location and reproductive phase.

The high salt content in unwashed seaweeds, owing to the seawater salinity and the preferential uptake of K^+ and Na^+ ions through the cell walls, can interfere with microbial fermentation processes [26]. Osmotic stress, particularly that induced by NaCl, has been shown to affect *Y. lipolytica* morphology, typically resulting in smaller and more rounded cells [44]. Tzirita et al. [45] reported that NaCl addition to the medium negatively affected *Y. lipolytica* biomass production, substrate assimilation, and lipids accumulation. Considering the influence of NaCl on *Y. lipolytica* metabolism, *P. palmata* was also washed to reduce its sodium content and evaluate the effect of lower salinity of seaweed hydrolysate on the production of *Y. lipolytica* biomass and bioproducts. This washing step was effective in reducing ash content (Table 1) and macro- and micro-element concentrations (Table 2). The higher protein and carbon content observed in washed *P. palmata* reflects a relative concentration effect, as the removal of ash increases the proportion of the remaining constituents. Fernandes et al. [46] washed *Ulva rigida*, achieving a 4.6-fold and 1.2-fold reduction in salt and ash content, respectively, to investigate the effect of salt removal on the growth of *Aspergillus ibericus* and enzyme production. Moreover, protein, carbon, and glucan content also increased after the washing step. Novoa-Garrido et al. [47] also reported that, after washing, the ash content of *Porphyra umbilicalis* decreased 1.4-fold, whereas the nitrogen content slightly increased. Nanofiltration of *P. palmata* hydrolysates successfully removed 64%–78% of K^+ and Na^+, and 44%–59% of Ca^{2+}, mitigating the inhibitory effects on *Clostridium beijerinckii* fermentation [26].

3.2. Hydrothermal pretreatment and enzymatic hydrolysis

Due to the absence of lignin, seaweed biomass can be pretreated under milder conditions than lignocellulosic materials, resulting in lower energy requirements [10]. The autoclave hydrothermal pretreatment of *F. vesiculosus* (unwashed) and *P. palmata* (unwashed and washed) was carried out at 121 °C for 30 min, corresponding to a severity factor of 2.26.

After enzymatic hydrolysis of the pretreated seaweeds with Cellic CTec3, the *P. palmata* hydrolysate (PH) was composed of 83 $g{\cdot}L^{-1}$ of total sugars (with xylose and glucose accounting for 84% and 16%, respectively), and formic acid (Table 3). This composition corresponds to saccharification yields of 100% for glucan and 76% for xylan. The enzymatic hydrolysis of the pretreated washed *P. palmata* produced a hydrolysate (WPH) with similar glucose and xylose concentrations, but with low formic acid concentration (Table 3). No glucooligosaccharides and xylooligosaccharides were detected in PH and WPH. Since *F. vesiculosus* has a more complex carbohydrate profile, its hydrolysate (FH) had a higher diversity of components: 28 $g{\cdot}L^{-1}$ of sugars and sugars alcohols (including besides glucose and xylose, 0.8 $g{\cdot}L^{-1}$ fucose, and 15 $g{\cdot}L^{-1}$ mannitol), 13 $g{\cdot}L^{-1}$ of uronic acids (glucuronic acid), and 2 $g{\cdot}L^{-1}$ of organic acids (formic acid and 0.5 $g{\cdot}L^{-1}$ acetic acid) (Table 3). As expected, the total sugar concentration in FH was lower than in PH, due to the lower carbohydrate content of *F. vesiculosus*. Despite the longer enzymatic hydrolysis applied to *F. vesiculosus* (96 h) compared to *P. palmata* (48 h), due to its more complex carbohydrate profile, FH had a higher concentration of oligosaccharides than PH (2 $g{\cdot}L^{-1}$ glucooligosaccharides, 4 $g{\cdot}L^{-1}$ xylooligosaccharides, and 14 $g{\cdot}L^{-1}$ fucooligosaccharides), suggesting incomplete hydrolysis of pretreated *F. vesiculosus*. All the mannitol and glucuronic acid from the crude *F. vesiculosus* were recovered in the hydrolysate. However, saccharification of glucan, xylan, and fucoidan was incomplete, with conversion yields reaching only 48%, 28%, and 4%, respectively. This low hydrolysis efficiency may be attributed to the presence of enzyme inhibitors in the hydrolysate, such as sugars, acids, soluble proteins, or the high sulfate content of fucoidans, which can impair enzymatic activity by competing with target polysaccharides or by binding directly to the enzymes [26]. Moreover, Cellic CTec3, an enzymatic cocktail rich in cellulases, hemicellulases, and β-glucosidases, may lack the specificity needed to effectively hydrolyze the various polysaccharides in *F. vesiculosus*, such as fucoidans, due to the absence of fucoidanase activity.

The nitrogen concentration in hydrolysates indicates that part of the nitrogen content of the seaweed was solubilized during the hydrothermal pretreatment and enzymatic hydrolysis steps (Table 3). As observed in the mineral composition of washed *P. palmata*, the mineral content of WPH confirmed that the washing step reduced sodium and potassium concentrations compared to PH. In all hydrolysates, magnesium, calcium, and phosphorus were below 1 $g{\cdot}L^{-1}$, and micronutrients concentration (Fe, Mn, Pb, and Zn) were less than 0.08 $g{\cdot}L^{-1}$.

Table 2
Mineral composition of *Fucus vesiculosus* and *Palmaria palmata*. Data are the average ± standard deviation of three independent analyses. Values followed by the same letter in line do not present statistically significant differences ($p \geq 0.05$).

Element ($g{\cdot}kg^{-1}$ dry seaweed)	*Fucus vesiculosus*	*Palmaria palmata*	Washed *Palmaria palmata*
Na	35.3 ± 1.2^a	71.5 ± 0.8^b	2.3 ± 0.1^c
K	40.3 ± 1.5^a	13.3 ± 1.1^b	0.5 ± 0.1^c
Mg	8.0 ± 0.1^a	7.1 ± 0.3^a	1.8 ± 0.1^b
Ca	9.5 ± 0.2^a	3.6 ± 0.1^b	2.4 ± 0.1^c
P	2.7 ± 0.2^a	2.6 ± 0.3^a	2.6 ± 0.2^a
Fe	0.41 ± 0.01^a	0.31 ± 0.01^b	0.27 ± 0.01^b
Mn	0.47 ± 0.03^a	0.13 ± 0.02^b	0.08 ± 0.01^b
Pb	0.11 ± 0.01^a	0.10 ± 0.01^a	0.03 ± 0.01^b
Zn	0.05 ± 0.01^a	0.02 ± 0.01^a	0.02 ± 0.01^a

Table 3
Main composition of *Palmaria palmata* and *Fucus vesiculosus* hydrolysates obtained by autoclave hydrothermal pretreatment followed by enzymatic hydrolysis. Data are the average ± standard deviation of two independent analyses. Values followed by the same letter in each line do not present statistically significant differences ($p \geq 0.05$).

Concentration ($g{\cdot}L^{-1}$)	*Palmaria palmata*	Washed *Palmaria palmata*	*Fucus vesiculosus*
Glucose	13.0 ± 0.6^a	15.3 ± 0.9^a	9.4 ± 0.6^b
Xylose	70.3 ± 1.3^a	72.7 ± 1.0^a	2.9 ± 0.2^b
Formic acid	4.7 ± 0.3^a	0.9 ± 0.2^b	1.7 ± 0.1^c
Nitrogen	1.4 ± 0.2^a	1.0 ± 0.2^{ab}	0.9 ± 0.1^b
Sodium	10.2 ± 0.7^a	1.9 ± 0.3^b	6.1 ± 0.5^c
Potassium	5.2 ± 0.5^a	0.4 ± 0.1^b	6.0 ± 0.3^a

3.3. Stirred tank bioreactor experiments

The ability of *Y. lipolytica* NCYC 2904 to grow in undiluted PH, WPH, and FH was evaluated in stirred-tank bioreactor experiments (Fig. 1). Considering the diversity and varying concentrations of carbon sources in the three hydrolysates, the C/N ratio was adjusted to approximately 20 in all of them by adding ammonium sulfate. This relatively low C/N ratio was selected to potentially enhance yeast growth [48] when using these less-explored and non-preferred carbon sources for *Yarrowia lipolytica*. After ammonium sulfate addition, the nitrogen concentrations in PH, WPH, and FH were 1.9 $g{\cdot}L^{-1}$, 1.6 $g{\cdot}L^{-1}$, and 1.1 $g{\cdot}L^{-1}$, respectively.

Yarrowia lipolytica is commonly classified as a non-xylose-utilizing yeast, despite naturally possessing a cryptic xylose utilization pathway with xylose-degrading enzymes, including D-xylose reductase (XR), xylitol dehydrogenase (XDH), and xylulokinase (XK), which have weak activity [49]. Approaches such as adaptation of the native xylose pathway, overexpression of native enzymes, or introduction of heterologous xylose pathways (e.g., isomerase pathways) have been employed in *Y. lipolytica* strains to enhance xylose assimilation [50,51]. Overexpression of XDH and XK is reported as sufficient to enable efficient xylose assimilation [52]. However, there are a few studies regarding sequential glucose-xylose consumption by *Y. lipolytica* wild-type strains in lignocellulosic biomass hydrolysates [19–21,53,54]. Dias et al. [19] observed that the wild-type *Y. lipolytica* NCYC 2904, when grown in olive tree prunings hydrolysate with a high glucose-to-xylose ratio (6.5), started xylose consumption only after the glucose concentration fell below 30 $g{\cdot}L^{-1}$. A similar behavior was observed for *Y. lipolytica* W29 cultivated in eucalyptus bark hydrolysate with a glucose-to-xylose ratio of 6.3 [20]. Contrary to those reports, in this study, xylose was simultaneously assimilated with glucose by *Y. lipolytica* NCYC 2904 in PH and WPH (Fig. 1A), where the glucose-to-xylose ratio was much lower (0.18 or 0.21). These findings suggest that xylose assimilation ability may be influenced by the glucose-to-xylose concentration ratio. A high glucose-to-xylose ratio appears to favor the preferential consumption of glucose. Simultaneous assimilation of both sugars is observed only after a decrease in glucose concentration, and consequently, in the glucose-to-xylose ratio. Glucose and xylose uptake rates were comparable in both the PH and WPH experiments. Remarkably, the uptake rate of xylose was approximately 3-fold higher than that of glucose (Table 4), a finding that, to the best of our knowledge, has not been previously reported. The assimilation of xylose by the wild-type strain NCYC 2904 suggests that the high xylose-to-glucose ratio in *P. palmata* hydrolysates may have favored activation of the cryptic xylose pathway. A control experiment performed in a synthetic medium mimicking washed *P. palmata* hydrolysate showed a similar xylose consumption profile (Supplementary Fig. S1), indicating that this behavior is independent of the medium's complexity and is influenced by the glucose-to-xylose ratio and the strain's metabolic capacity. This novel observation challenges the conventional understanding of xylose utilization by wild-type *Y. lipolytica* strains, particularly the NCYC 2094 strain that has an efficient xylose metabolic pathway and may have significant implications for the optimization of bioprocesses involving xylose-rich substrates. Thus, this fact opens new perspectives for studies on the expression of the genes encoding the key enzymes of the pathway in this strain [51]. In both experiments, glucose (13 $g{\cdot}L^{-1}$ - 15 $g{\cdot}L^{-1}$) was consumed within 72 h, and formic acid (0.9 $g{\cdot}L^{-1}$–4.7 $g{\cdot}L^{-1}$) in 24 h. An impressive amount of xylose (56 $g{\cdot}L^{-1}$ in PH and 59 $g{\cdot}L^{-1}$ in WPH experiments) was assimilated within 120 h. Despite similar carbon source consumption patterns, biomass production in WPH was 1.3-fold higher than in PH, leading to a higher biomass yield (Table 4). The removal of salts and minerals by *P. palmata* washing seems to favor *Y. lipolytica* biomass production.

Y. lipolytica synthesized erythritol from *P. palmata* hydrolysates, a feature never reported before. Erythritol is a low-calorie polyol widely used as a sugar substitute in food formulations, with an estimated market value of approximately USD 449.6 billion by 2033 [55]. Erythritol synthesis was a growth-coupled process, achieving the maximum value at 96 h in both PH and WPH experiments (Fig. 1B). The polyol

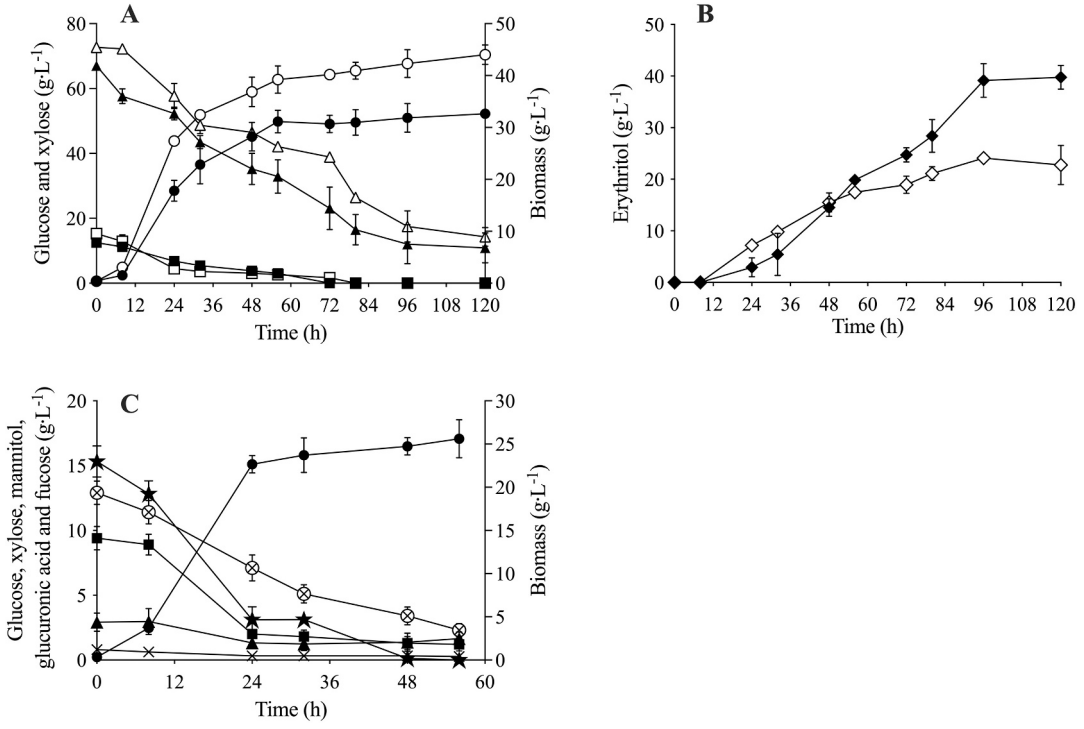


Fig. 1. Variation through time of the concentration of biomass (●, ○), glucose (■, □), xylose (▲, △), fucose (×), mannitol (★), glucuronic acid (⊗) and erythritol (◆, ◇) in *Y. lipolytica* NCYC 2904 batch cultures carried out in STR operating at atmospheric pressure with *P. palmata* hydrolysate (A, B, closed symbols), washed *P. palmata* hydrolysate (A, B, open symbols), and *F. vesiculosus* hydrolysate (C). Data represent the mean of two independent biological replicates, with triplicate measurements performed at each time point. Error bars indicate standard deviation.

Table 4
Values of biomass yield, glucose and xylose uptake rates, and erythritol yield obtained in batch cultures using *P. palmata* and *F. vesiculosus* hydrolysates in STR experiments. Data are the average ± standard deviation of two independent biological replicates, with triplicate measurements performed at each sample. Values followed by the same letter in each column do not present statistically significant differences ($p \geq 0.05$).

Culture medium	Biomass yield ($g \cdot g^{-1}$)	Glucose uptake rate ($g \cdot L^{-1} \cdot h^{-1}$)	Xylose uptake rate ($g \cdot L^{-1} \cdot h^{-1}$)	Erythritol yield ($g \cdot g^{-1}$)
PH	0.44 ± 0.03[a]	0.17 ± 0.01[a]	0.56 ± 0.02[a]	0.54 ± 0.03[a]
WPH	0.58 ± 0.02[b]	0.17 ± 0.01[a]	0.57 ± 0.01[a]	0.31 ± 0.02[b]
FH	0.67 ± 0.02[b]	0.32 ± 0.02[b]	0.06 ± 0.01[b]	–

production and yield in PH were 1.7-fold higher than in WPH, a result linked to the higher sodium concentration in PH. Erythritol synthesis in *Y. lipolytica* is typically induced by osmotic stress, functioning as an adaptive response to maintain cellular osmotic balance [56,57]. Several studies have reported that high NaCl concentrations in a medium containing glucose [55], glycerol [58], or waste cooking oils [24] significantly enhance erythritol production by *Y. lipolytica* strains. Rzechonek et al. [59] reported that an increase in osmotic pressure enhances erythritol production while reducing side product formation. Accordingly, our results demonstrated that PH (with high sodium concentration) favored erythritol synthesis, whereas WPH (with low sodium concentration) promoted the highest biomass production.

In the experiments with *F. vesiculosus* hydrolysates, *Y. lipolytica* also assimilated all sugars and other compounds of FH (Fig. 1C). Mannitol and glucose were faster assimilated, with uptake rates of 0.34 $g \cdot L^{-1} \cdot h^{-1}$ and 0.32 $g \cdot L^{-1} \cdot h^{-1}$, respectively, followed by glucuronic acid at 0.19 $g \cdot L^{-1} \cdot h^{-1}$. Glucose and mannitol are readily assimilable carbon sources that can be directly channeled into central carbon metabolism through glycolysis [60]. Xylose and fucose were consumed more slowly, at 0.06 $g \cdot L^{-1} \cdot h^{-1}$ and 0.02 $g \cdot L^{-1} \cdot h^{-1}$, respectively. The uptake rate of xylose observed in FH experiments supports the results found in the *P. palmata* hydrolysates, which indicate that xylose assimilation depends on the glucose-to-xylose ratio. Indeed, in the FH experiments, where the glucose-to-xylose ratio was 3.2, significantly higher than in the PH experiments, glucose was preferentially consumed. Xylose assimilation began after a decrease in glucose concentration, indicating a clear preference for glucose utilization under these conditions. Besides these compounds, the yeast assimilated all acetic and formic acids after 24 h and 48 h, respectively. The experiments with FH were completed earlier compared to PH and WPH experiments (56 h vs. 120 h), as the stationary phase was reached at 24 h and carbon sources in the medium dropped below 3 $g \cdot L^{-1}$. Indeed, at the end of the experiments, residual concentrations of 2.3 $g \cdot L^{-1}$ glucuronic acid, 1.2 $g \cdot L^{-1}$ glucose, and 1.3 $g \cdot L^{-1}$ xylose remained in the FH medium. Since *Y. lipolytica* possesses the ability to assimilate mannitol from the medium, although the process is relatively inefficient, several studies have engineered *Y. lipolytica* strains to improve mannitol utilization [61]. However, the assimilation of mannitol by wild-type *Y. lipolytica* strains remains a less explored topic in the literature. Similarly, the use of glucuronic acid and fucose as carbon sources by *Y. lipolytica*, particularly in wild-type strains, has also been less explored. Therefore, the results obtained with *F. vesiculosus* hydrolysate offer valuable insights into the potential of *Y. lipolytica* to utilize alternative substrates.

Biomass concentration in FH was lower than in WPH but similar to that obtained in PH. However, the biomass yield in FH was the highest among the seaweed hydrolysates, indicating that the assimilated carbon source was directed toward biomass production, as no other metabolites, such as erythritol, were produced. The absence of erythritol production in FH could not be attributed to insufficient osmotic stress, as the concentration of sodium and potassium was higher than in WPH, where erythritol was produced. Instead, the presence of mannitol in the FH may explain this outcome. Polyols such as erythritol, mannitol, and arabitol, synthesized by *Y. lipolytica* under high salinity, act as osmoprotectants to help maintain cellular osmotic balance [61]. The mannitol already present in the FH may have fulfilled the cell's osmotic needs, potentially downregulating the metabolic machinery required for de novo erythritol synthesis. Additionally, the assimilation of mannitol may introduce metabolic competition for key precursors (intermediates of glycolysis or the pentose phosphate pathway) and cofactors (NADPH), which are essential for both mannitol metabolism and erythritol biosynthesis [60].

3.4. Characterization of *Y. lipolytica* biomass

The lipids content of the final *Y. lipolytica* biomass obtained in both PH and WPH was low (Table 5) and remained similar throughout the cultivation in both cases. Although the NCYC 2904 strain is recognized as a high lipid producer, achieving contents ranging from 25% to 42% [19,23,62], these values were not reached in this study, likely due to the high concentrations of xylose in the hydrolysates and the selected C/N ratio, which favored yeast growth but limited lipids accumulation. High nitrogen concentrations, and consequently a low C/N ratio, are typically associated with enhanced growth rather than lipids accumulation in *Y. lipolytica* [48]. The lipids content reached in PH and WPH was 6- and 9-fold higher, respectively, than the content in *P. palmata* seaweed (2%), demonstrating the potential of *Y. lipolytica* NCYC 2904 for seaweed valorization through lipids enrichment, even at relatively low levels. The slightly higher lipids content observed in WPH compared to PH may be attributed to a lower metabolic flux toward erythritol synthesis in the final stages of cultivation, which may have redirected carbon toward lipids accumulation. Indeed, under nitrogen-limited conditions, *Y. lipolytica* is known to prioritize lipids biosynthesis but can also shift metabolism toward the secretion of polyols, mainly erythritol and mannitol [63]. Since both lipids and polyols are synthesized under similar nutrient conditions, which commonly occur in the late stages of the experiments, lipids production can shift metabolism away from erythritol synthesis and vice versa. In FH experiments, lipids content was even lower than that observed in PH and WPH medium. Nevertheless, as with *P. palmata*, the lipids content accumulated by *Y. lipolytica* in FH was 7-fold higher than that found in the crude *F. vesiculosus* (1%). *Y. lipolytica* A101 cultivated on *F. vesiculosus* hydrolysate also produced low amounts of lipids, approximately 5% [17].

The fatty acid profile of lipids produced in WPH and FH was similar, being mostly composed of oleic acid (C18:1), followed by palmitoleic acid (C16:1) and linoleic acid (C18:2), with the lowest contents of heptadecenoic acid (C17:1) and myristic acid (C14:0) (Table 5). In contrast, lipids obtained from *Y. lipolytica* cultivated in PH had a lower content of oleic acid than in the other cases, and close to the content of palmitoleic and linoleic acids. Heptadecenoic and myristic acids content were highest in PH-derived lipids. These variations indicate that the composition of the hydrolysate, mainly salt composition, can significantly influence lipids composition. Indeed, osmotic stress is known to affect membrane fluidity and, depending on the yeast, can trigger various adaptive responses, such as increased or decreased fatty acid unsaturation, changes in acyl chain length, reductions in phospholipid and sterol content, and alterations in the sterol-to-phospholipid ratio [64]. Similar to the results observed in this study, an increase in myristic acid and a reduction in oleic acid have also been reported in *Y. lipolytica* [65] and *R. glutinis* [66] under high salinity conditions. Despite these variations in fatty acid profile, the overall content of monounsaturated fatty acids (MUFA, 71%–78%) and polyunsaturated fatty acids (PUFA, 20%–22%) was similar across PH, WPH, and FH experiments.

Protein synthesis in microorganisms requires assimilable nitrogen sources [67]. The protein content of yeast biomass was significantly higher when grown in PH compared to WPH and FH (Table 6), which

Table 5
Lipids content, respective fatty acids composition, and contents of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) in *Y. lipolytica* NCYC 2904 final biomass obtained in batch cultures using *P. palmata* and *F. vesiculosus* hydrolysates. Data are the average ± standard deviation of two independent biological replicates, with triplicate measurements performed at each sample. Values followed by the same letter in each column do not present statistically significant differences ($p \geq 0.05$).

Culture medium	Lipids content (%, w/w)	Relative fatty acid content (%)							
		C14:0	C16:1	C17:1	C18:1	C18:2	SFA	MUFA	PUFA
PH	10.8 ± 0.3^{a}	7.0 ± 1.4^{a}	30.2 ± 0.9^{a}	14.9 ± 0.2^{a}	25.9 ± 0.6^{a}	22.0 ± 1.5^{a}	7.0 ± 1.4^{a}	71.0 ± 1.2^{a}	22.0 ± 1.5^{a}
WPH	14.9 ± 0.6^{b}	1.8 ± 0.4^{b}	26.6 ± 1.1^{a}	5.8 ± 1.3^{b}	46.0 ± 0.7^{b}	19.7 ± 2.5^{a}	1.8 ± 0.4^{b}	78.4 ± 1.0^{b}	19.7 ± 2.5^{a}
FH	7.1 ± 1.3^{c}	2.8 ± 0.6^{b}	24.9 ± 2.3^{a}	8.9 ± 0.4^{b}	44.0 ± 1.5^{b}	20.4 ± 1.8^{a}	2.8 ± 0.6^{b}	78.0 ± 1.6^{b}	20.4 ± 1.8^{a}

Table 6
Values of protein content and respective amino acid profile of *Y. lipolytica* NCYC 2904 final biomass obtained in batch cultures using *P. palmata* and *F. vesiculosus* hydrolysates. Data are the average ± standard deviation of two independent biological replicates, with triplicate measurements performed at each sample. Values followed by the same letter in each line do not present statistically significant differences ($p \geq 0.05$).

			Culture medium		
			PH	WPH	FH
Protein content (%, w/w)			29.1 ± 1.4^{a}	20.5 ± 0.9^{b}	12.7 ± 1.2^{c}
Amino acid (mg·g^{-1} of protein)	Essential amino acid	Val	14.4 ± 2.3^{a}	11.8 ± 1.9^{a}	2.6 ± 0.7^{b}
		Trp	11.3 ± 0.9^{ab}	15.7 ± 1.6^{a}	8.9 ± 0.7^{b}
		Phe	16.9 ± 1.7^{a}	15.2 ± 1.0^{a}	3.5 ± 0.4^{b}
		Ile	1.7 ± 0.9^{a}	3.4 ± 0.8^{ab}	6.3 ± 0.7^{b}
		Leu	15.2 ± 1.5^{a}	19.3 ± 3.0^{a}	40.8 ± 4.6^{b}
		Thr	49.4 ± 1.8^{a}	40.1 ± 4.6^{a}	n.d.
		Met	17.0 ± 1.7^{a}	22.7 ± 1.5^{a}	38.4 ± 2.7^{b}
	Non-essential amino acid	Glu	31.9 ± 2.1^{a}	38.3 ± 4.2^{a}	3.2 ± 0.5^{b}
		Asn	3.1 ± 0.8^{a}	4.8 ± 1.0^{a}	10.6 ± 0.7^{b}
		Ser	23.8 ± 3.3^{a}	20.6 ± 3.0^{a}	8.1 ± 0.5^{b}
		Gln	1.1 ± 0.5^{a}	1.4 ± 0.6^{a}	2.7 ± 0.5^{a}
		Gly	18.5 ± 1.8^{a}	20.6 ± 2.7^{a}	25.0 ± 1.8^{a}
		Cys	4.4 ± 1.1^{a}	7.5 ± 2.0^{a}	3.8 ± 1.0^{a}
		Arg	24.0 ± 3.6^{a}	27.1 ± 2.0^{a}	9.6 ± 0.9^{b}

n.d. – not detected.

positively correlates with the nitrogen concentration in the culture medium. Indeed, the nitrogen content at the beginning of the experiments decreased from PH to WPH and then to FH, and protein content in *Y. lipolytica* biomass followed the same trend. Additionally, the higher salt content in PH may have contributed to the observed differences in protein synthesis. Indeed, potassium plays a critical role in maintaining ribosomal stability and function, and its presence is essential for efficient protein synthesis [68,69]. The protein content of *Y. lipolytica* biomass in PH experiments was 2.1-fold higher than that of unwashed *P. palmata*. However, a mass balance based on 100 g of unwashed *P. palmata* demonstrated that the total protein recovered in the final yeast biomass was similar to the protein originally present in the seaweed. Although the similar potential for protein-enriched food supplements, the yeast biomass represents a more suitable alternative for food supplements, as it contains significantly higher lipids content and its production process also enables the co-production of erythritol, increasing the overall value of the process. By contrast, the total protein recovered in the final yeast biomass grown in WPH and FH was slightly lower than the protein of washed *P. palmata* and unwashed *F. vesiculosus*, respectively.

The amino acid profile of *Y. lipolytica* biomass was well balanced, including essential amino acids such as valine, tryptophan, phenylalanine, isoleucine, leucine, threonine, and methionine (Table 6). Similar amino acid profiles were observed in PH and WPH. The cells' protein obtained in FH had a different amino acid profile from those found in PH and WPH. The amino acid threonine was not part of the protein composition in this condition. The amount of valine, phenylalanine, glutamic acid, serine, and arginine was lower than that observed in PH and WPH. However, leucine, methionine, and asparagine contents were higher. These results demonstrate that the amino acid composition of *Y. lipolytica* biomass is highly dependent on the specific nutrient profile of the seaweed hydrolysate, highlighting the potential to tailor biomass quality through substrate selection. Despite the variability among hydrolysates, the amino acid contents identified were within the range previously reported for *Y. lipolytica* biomass [18].

3.5. Global discussion

The results demonstrate that *P. palmata* and *F. vesiculosus* hydrolysates are suitable substrates for biomass production by *Y. lipolytica* NCYC 2904. This is the first study reporting the consumption of glucuronic acid and fucose by a wild-type *Y. lipolytica* strain. Furthermore, it was observed that *Y. lipolytica* NCYC 2904 has a considerable ability to consume significant amounts of xylose (≈ 60 g·L^{-1}) with an uptake rate higher than that of glucose, which was never reported. These findings represent a significant advance in the understanding of *Y. lipolytica*'s metabolic versatility and its potential for biotechnological applications using seaweed-derived substrates.

The composition of each hydrolysate significantly influenced the metabolites produced by *Y. lipolytica*. *P. palmata* hydrolysate favored erythritol production. The substrates commonly used for erythritol production by *Y. lipolytica* include glucose, fructose, and glycerol, and the use of xylose remains unexplored [55]. However, the *Y. lipolytica* genome contains genes encoding the three key enzymes required for xylose metabolism: xylose reductase (converts D-xylose into xylitol using NADPH), xylitol dehydrogenase (converts xylitol into D-xylulose with NADH generation), and xylulose kinase (converts D-xylulose into D-xylulose-5-phosphate using ATP) [49,70]. D-xylulose-5-phosphate is a crucial intermediate of the pentose phosphate pathway (PPP), the central metabolic route for erythritol biosynthesis in *Y. lipolytica* [57]. In the wild-type strain *Y. lipolytica* NCYC 2904, the observed assimilation of xylose suggests that these enzymes are expressed with appropriate activity, particularly XDH, which has previously been reported as the rate-limiting step in xylose utilization [51]. Thus, xylose may serve as a carbon source for growth and erythritol synthesis (Fig. 2), a significant insight for the utilization of xylose-rich seaweed hydrolysates. Furthermore, co-consumption of glucose may help supply essential cofactors, supporting XR, XDH, and XK activity and increasing flux through the PPP. The maximum erythritol concentrations obtained in this work (40 g·L^{-1}) were comparable to those reported in studies using wild-type *Y. lipolytica* strains in glycerol-based medium [71] and waste cooking oil-based medium [24]. To the best of our knowledge, this is the first

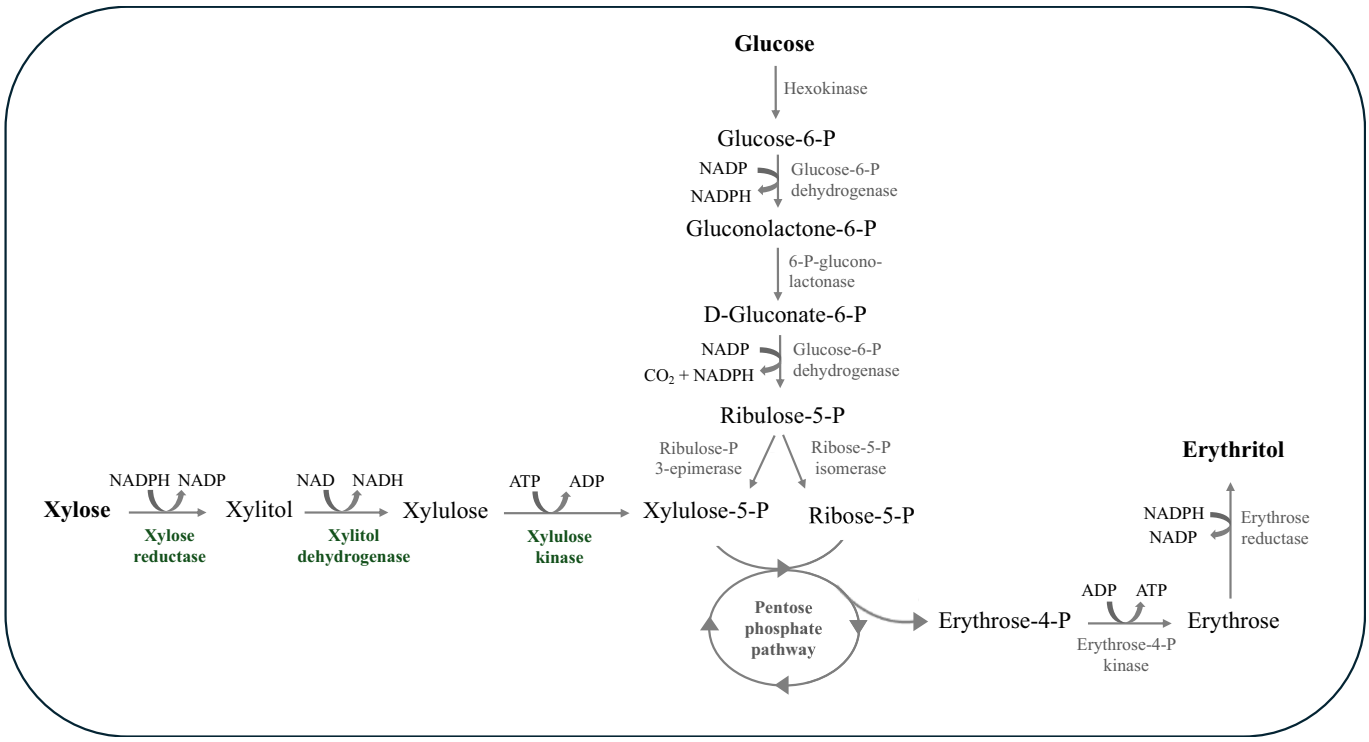


Fig. 2. Overview of the principal metabolic pathways for erythritol synthesis in *Y. lipolytica* NCYC 2904 from glucose and xylose.

report of erythritol production from seaweed hydrolysates. Notably, since the osmotic conditions were not optimized, these results represent a significant advancement in the valorization of seaweed biomass for sustainable polyol production and are particularly promising for future scale-up and process optimization efforts.

The *P. palmata* hydrolysate also supported the highest production of protein-rich biomass in this study. *Y. lipolytica* biomass and its metabolites have been granted Generally Recognized as Safe (GRAS) status by the Food and Drug Administration (FDA) in 2019, and the European Food Safety Authority (EFSA) has approved its biomass as an innovative novel food. Given its protein content and its well-balanced amino acid profile, including essential amino acids, the *Y. lipolytica* biomass produced in this study represents a suitable alternative for food and feed applications [18]. The washed *P. palmata* hydrolysate yielded the highest lipids accumulation by *Y. lipolytica*, representing an 8.3-fold increase compared to the lipids content in crude seaweed. Due to its rich composition in mono- and polyunsaturated fatty acids such as oleic, linoleic, and palmitoleic acids, these lipids have promising applications in the food, feed, and nutraceutical sectors, as functional ingredients or alternatives to conventional oils [72].

This study underscores the biotechnological potential of seaweed hydrolysates, demonstrating a viable process for the sustainable production of biomass, protein, lipids, and erythritol by *Y. lipolytica*, with strong potential for industrial scale-up. Notably, the two seaweed species explored offer distinct valorization opportunities: *P. palmata* hydrolysates were an efficient substrate for erythritol production by *Y. lipolytica*, whereas *F. vesiculosus* was naturally enriched in another polyol, mannitol, which was fully recovered in the hydrolysates. Similar to erythritol, mannitol is a valuable polyol widely employed as a low-calorie sweetener and as an excipient in pharmaceutical formulations [63]. These differentiated product profiles highlight the importance of strategic seaweed selection within blue biorefinery frameworks, allowing tailored approaches to meet specific market demands while maximizing resource efficiency.

4. Conclusion

F. vesiculosus and *P. palmata* hydrolysates are suitable substrates for biomass production by *Y. lipolytica* NCYC 2904. The yeast assimilates a variety of sugars, including glucose, xylose, and fucose, as well as other compounds found in hydrolysates, such as mannitol, glucuronic acid, formic acid, and acetic acid. Notably, the consumption of fucose and glucuronic acid by *Y. lipolytica* has not yet been thoroughly investigated in the literature. Furthermore, it was demonstrated the high ability of yeast to consume significant amounts of xylose in conditions of low glucose-to-xylose ratio. Unwashed *P. palmata* hydrolysate favored erythritol and protein synthesis, while the highest lipids content was obtained in washed *P. palmata* hydrolysate. To the best of our knowledge, this is the first report on erythritol production from seaweed hydrolysates. In addition, the pretreatment and hydrolysis steps enabled the liberation of all mannitol present in the crude *F. vesiculosus* biomass, further expanding its valorization potential. This study highlights the versatility of *Y. lipolytica* as a microbial platform for the valorization of seaweed biomass into several high-value products, including protein-rich biomass (approved by EFSA for human consumption), erythritol, and lipids, supporting the food and feed industry. Yet, differences in the seaweeds composition may lead to hydrolysates with slightly different concentrations of sugars and other compounds, that consequently may cause differences in the yeast performance. The targeted selection of hydrolysate can optimize compound production within the biorefinery context. By aligning marine biomass valorization with advanced fermentation technologies, these findings contribute significantly to the expansion of the blue bioeconomy and support the global transition toward circular economy strategies.

CRediT authorship contribution statement

Bruna Dias: Writing – original draft, Methodology, Investigation, Formal analysis. **Marlene Lopes:** Writing – review & editing, Supervision, Formal analysis, Conceptualization. **Isabel Belo:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This study was supported by the Portuguese Foundation for Science and Technology (FCT) through the project "MB4AQUA-Macroalgae biorefinery: a novel approach to produce sustainable feedstuffs and functional additives towards low carbon footprint aquafeeds," (2022.06587.PTDC) with DOI 10.54499/CEECINST/00064/2021/CP2812/CT0001; under the scope of the strategic funding of UID/04469: Centre of Biological Engineering of the University of Minho, LABBELS – Associate Laboratory in Biotechnology, Bioengineering and Microelectromechanical Systems, LA/P/0029/2020; and the Doctoral grant (2021.05799.BD) (https://doi.org/10.54499/2021.05799.BD).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.algal.2026.104610.

Data availability

Data will be made available on request.

References

[1] ResearchAndMarkets, Seaweed Cultivation Market Report 2025, 2025.
[2] A. Sharma, S. Dubey, K. Singh, R. Mittal, P. Quille, G. Rajauria, Innovative processing and industrial applications of seaweed, Phycology 5 (2025) 1–27, https://doi.org/10.3390/phycology5010010.
[3] N. Khan, K. Sudhakar, R. Mamat, Macroalgae farming for sustainable future: navigating opportunities and driving innovation, Heliyon 10 (2024) e28208, https://doi.org/10.1016/j.heliyon.2024.e28208 (1–28).
[4] O. Babich, S. Ivanova, P. Michaud, E. Budenkova, E. Kashirskikh, V. Anokhova, S. Sukhikh, Fermentation of micro- and macroalgae as a way to produce value-added products, Biotechnol. Rep. 41 (2024) 1–11, https://doi.org/10.1016/j.btre.2023.e00827.
[5] S. Kammler, A. Malvis Romero, C. Burkhardt, L. Baruth, G. Antranikian, A. Liese, M. Kaltschmitt, Macroalgae valorization for the production of polymers, chemicals, and energy, Biomass Bioenergy 183 (2024), https://doi.org/10.1016/j.biombioe.2024.107105.
[6] M.A. Manan, C. Webb, Modern microbial solid state fermentation technology for future biorefineries for the production of added-value products, Biofuel Res. J. 16 (2017) 730–740, https://doi.org/10.18331/BRJ2017.4.4.5.
[7] M. Ferreira, J.M. Salgado, H. Fernandes, H. Peres, I. Belo, Potential of red, green and brown seaweeds as substrates for solid state fermentation to increase their nutritional value and to produce enzymes, Foods 11 (2022) 1–6, https://doi.org/10.3390/foods11233864.
[8] L. Pereira, Non-indigenous seaweeds in the Iberian Peninsula, Macaronesia Islands (Madeira, Azores, Canary Islands) and Balearic Islands: biodiversity, ecological impact, invasion dynamics, and potential industrial applications, Algal Res. 78 (2024) 1–26, https://doi.org/10.1016/j.algal.2024.103407.
[9] A. Romero-Vargas, A. Gallé, A. Blandino, L.I. Romero-García, Use of macroalgal waste from the carrageenan industry as feedstock for the production of polyhydroxybutyrate, Biofuels Bioprod. Biorefin. 17 (2023) 1290–1302, https://doi.org/10.1002/bbb.2508.
[10] M. Farghali, I.M.A. Mohamed, A.I. Osman, D.W. Rooney, Seaweed for climate mitigation, wastewater treatment, bioenergy, bioplastic, biochar, food, pharmaceuticals, and cosmetics: a review, Environ. Chem. Lett. 21 (2023) 97–152, https://doi.org/10.1007/s10311-022-01520-y.
[11] A. Moenaert, A.M. López-Contreras, M. Budde, L. Allahgholi, X. Hou, A.B. Bjerre, J. Örlygsson, E.N. Karlsson, Ó.H. Friðjónsson, G.Ó. Hreggviðsson, Evaluation of *Laminaria digitata* hydrolysate for the production of bioethanol and butanol by fermentation, Fermentation 9 (2023), https://doi.org/10.3390/fermentation9010059.
[12] V. Alfonsín, R. Maceiras, C. Gutiérrez, Bioethanol production from industrial algae waste, Waste Manag. 87 (2019) 791–797, https://doi.org/10.1016/j.wasman.2019.03.019.
[13] T.H. Nguyen, I.Y. Sunwoo, G.T. Jeong, S.K. Kim, Detoxification of hydrolysates of the red seaweed *Gelidium amansii* for improved bioethanol production, Appl. Biochem. Biotechnol. 188 (2019) 977–990, https://doi.org/10.1007/s12010-019-02970-x.
[14] J.H. Park, J.Y. Hong, H.C. Jang, S.G. Oh, S.H. Kim, J.J. Yoon, Y.J. Kim, Use of *Gelidium amansii* as a promising resource for bioethanol: a practical approach for continuous dilute-acid hydrolysis and fermentation, Bioresour. Technol. 108 (2012) 83–88, https://doi.org/10.1016/j.biortech.2011.12.065.
[15] D. Greetham, J.M. Adams, C. Du, The utilization of seawater for the hydrolysis of macroalgae and subsequent bioethanol fermentation, Sci. Rep. 10 (2020), https://doi.org/10.1038/s41598-020-66610-9.
[16] S. Sharma, L.D. Hansen, J.O. Hansen, L.T. Mydland, S.J. Horn, M. Øverland, V.G. H. Eijsink, K.S. Vuoristo, Microbial protein produced from brown seaweed and spruce wood as a feed ingredient, J. Agric. Food Chem. 66 (2018) 8328–8335, https://doi.org/10.1021/acs.jafc.8b01835.
[17] A. Dobrowolski, W. Nawijn, A.M. Mirończuk, Brown seaweed hydrolysate as a promising growth substrate for biomass and lipid synthesis of the yeast *Yarrowia lipolytica*, Front. Bioeng. Biotechnol. 10 (2022), https://doi.org/10.3389/fbioe.2022.944228.
[18] M.E. Jach, A. Malm, *Yarrowia lipolytica* as an alternative and valuable source of nutritional and bioactive compounds for humans, Molecules 27 (2022) 1–22, https://doi.org/10.3390/molecules27072300.
[19] B. Dias, R.M. Rodríguez-Jasso, I. Belo, M. Lopes, *Yarrowia lipolytica* uses olive tree prunings hydrolysates for microbial lipids as biodiesel feedstock, Chem. Eng. Sci. 305 (2025), https://doi.org/10.1016/j.ces.2024.121150.
[20] B. Dias, M. Lopes, H. Fernandes, S. Marques, F. Gírio, I. Belo, Biomass and microbial lipids production by *Yarrowia lipolytica* W29 from eucalyptus bark hydrolysate, Renew. Energy 224 (2024) 120173, https://doi.org/10.1016/j.renene.2024.120173.
[21] B. Dias, M. Lopes, S. Marques, F. Gírio, I. Belo, Alternative feedstocks for high-quality biodiesel: lipid production from eucalyptus bark hydrolysate by *Yarrowia lipolytica* W29 using different cultivation modes, biofuels, Bioprod. Biorefin. (2025), https://doi.org/10.1002/bbb.2786.
[22] P. Ferreira, M. Lopes, I. Belo, Use of pressurized and airlift bioreactors for citric acid production by *Yarrowia lipolytica* from crude glycerol, Fermentation 8 (2022) 1–10, https://doi.org/10.3390/fermentation8120700.
[23] A.S. Pereira, M. Lopes, I. Belo, From crude glycerol and volatile fatty acids to biodiesel and other bioproducts using *Yarrowia lipolytica* NCYC 2904 as a cell factory, Sustain. Energy Fuel 7 (2023) 4687–4696, https://doi.org/10.1039/d3se00682d.
[24] X. Liu, J. Lv, J. Xu, J. Xia, A. He, T. Zhang, X. Li, J. Xu, Effects of osmotic pressure and pH on citric acid and erythritol production from waste cooking oil by *Yarrowia lipolytica*, Eng. Life Sci. 18 (2018) 344–352, https://doi.org/10.1002/elsc.201700114.
[25] A. Braga, D.P. Mesquita, A. Cordeiro, I. Belo, E.C. Ferreira, A.L. Amaral, Monitoring biotechnological processes through quantitative image analysis: application to 2-phenylethanol production by *Yarrowia lipolytica*, Process Biochem. 129 (2023) 221–229, https://doi.org/10.1016/j.procbio.2023.03.019.
[26] K. Dussan, W.J.J. Huijgen, T. de Vrije, H. van der Wal, H. Capella, J. van Doorn, A. M. López-Contreras, J.W. van Hal, Biorefinery of red seaweed *Palmaria palmata* for production of bio-based chemicals and biofuels, Algal Res. 82 (2024), https://doi.org/10.1016/j.algal.2024.103608.
[27] I. Maribu, K. Elvevold, K.E. Eilertsen, M.J. Blikra, Seasonal variation in the nutritional composition and potentially toxic elements of the red macroalga *Palmaria palmata* in the Arctic, Food Res. Int. 212 (2025) 1–10, https://doi.org/10.1016/j.foodres.2025.116477.
[28] E.D. Obluchinskaya, O.N. Pozharitskaya, D.V. Zakharov, E.V. Flisyuk, I.I. Terninko, Y.E. Generalova, I.E. Smekhova, A.N. Shikov, The biochemical composition and antioxidant properties of *Fucus vesiculosus* from the arctic region, Mar. Drugs 20 (2022), https://doi.org/10.3390/md20030193.
[29] A.R. Circuncisão, S.S. Ferreira, A.M.S. Silva, M.A. Coimbra, S.M. Cardoso, *Fucus vesiculosus*-rich extracts as potential functional food ingredients: a holistic extraction approach, Foods 13 (2024), https://doi.org/10.3390/foods13040540.
[30] S. Mutripah, M.D.N. Meinita, J.Y. Kang, G.T. Jeong, A.B. Susanto, R.E. Prabowo, Y. K. Hong, Bioethanol production from the hydrolysate of *Palmaria palmata* using sulfuric acid and fermentation with brewer's yeast, J. Appl. Phycol. 26 (2014) 687–693, https://doi.org/10.1007/s10811-013-0068-6.
[31] A. Sluiter, B. Hames, D. Hyman, C. Payne, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, J.W. Nrel, Determination of Ash in Biomass, NREL/TP-510-42622, 2008.
[32] M. Ferreira, H. Fernandes, H. Peres, A. Oliva-Teles, I. Belo, J.M. Salgado, Bio-enrichment of oilseed cakes by *Mortierella alpina* under solid-state fermentation, Lwt 134 (2020), https://doi.org/10.1016/j.lwt.2020.109981.
[33] A. Sluiter, B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, D. Crocker, Determination of Structural Carbohydrates and Lignin in Biomass, NREL/TP-510-42618, National Renewable Energy Laboratory, USA, 2012, pp. 1–15. http://www.nrel.gov/docs/gen/fy13/42618.pdf.
[34] M. Lopes, S.M. Miranda, J.M. Alves, A.S. Pereira, I. Belo, Waste cooking oils as feedstock for lipase and lipid-rich biomass production, Eur. J. Lipid Sci. Technol. 121 (2019) 1–9, https://doi.org/10.1002/ejlt.201800188.
[35] M. Hakyakawa, High-speed simultaneous analysis of amino acids by pre-column derivatization using automatic pretreatment function. http://www.shimadzu.com/about/trademarks/index.html, 2022.
[36] A.R. Angell, L. Mata, R. de Nys, N.A. Paul, The protein content of seaweeds: a universal nitrogen-to-protein conversion factor of five, J. Appl. Phycol. 28 (2016) 511–524, https://doi.org/10.1007/s10811-015-0650-1.
[37] H.K. Mæhre, M.K. Malde, K.E. Eilertsen, E.O. Elvevoll, Characterization of protein, lipid and mineral contents in common Norwegian seaweeds and evaluation of their potential as food and feed, J. Sci. Food Agric. 94 (2014) 3281–3290, https://doi.org/10.1002/jsfa.6681.

[38] K.C. Morgan, J.L.C. Wright, F.J. Simpson, Review of chemical constituents of the red alga Palmaria palmata (dulse), Econ. Bot. 34 (1980) 27–50, https://doi.org/10.1007/BF02859553.
[39] M. Narayanan, Promising biorefinery products from marine macro and microalgal biomass: a review, Renew. Sust. Energ. Rev. 190 (2024) 1–17, https://doi.org/10.1016/j.rser.2023.114081.
[40] C. Teixeira-Guedes, J.S. Gomes-Dias, S.A. Cunha, M.E. Pintado, R.N. Pereira, J. A. Teixeira, C.M.R. Rocha, Enzymatic approach for the extraction of bioactive fractions from red, green and brown seaweeds, Food Bioprod. Process. 138 (2023) 25–39, https://doi.org/10.1016/j.fbp.2022.12.005.
[41] M. Xu, Y. Zhang, B. Wu, Y. Zhang, M. Qiao, G. Singh, E.S. Ólafsdóttir, S. Pálsson, S. Heiðmarsson, H. de Boer, M. Þorsteinsdóttir, G. Þorkelsson, B.V. Aðalbjörnsson, A critical review of the edible seaweed *Palmaria palmata* (L.) Weber & Mohr and its bioactive compounds in the "omics" era, Algal Res. 82 (2024), https://doi.org/10.1016/j.algal.2024.103606.
[42] P. Nova, A. Pimenta-Martins, É. Maricato, C. Nunes, H. Abreu, M.A. Coimbra, A. C. Freitas, A.M. Gomes, Chemical composition and antioxidant potential of five algae cultivated in fully controlled closed systems, Molecules 28 (2023) 1–19, https://doi.org/10.3390/molecules28124588.
[43] B. Lafeuille, É. Tamigneaux, K. Berger, V. Provencher, L. Beaulieu, Impact of harvest month and drying process on the nutritional and bioactive properties of wild *Palmaria palmata* from Atlantic Canada, Mar. Drugs 21 (2023), https://doi.org/10.3390/md21070392.
[44] A. Timoumi, S.E. Guillouet, C. Molina-Jouve, L. Fillaudeau, N. Gorret, Impacts of environmental conditions on product formation and morphology of *Yarrowia lipolytica*, Appl. Microbiol. Biotechnol. 102 (2018) 3831–3848, https://doi.org/10.1007/s00253-018-8870-3.
[45] M. Tzirita, M. Kremmyda, D. Sarris, A.A. Koutinas, S. Papanikolaou, Effect of salt addition upon the production of metabolic compounds by *Yarrowia lipolytica* cultivated on biodiesel-derived glycerol diluted with olive-mill wastewaters, Energies (Basel) 12 (2019) 1–19, https://doi.org/10.3390/en12193649.
[46] H. Fernandes, J.M. Salgado, N. Martins, H. Peres, A. Oliva-Teles, I. Belo, Sequential bioprocessing of *Ulva rigida* to produce lignocellulolytic enzymes and to improve its nutritional value as aquaculture feed, Bioresour. Technol. 281 (2019) 277–285, https://doi.org/10.1016/j.biortech.2019.02.068.
[47] M. Novoa-Garrido, C. Marcos, M. Travieso, E. Alcaide, M. Larsen, M. Weisbjerg, Preserving *Porphyra umbilicalis* and *Saccharina latissima* as silages for ruminant feeding, Animals 10 (2020) 1–19, https://doi.org/10.3390/ani10111957.
[48] P. Hapeta, E.J. Kerkhoven, Z. Lazar, Nitrogen as the major factor influencing gene expression in *Yarrowia lipolytica*, Biotechnol. Rep. 27 (2020), https://doi.org/10.1016/j.btre.2020.e00521.
[49] G.M. Rodriguez, M.S. Hussain, L. Gambill, D. Gao, A. Yaguchi, M. Blenner, Engineering xylose utilization in *Yarrowia lipolytica* by understanding its cryptic xylose pathway, Biotechnol. Biofuels 9 (2016) 1–15, https://doi.org/10.1186/s13068-016-0562-6.
[50] I. Torre, M. Acedos, J. Cestero, J. Barriuso, J. García, Engineering xylose isomerase and reductase pathways in *Yarrowia lipolytica* for efficient lipid production, Microb. Biotechnol. 18 (2025) 1–13, https://doi.org/10.1111/1751-7915.70127.
[51] M. Hussain, G. Rodriguez, D. Gao, M. Spagnuolo, L. Gambill, M. Blenner, Recent advances in bioengineering of the oleaginous yeast *Yarrowia lipolytica*, AIMS Bioeng. 3 (2016) 493–514, https://doi.org/10.3934/bioeng.2016.4.493.
[52] K. Drzymała-Kapinos, A.M. Mirończuk, A. Dobrowolski, Lipid production from lignocellulosic biomass using an engineered *Yarrowia lipolytica* strain, Microb. Cell Factories 21 (2022) 1–11, https://doi.org/10.1186/s12934-022-01951-w.
[53] X. Niehus, A.M. Crutz-Le Coq, G. Sandoval, J.M. Nicaud, R. Ledesma-Amaro, Engineering *Yarrowia lipolytica* to enhance lipid production from lignocellulosic materials, Biotechnol. Biofuels 11 (2018), https://doi.org/10.1186/s13068-018-1010-6.
[54] C. Walker, B. Dien, R.J. Giannone, P. Slininger, S.R. Thompson, C.T. Trinh, Exploring proteomes of robust *Yarrowia lipolytica* isolates cultivated in biomass hydrolysate reveals key processes impacting mixed sugar utilization, lipid accumulation, and degradation, MSystems 6 (2021) 1–15, https://doi.org/10.1128/msystems.00443-21.
[55] A. Rywińska, L. Tomaszewska-Hetman, P. Juszczyk, M. Rakicka-Pustułka, A. Bogusz, W. Rymowicz, Enhanced production of erythritol from glucose by the newly obtained UV mutant *Yarrowia lipolytica* K1UV15, Molecules 29 (2024) 1–15, https://doi.org/10.3390/molecules29102187.
[56] M. Szczepańczyk, D.A. Rzechonek, C. Neuvéglise, A.M. Mirończuk, In-depth analysis of erythrose reductase homologs in *Yarrowia lipolytica*, Sci. Rep. 13 (2023) 1–15, https://doi.org/10.1038/s41598-023-36152-x.
[57] A.M. Mirończuk, A. Biegalska, A. Dobrowolski, Functional overexpression of genes involved in erythritol synthesis in the yeast *Yarrowia lipolytica*, Biotechnol. Biofuels 10 (2017) 1–12, https://doi.org/10.1186/s13068-017-0772-6.
[58] L. Tomaszewska-Hetman, A. Rywińska, Erythritol biosynthesis from glycerol by *Yarrowia lipolytica* yeast: effect of osmotic pressure, Chem. Pap. 70 (2016) 272–283, https://doi.org/10.1515/chempap-2015-0201.
[59] D. Rzechonek, A. Dobrowolski, W. Rymowicz, A. Mirończuk, Recent advances in biological production of erythritol, Crit. Rev. Biotechnol. 38 (2017) 620–633, https://doi.org/10.1080/07388551.2017.1380598.
[60] N. Wang, P. Chi, Y. Zou, Y. Xu, S. Xu, M. Bilal, P. Fickers, H. Cheng, Metabolic engineering of *Yarrowia lipolytica* for thermoresistance and enhanced erythritol productivity, Biotechnol. Biofuels 13 (2020) 1–20, https://doi.org/10.1186/s13068-020-01815-8.
[61] M. Szczepańczyk, D.A. Rzechonek, A. Dobrowolski, A.M. Mirończuk, Engineered yeast *Yarrowia lipolytica* as a chassis for biosynthesis of fatty acids from mannitol and macroalgal biomass extracts, Microb. Cell Factories 24 (2025) 1–13, https://doi.org/10.1186/s12934-025-02699-9.
[62] A.S. Pereira, S.M. Miranda, M. Lopes, I. Belo, Factors affecting microbial lipids production by *Yarrowia lipolytica* strains from volatile fatty acids: effect of co-substrates, operation mode and oxygen, J. Biotechnol. 331 (2021) 37–47, https://doi.org/10.1016/j.jbiotec.2021.02.014.
[63] P. Diamantopoulou, S. Papanikolaou, Biotechnological production of sugar-alcohols: focus on *Yarrowia lipolytica* and edible/medicinal mushrooms, Process Biochem. 124 (2023) 113–131, https://doi.org/10.1016/j.procbio.2022.11.008.
[64] M. Turk, V. Montiel, D. Žigon, A. Plemenitaš, J. Ramos, Plasma membrane composition of *Debaryomyces hansenii* adapts to changes in pH and external salinity, Microbiology (N.Y.) 153 (2007) 3586–3592, https://doi.org/10.1099/mic.0.2007/009563-0.
[65] E. Andreishcheva, E. Isakova, N. Sidorov, N. Abramova, N. Ushakova, G. Shaposhnikov, M. Soares, R. Zvyagilskaya, Adaptation to salt stress in a salt-tolerant strain of the yeast *Yarrowia lipolytica*, Biochemistry 64 (1999) 1259–1266.
[66] K. Sartaj, S. Tripathi, A. Patel, K. Mohan Poluri, R. Prasad, Detailed mechanistic investigation of stress-induced lipogenesis in oleaginous yeast for value-added metabolites, Chem. Eng. J. 471 (2023) 1–17, https://doi.org/10.1016/j.cej.2023.144562.
[67] M. Sharif, M.H. Zafar, A.I. Aqib, M. Saeed, M.R. Farag, M. Alagawany, Single cell protein: sources, mechanism of production, nutritional value and its uses in aquaculture nutrition, Aquaculture 531 (2021), https://doi.org/10.1016/j.aquaculture.2020.735885.
[68] A. Rozov, I. Khusainov, K. El Omari, R. Duman, V. Mykhaylyk, M. Yusupov, E. Westhof, A. Wagner, G. Yusupova, Importance of potassium ions for ribosome structure and function revealed by long-wavelength X-ray diffraction, Nat. Commun. 10 (2019) 1–12, https://doi.org/10.1038/s41467-019-10409-4.
[69] L. Yenush, Potassium and sodium transport in yeast, in: Yeast Membrane Transport, Advances in Experimental Medicine and Biology, Springer International Publishing Switzerland, 2016, pp. 187–228, https://doi.org/10.1007/978-3-319-25304-6_8.
[70] O. Konzock, S. Zaghen, J. Norbeck, Tolerance of *Yarrowia lipolytica* to inhibitors commonly found in lignocellulosic hydrolysates, BMC Microbiol. 21 (2021) 1–10, https://doi.org/10.1186/s12866-021-02126-0.
[71] S. Papanikolaou, E. Kampisopoulou, F. Blanchard, E. Rondags, C. Gardeli, A. A. Koutinas, I. Chevalot, G. Aggelis, Production of secondary metabolites through glycerol fermentation under carbon-excess conditions by the yeasts *Yarrowia lipolytica* and *Rhodosporidium toruloides*, Eur. J. Lipid Sci. Technol. 119 (2017), https://doi.org/10.1002/ejlt.201600507.
[72] V. Shapaval, J. Brandenburg, J. Blomqvist, V. Tafintseva, V. Passoth, M. Sandgren, A. Kohler, Biochemical profiling, prediction of total lipid content and fatty acid profile in oleaginous yeasts by FTIR spectroscopy, Biotechnol. Biofuels 12 (2019), https://doi.org/10.1186/s13068-019-1481-0.