# Impact of Spirulina Biomass Physical Form on Texture, Cooking Properties, and Sensory Profile of Enriched Pasta and Meat Analogues

Mathieu De Rijdt , Jeanne Verhaegen , Dorian Dohogne , Yves Brostaux , Eric Haubruge , Dorothée Goffin

PII: S2772-5022(25)00633-X
DOI: https://doi.org/10.1016/j.afres.2025.101328
Reference: AFRES 101328

To appear in: *Applied Food Research*

Received date: 28 April 2025
Revised date: 20 August 2025
Accepted date: 4 September 2025

Please cite this article as: Mathieu De Rijdt , Jeanne Verhaegen , Dorian Dohogne , Yves Brostaux , Eric Haubruge , Dorothée Goffin , Impact of Spirulina Biomass Physical Form on Texture, Cooking Properties, and Sensory Profile of Enriched Pasta and Meat Analogues, *Applied Food Research* (2025), doi: https://doi.org/10.1016/j.afres.2025.101328

## Highlights

- Drying spirulina generates off-flavours, limiting real enrichment in food products.
- The physical form of spirulina incorporated in pasta significantly impacts its sensory profile.
- Spirulina quantity affects the sensory profile of meat analogues more than its physical form.
- Texture of pasta and meat analogues enriched in fresh spirulina is not impacted.
- Spirulina's impact varies by food model, influencing cooking properties.

# Impact of Spirulina Biomass Physical Form on Texture, Cooking Properties, and Sensory Profile of Enriched Pasta and Meat Analogues

Mathieu De Rijdt[1a]*, Jeanne Verhaegen[1a]*, Dorian Dohogne[a,] Yves Brostaux[b], Eric Haubruge[a], Dorothée Goffin[a]

*Corresponding author: mderijdt@uliege.be
[1] These authors contributed equally to the study.
[a]University of Liege, Gembloux Agro-Bio Tech, Laboratory of Gastronomic Sciences, Belgium
[b]University of Liege, Gembloux Agro-Bio Tech, Modélisation et développement, Belgium

[ab]Passage des déportés 2
5030 Gembloux
Belgium

jeanne.verhaegen@uliege.be
y.brostaux@uliege.be
Dorian.Dohogne@uliege.be
dorothee.goffin@uliege.be
e.haubruge@uliege.be

**Keywords:** Spirulina, Fresh Biomass, Meat Analogue, Volatile Organic Compounds (VOCs), Sensory analysis, Cooking properties, Pasta

## Abstract

The global rising demand for proteins requires sustainable alternatives. With bacterial proteins attracting increasing interest, this study compares the incorporation of fresh frozen and dried spirulina biomass into pasta and meat analogues. In addition to characterising the biomass, the experimental protocol examines different levels of spirulina in fresh or rehydrated dried form and explores the evaluation of cooking quality parameters, textural properties and the sensory profile of the corresponding products. Seven volatile molecules were detected in the fresh form, compared to twenty-four in the dried form. When fresh spirulina is incorporated into pasta at levels of 6% and 10% dry equivalent, the swelling index and weight increase are greater than those of equivalent pasta made with rehydrated dried biomass. In addition, the texture and sensory profile remain closer to spirulina-free pasta. For meat analogues, texture and cooking properties are not affected by the type of biomass. The sensory profile is influenced by the level of incorporation, although at high incorporation percentage, products made with fresh spirulina are perceived as less fishy. The results highlight the potential of fresh frozen spirulina as an ingredient that can significantly enrich food products with protein without compromising organoleptic aspect and textural properties, as is the case with dried spirulina.

## 1. Introduction

Population growth is driving an increase in protein requirements. As a result, the development of protein-rich products and nutritious alternatives to animal and plant-based protein has become a rapidly expanding area of research (Smith et al., 2024). Given concerns about the environmental, resource efficiency and biodiversity, it is essential to explore sustainable, nutritious, affordable and consumer-acceptable alternatives to conventional proteins (FAO, 2021).

Spirulina, more specifically *Arthrospira platensis*, is a filamentous photosynthetic cyanobacteria that represents a promising solution to meet these expectations (Becker, 2007). Spirulina cultivation offers a more sustainable approach than animal production, as it relies on photosynthesis, uses minimal arable land and reduces environmental impact (Ramírez-Rodrigues et al., 2021). With a protein content of 50–70% (on a dry basis), spirulina also provides a complete amino acid profile, is highly digestible and is rich in vitamins, minerals and antioxidants (Batista et al., 2017; Becker, 2007). These nutritional and environmental benefits make spirulina an attractive option for addressing protein supply challenges in modern food systems.

Currently, spirulina is mainly consumed in dried form as a dietary supplement or to enrich food products (Wang et al., 2023) and has already been widely studied. For instance, dried spirulina has been incorporated into pasta (De Marco Castro et al., 2019; Fradique et al., 2010; Raczyk et al., 2022 ; Zouari et al., 2011), meat substitutes (Palanisamy et al., 2019; Zhao et al., 2024) and other food products such as in cookies (Batista et al., 2017) and ice cream (Boyanova et al., 2022). However, these formulations often contain limited percentages of biomass (from 0.3% to rarely more than 10%) and exhibit low acceptance from consumer as dried spirulina presents unpleasant off-flavours and odours. For instance, Fradinho et al. (2020) report that pasta containing 4% and 5% dried spirulina presents a strong fishy flavour. Raczyk et al. (2022) concluded that enriching pasta with dried spirulina has a positive impact on its nutritional quality (particularly in terms of protein and essential amino acid content) but emphasised that the main challenge remains in consumer sensory acceptance.

Fresh spirulina, i.e. undried spirulina, sometimes referred to as “wet spirulina” is a form that is rarely explored for product enrichment despite its advantages, namely the preservation of its nutritional qualities without unpleasant odours or flavours (Ma et al., 2019; Stunda-Zujeva and Berele, 2024). Indeed, while drying ensures microbiological safety, extended shelf life and transportability, it has a significant impact on its nutritional and sensory properties, degrading compounds such as antioxidants (e.g. phycocyanin), other proteins and sugars to varying degrees depending on the drying method (Stunda-Zujeva & Berele, 2024). Fresh spirulina, even if it still needs to be frozen for storage and transport, could be more suitable for significantly enriching food products with protein.

Among the limited number of studies that have examined the incorporation of fresh spirulina into food products, the work of Bchir et al. (2019) can be cited. Fresh spirulina, at low concentrations (0.1, 0.3 and 0.5%), was added to yoghurt and compared to equivalent products enriched with dried spirulina. The incorporation of fresh spirulina slightly decreased the viability of the ferments, induced a colour modification and reduced viscosity compared to products enriched with dried spirulina. In addition, the product enriched with 0.3% of fresh spirulina was more appreciated than others. Özyurt et al. (2015) incorporated fresh spirulina into pasta (5% and 10%) but the water content of the biomass used is not specified. Lemes et al. (2012) who tested concentrations of 5 to 10% in fresh pasta, showed that the sensory and technological aspects were satisfactory compared to products without spirulina. The work of Koli et al. (2021) is also worth highlighting, as it demonstrated that incorporating 10 to 20% of fresh spirulina (moisture content not specified) into dehydrated pasta improved its nutritional profile by increasing their protein, lipids, flavonoids, and essential minerals such as iron, zinc, and calcium contents. Furthermore, the sensory and textural aspects were not negatively affected by fresh spirulina incorporation compared to the product without spirulina.

Based on current knowledge, no studies have been conducted to directly compare fresh and dried spirulina at significant level of enrichment in food products that can be easily prepared by consumers without technological or industrial equipment. This article explores the use of frozen fresh spirulina to overcome the disadvantages of the drying process namely preserving nutritional quality and neutral taste, to achieve higher enrichment levels. First, both forms of biomass will be characterized in terms of proteins content, antioxidant capacity, viscosity, colour and aroma. Secondly, given that incorporating specific ingredients into everyday products is an interesting strategy for designing and studying the acceptability of fortified foods, two widely available and popular food models were selected: (1) Fresh pasta, considered a frequently consumed, popular staple food with a high water content, which allows it to be used as a carrier for specific compounds (Özyurt et al., 2015). (2) Meat analogue such as alternative "burger" are products commonly developed by western European industries as direct substitutes for animal proteins (Oliviero and Fogliano, 2016) and have never been highly fortified with fresh spirulina. Products will be enriched with fresh frozen or dehydrated spirulina rehydrated to an equivalent water content. Different incorporation percentages will be tested. For the comparative study, the focus will be set on sensory profile, textural properties and cooking quality parameters considering the specific cooking method for the food model considered. With the exception of the protein content of the biomass, nutritional analysis will not be examined in this article, as the impact of the drying process has already been extensively discussed in the literature (Becker, 2007; Ma et al., 2019), although the impact of the cooking methods on the nutritional value of the product could be of interest.

## 2. Materials and Methods

### 2.1. Spirulina biomass characterisation

Both forms of spirulina are supplied by Etika Spirulina (France). According to Etika's process, after harvest, the biomass is filtered, rinsed, re-filtered and pressed. The fresh form is then frozen at -18°C, with a water content of 77%. To obtain the dried form, the fresh form is dehydrated under hot air at temperatures below 42°C by the supplier. To be comparable and prior to any use (direct analysis or for formulation), dried spirulina was rehydrated with mineral water (Ordal) at room temperature for at least 30 minutes to reach the moisture content of the fresh form.

#### 2.1.1. Instrumental colour, viscosity and water activity

The two forms of biomass were subsequently characterised for their colour with the CIELAB colour space $L*$ (lightness/brightness) $a*$ (redness/greenness) $b*$ (yellowness/blueness) system method (ColorFlex EZ spectrophotometer from HunterLab), their viscosity with a rotational viscosimeter (ViscoQC300-L from Anton Paar) and water activity (Aqualab® Decagon CX3) by using the chilled-mirror dew point technique.

#### 2.1.2. Volatile organic compounds

The volatile organic compounds (VOCs) were characterised as follow: 2 grams of spirulina (defrosted fresh biomass or rehydrated dried biomass), were placed in a 20 ml vial, which was incubated in water at 50°C for 30 minutes. To capture the VOCs, the headspace solid-phase microextraction (HS-SPME) technique was used. After incubation, the SPME fibre assembly (Divinylbenzene/Carboxen/Polydimethylsiloxane, DVB/CAR/PDMS) from Sigma Aldrich was exposed to the biomass at 50°C in the vial headspace for 30 minutes. The extracted compounds were then analysed using gas chromatography coupled with mass spectrometry (Agilent Technologies 5975C). The column used was a HP-5MS with dimensions 30 m x 250 µm x 0.25 µm. The analysis for each sample lasted 48 minutes and 20 seconds, starting at an initial temperature of 40°C, increasing to 300°C at a rate of 6°C per minute, and holding at 300°C for 5 minutes. The injection was performed in the split-less mode at a flowrate of 1.5ml/min and helium was used as gas carrier.

The collected data were analysed with the software Agilent Masshunter (Unknown analysis B.08.00). The method used for the peak detection was the analysis of the total ion chromatogram (TIC) with a minimum match factor of 0.85. NIST17.L and WILEY275.L were used as spectra database libraries for identification. The linear retention indexes (RI) were assessed based on retention time data obtained by analyzing a series of normal alkanes C6-C30 (Sigma Aldrich, Darmstadt, Germany). Both methods, mass spectra and the comparison of RI in the literature, were used to correctly identify molecules. Results are expressed in relative proportions (%).

### 2.1.3. Protein content

The concentration of proteins in each biomass has been determined with the modified Lowry method (Markwell et al., 1978; Spanoghe et al., 2021), using a spectrophotometer (SpectraMax ABS Plus molecular devices).

### 2.1.4 Total antioxidant capacity

Total antioxidant capacity (TAC) of each biomass was determined by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method with slight modifications (Ismaiel et al., 2016). Extraction was assisted with an ultrasonic probe (Sonicprep Ultrasonic Homogenizer, PolyScience) on biomass diluted in distilled water (1:2 w/w). 200 mg of sonicated suspension was mixed with 9.8 ml of methanol for 2 hours, gently mixed and centrifuged 10 min at 5433g. Supernatants were collected for TAC analysis. 4 mg of DPPH (Sigma-Aldrich, St Louis, USA) was dissolved in 100mL of methanol. 100 µl of each biomass methanolic extract were added to 100 µl of DPPH solution. Due to the coloration of the extracts, a background blank was prepared, which consisted of 100µL of each extract added to 100µl of methanol. The absorbance was measured after 30 min at 517nm using a spectrophotometer (SpectraMax ABS Plus molecular devices). The radical scavenging activity of methanolic extracts is expressed as the inhibition percentage of the DPPH in solution using the following equation:

$$Radical\ scavenging\ activity\ (\%) = (1 - ((As - Ab)/Ad))$$

Where As: Absorbance of the sample in contact with DPPH, Ab: Absorbance of sample background and Ad: Absorbance of DPPH in solution.

A calibration curve varying from 0.001 mM to 0.04 mM of Trolox (Sigma Aldrich, Switzerland) was made to link the obtained scavenging activity of spirulina biomass to a Trolox concentration. Results are expressed in µmol of Trolox equivalent (TE) per gram of biomass dry matter (DM).

## 2.2. Formulations and production process

The dried spirulina was rehydrated at room temperature for 30 minutes with mineral water (Ordal) and fresh spirulina was thawed at room temperature.

For pasta, in addition to spirulina, the formulations included mineral water (Ordal), type T00 soft wheat flour and salt (Table 1). The dry matter content in the pasta dough was set at 67%. The experimental design included 2 main variables: the form of spirulina biomass (fresh or dried) and the spirulina incorporation level (2%, 6% and 10% of dried spirulina equivalent). A control formulation without biomass was also produced.

The production process is as follows: all ingredients were mixed in a Thermomix® TM5 and kneaded for 5 minutes (kneading mode). The dough was covered with a plastic film and refrigerated at 4°C for 30 minutes before being laminated out using a Pasta Maker Imperia. The type of pasta chosen was tagliatelle. Ten grams of pasta were cooked for 3 minutes in 100 ml of boiling water. The pasta was weighed raw (before cooking) and cooked (after cooking) after being drained using a sieve. The cooking water was also collected and weighed post-cooking.

Table 1. Ingredients and proportions (expressed in weight percentage) of the pasta's formulations enriched in spirulina.

| | **SP 2%** | | **SP 6%** | | **SP 10%** | | **Control 0%** |
|---|---|---|---|---|---|---|---|
| **Ingredients (% w/w)** | Dried | Fresh | Dried | Fresh | Dried | Fresh | |
| **Water** | 33.3 | 26.6 | 33.3 | 13.2 | 33.3 | 0.0 | 33.3 |
| **Wheat flour** | 63.7 | 63.7 | 59.7 | 59.7 | 55.7 | 55 | 65.7 |
| **Salt** | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| **Fresh biomass** | 0 | 8.7 | 0 | 26.1 | 0 | 44 | 0 |
| **Dried biomass** | 2 | 0 | 6 | 0 | 10 | 0 | 0 |

SP: spirulina. Fresh corresponds to the use of fresh spirulina; Dried corresponds to the use of dried spirulina.

For burgers, seven formulations were studied (Table 2): one without spirulina (Control 0%), two with 7.5% spirulina (fresh and dried, SP 7.5%), two with 12.5% spirulina (fresh and dried, SP 12.5%), and two with 17.5% spirulina (fresh and dried, SP 17.5%). The dry mix (oat flakes, wheat flour and textured wheat protein) was adjusted to maintain a similar water content across all the formulations. Water and spirulina were added to the dry ingredients and mixed for one minute using a stand mixer (KitchenAid) to obtain a homogeneous patty which was left for one hour in the refrigerator for complete hydration.

The burgers were shaped manually using a burger press and a 90 mm cookie cutter to obtain a cylindrical "burger-like" shapes. Meat analogues were baked at 180°C for 12 minutes (Rational dry heat oven), flipping them after 6 minutes for even cooking.

Table 2. Ingredients used and proportions (expressed in weight percentage) of the burger's formulations enriched in spirulina.

| | **SP 7.5%** | | **SP 12.5%** | | **SP 17.5%** | | **Control 0%** |
|---|---|---|---|---|---|---|---|
| **Ingredients (% w/w)** | Dried | Fresh | Dried | Fresh | Dried | Fresh | |
| **Wheat flour** | 10 | 10 | 8.75 | 8.75 | 7.25 | 7.25 | 11.75 |
| **Salt** | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| **Water** | 51.5 | 29 | 51.5 | 14 | 52.5 | 0 | 52 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Ground oat flakes** | 10 | 10 | 8.75 | 8.75 | 7.25 | 7.25 | 11.75 |
| **Textured wheat protein** | 20 | 20 | 17.5 | 17.5 | 14.5 | 14.5 | 23,5 |
| **Fresh biomass** | 0 | 30 | 0 | 50 | 0 | 70 | 0 |
| **Dried biomass** | 7.5 | 0 | 12.5 | 0 | 17.5 | 0 | 0 |

SP: spirulina. Fresh corresponds to the use of fresh spirulina; Dried corresponds to the use of dried spirulina.

## 2.3. Products characterisation

### 2.3.1. Cooking quality parameters

Dry matter content was determined by gravimetric method using an air oven at 130°C for 3 hours (Afyounizadeh Esfahani & Goli, 2022). The moisture content and the water loss were calculated using the following formula:

$$Moisture\ (\%) = 100 - Dry\ Matter\ (\%)$$

$$Water\ loss\ (\%) = Moisture\ raw\ product\ (\%) - Moisture\ cooked\ product\ (\%)$$

The quality of pasta and burgers was assessed using specific criteria (Rodríguez De Marco et al., 2014). For pasta, 4 key parameters were evaluated: cooking losses, swelling index, weight increase and colour. For burgers: water loss and baking yield were determined.

Cooking losses (CL) were quantified by determining the solid residues in cooking water. The results are expressed as a percentage of the weight of the uncooked fresh pasta:

$$CL\ (\%) = (weight\ of\ cooking\ water\ residues/\ weight\ of\ uncooked\ pasta) \times 100$$

The swelling index (SI) of cooked pasta was evaluated by drying pasta samples to a constant weight at 130°C for 3 hours. The difference in weight between cooked and dried pasta was converted into volume (ρ = 1000 kg.m$^{-3}$) using the following expression:

$$SI = (cooked\ pasta\ weight - cooked\ pasta\ weight\ after\ drying\ ) /(cooked\ pasta\ weight\ after\ drying)$$

The weight increase (WI) and baking yield were calculated using the following equations (Cho et al., 2023; Rodríguez De Marco et al., 2014):

$$WI\ (\%) = 100 * (weight\ of\ cooked\ pasta - weight\ of\ uncooked\ pasta\ )/(weight\ of\ uncooked\ pasta)$$

$$Baking\ yield\ (\%) = 100 * (weight\ after\ baking\ )/(weight\ before\ baking)$$

Percentages are always expressed on weight basis.

CIELAB colour space was used to express the instrumental colour of the residual cooking water for pasta (ColorFlex EZ spectrophotometer from HunterLab).

### 2.3.2. Texture profile analysis (TPA)

Texture profile analysis (TPA), a widely used instrumental method for characterising the textural properties of food products, is a technique that relies on bi-cyclic compression of a sample to extract mechanical parameters correlated with human sensory perceptions (Mirani & Goli., 2021; Twarogowska et al., 2022.). The TPA of cooked products was performed using a TA.XTPlus texture analyzer (Ametek TA1 Lloyd). For pasta, the test was conducted with a P/35 probe speed of 0.8 mm/s, reaching a depth corresponding to 65% of the pasta's height. A waiting time of 2 seconds was observed between the two successive compressions. Four pasta strands were placed in parallel. Two parameters were evaluated*:* firmness, defined as the maximum force recorded during the first compression cycle, and chewiness, calculated as the product of firmness, springiness, and cohesiveness. For burgers, a sample of 24 mm diameter was prepared. A cylindrical probe (50.8 mm diameter and 50 mm height) was used at a speed of 2.5 mm/s. The burger height compression was 40%. The more significant textural parameters for meat analogues products are the hardness, chewiness, the cohesiveness, the resilience and the springiness (Vu et al., 2022). In both cases, a precharge of 1N was applied.

### 2.3.3. Sensory analysis: flash profile

The sensory profile of the products was evaluated using the modified flash profile analysis method, a rapid sensory method described by Dairou & Sieffermann. (2002) and Delarue & Sieffermann. (2004), with two panels of 12 judges. The panel members were aged between twenty and forty-five years old, had knowledge of sensory sciences and were accustomed to describing food products. The first panel evaluated and ranked five pasta formulations (Fresh 2%, Fresh 10%, Dried 2%, Dried 10%, and Control) according to the perceived intensity of sensory descriptors (pre-established or defined during the analysis). The second panel evaluated five burger formulations (SP 7.5% fresh, SP 7.5% dried, SP 17.5% fresh, SP 17.5% dried, and control) using the same method.

## 2.4. Statistical analysis

3 food products have been made independently for each formulation of both food models. All the measurements have been performed 3 times. Data are expressed as mean value ± standard deviation. A linear mixed model was applied and estimated marginal means were calculated and used for statistical tests when measures couldn't be considered as independent. If measures could be considered as independent, the one-way analysis of variance (ANOVA) followed by post-hoc test Tukey or a t-test have been applied to assess significant differences (p-values < 0.05) across formulations when conditions of applications were met. The software RStudio and Microsoft Excel were used for statistical calculations. For the flash profile, a principal component analysis (PCA) has been applied on the ranking results given by panellists and data were treated with XLSTAT.

# 3. Results and Discussion

## 3.1.Spirulina biomass characterisation

Fresh biomass and rehydrated biomass are physically different, even at same moisture content (Table 3). Rehydrated dried biomass exhibits a darker colour (L*) and is less green (a*) than fresh biomass. This difference could be attributed to variations in pigment composition, such as carotenoids and phycocyanin (Ben Atitallah et al., 2019). These pigments could be degraded or oxidized during the drying process due to exposure to hot air (Palanisamy et al., 2019; Rezvani et al., 2022) which could explain the nearly twofold lower TAC (Total antioxidant capacity) for dried spirulina compared to fresh spirulina, as these pigments have antioxidant properties. The difference in color could be a barrier to consumption as brighter green colour has been shown to improve the acceptability of spirulina-enriched product (Koli et al., 2021). In addition, water activity of rehydrated dried and fresh biomass gives the same results. Viscosity is higher for rehydrated spirulina, which shows that interactions between biomass and water are different due to drying process. Protein content is close but remains different.

Table 3. Water activity, dry matter content, colour (*L*a*b**), protein content, TAC and viscosity of spirulina biomass.

| Spirulina biomass | Water activity | Dry matter content (g/100g) | Colour | | | Protein content (g/100g of DM) | TAC (µmol TE/g of DM) | Viscosity at 10°C and 30 RPM (cP) |
|---|---|---|---|---|---|---|---|---|
| | | | *L** | *a** | *b** | | | |
| Fresh | 0.99$^a$ | 22.91$^a$ ± 0.17 | 13.36$^a$ ± 0.13 | -8.74$^b$ ± 0.1 | 5.70$^a$ ± 0.2 | 73.47$^b$ ± 1.78 | 22.25$^a$ ± 2.71 | 12333.33$^b$ ± 718.42 |
| Rehydrated dried | 0.99$^a$ | 23.00$^a$ ± 0.15 | 2.62$^b$ ± 0.03 | 3.24$^a$ ± 0.07 | 1.92$^b$ ± 0.03 | 79.7$^a$ ± 1.75 | 12.50$^b$ ± 0.36 | 17993.33$^a$ ± 533.666 |

TAC: Total antioxidant capacity; TE: Trolox equivalent; DM: Dry matter; RPM: Revolutions per minute. Values are expressed as mean ± standard deviation (n = 3). For each column, different letters indicate significant differences according to Tukey's HSD post hoc test on the one-way Anova ($p < 0.05$).

A larger number of VOCs were detected in rehydrated dried spirulina compared to fresh spirulina (Table 4). Among the 26 VOCs identified, only 7 were found in the fresh biomass, while 24 were detected in the dried spirulina. Only 4 molecules are present in both forms of biomass, but they do not contribute to the final aroma, as they are mainly alkanes or alkene. β-ionone, oct-1-en-3-ol, hexanal, benzaldehyde, hexan-1-ol, β-cyclocitral and 2-pentylfuran, which are only present in dried biomass, have an olfactory perception threshold in water of less than 0.01 mg/kg and could therefore have a significant impact on the overall aroma (Moran et al., 2022; Ughetti et al., 2024).

VOCs emitted by *Athrospira platensis* are influenced by various factors inherent to the production process, such as light exposure, salinity, stress, carbon and nitrogen sources, and growth stage (Prasetiyo Himawan et al., 2024). On top, downstream processes, such as drying and storage conditions, can also have a great influence on this profile (Jia et al., 2024; Ughetti et al., 2024). Given these sources of variation, various studies have already been conducted, mainly on dried spirulina. For example, Paraskevopoulou et al. (2024) analysed the VOCs of 17 commercial dietary supplements containing dried spirulina from different countries. 128 molecules were identified, 68 of which were present in at least 4 samples. Jia et al. (2024) compared the VOCs profile of spirulina at different stages of processing and found that the mud, i.e. the product before drying, was more neutral in terms of odour than the corresponding powder form, which is entirely consistent with the results presented in this study.

Table 4. Volatile organic compounds detected in dried (rehydrated) and fresh spirulina biomass heated at 50°C.

| Chemical group | Compound Name | Dried spirulina | Fresh spirulina | Aroma (Acree & Arn, 2004) |
|---|---|---|---|---|
| Alcohol | heptan-1-ol | 1 | ND | Chemical, green |
| | hexan-1-ol | 5 | ND | Resin, flower, green |
| | oct-1-en-3-ol | 3 | ND | Earthy, green, fungal, fatty |
| | 1-methyl-4-propan-2-ylcyclohex-3-en-1-ol | 1 | ND | Must |
| | (Z)-oct-2-en-1-ol | 1 | ND | Soap, plastic |
| | 2,6-dimethylcyclohexan-1-ol | 12 | ND | peppermint-like |
| Aldehyde | (E)-2-ethylhex-2-enal | 1 | ND | |
| | benzaldehyde | 2 | ND | Almond, burnt sugar |
| | hexanal | 3 | ND | Grassy, green, fatty |
| Alkane | heptadecane | 30 | 61 | Alkane |
| | hexadecane | 3 | 9 | Alkane |
| | pentadecane | 4 | 13 | Alkane |
| Alkene | (E)-heptadec-3-ene | 1 | 3 | |
| Carboxylic Acid | S-methyl ethanethioate | ND | 1 | |
| Furan | 2-pentylfuran | 4 | ND | Green bean, butter |
| Ketone | heptan-2-one | 1 | ND | Fruity, soapy |
| | butane-2,3-dione | ND | 12 | Butter |
| | (7aR)-4,4,7a-trimethyl-6,7-dihydro-5H-1-benzofuran-2-one | 3 | ND | Sweet, fruity, woody |
| | 3-hydroxybutan-2-one | ND | 1 | Butter, cream |
| | 6-methylhept-5-en-2-one | 2 | ND | Sweet, green |
| | 2,2,6-trimethylcyclohexan-1-one | 2 | ND | Characteristic of cyanobacteria |
| Pyrazine | 2,3-dimethylpyrazine | 1 | ND | Nutty, peanut |
| | 2,3,5,6-tetramethylpyrazine | 1 | ND | Cocoa, roasted nutty |
| | 2,3,5-trimethylpyrazine | 2 | ND | Cocoa, roasted nutty |
| Terpene | β-cyclocitral | 3 | ND | Fruits, vegetables, plants |
| | β-ionone | 11 | ND | Woody, floral, alkali |
| | Safranal | 1 | ND | Fresh herbal, spicy |

Results are expressed in relative proportions (area %). ND: not detected.

Alkanes, particularly heptadecane, are abundant in both forms of biomass, with relative abundances of 83% in fresh spirulina and 37% in dried spirulina. These molecules do not influence the sensory profile of the biomass. Their abundance is linked to the intrinsic metabolism of fatty acid through two pathways: the conversion of fatty acids into aldehydes and subsequently into alkanes, and the elongation of fatty acid followed by decarboxylation (Paraskevopoulou et al., 2024).

No alcohols were found in the fresh biomass and 7 were detected in the dried biomass. The alcohols originate from lipid peroxidation due to oxidative stress associated with significant exposure to air during the drying process (Ughetti et al., 2024). However, the perception threshold for alcohols is generally higher than that for the corresponding aldehydes.

No aldehydes were detected in the fresh biomass, but benzaldehyde, hexanal and safranal were present in the dried biomass. These three molecules were found in almost all of the 17 commercial spirulina samples analysed by Paraskevopoulou et al. (2024). Benzaldehyde contributes to bitter almond-like notes, safranal evokes aromas of herbs and tobacco, and hexanal contributes to grassy and tallow-like notes (Czerny et al.. 2008). Linear aldehydes such as hexanal may originate from lipid peroxidation due to exposure to light and oxygen. Strecker degradation of amino acids into aldehydes with a side chain such as 3-methyldbutanal or 2-methylbutanal does not appear to occur here, as Maillard reaction and subsequent Strecker degradation need higher temperature than that was used by the biomass supplier (below 42°C). These Strecker aldehydes have often been observed in various studies already cited, mainly when oven drying or spray-drying was used, which involves a higher drying temperature.

No pyrazines were detected in the fresh biomass, while three were identified in the dried biomass namely 2.5-dimethylpyrazine, 2.3.5-trimethylpyrazine and 2.3.5.6-tetramethylpyrazine, as previously reported in the literature (Martelli et al., 2021). These molecules generally originate from the Maillard reactions or a subsequent fermentation process during storage (Ughetti et al., 2024). Since the temperature used for the drying process and for the experiment were 42°C and 50°C, respectively, which is not sufficient to trigger the Maillard reactions, the origin could be a fermentation process during storage at room temperature as explained by Ughetti et al. (2024). Nevertheless, the Maillard reactions can occur slowly at room temperature, which is probably not the case here, as the $a_w$ of the powder is low (Feiner, 2006).

In addition to lipids that can be oxidised due to the significant exposure to hot air, certain molecules such as β-cyclocitral, β-ionone and sulcatone (6-methylhept-5-en-2-one) could originate from the degradation of carotenoids such as β-carotene (Ughetti et al., 2024). This hypothesis is consistent with the measured colour differences and TAC (Table 3).

The drying method therefore has significant impact on the aromatic profile of the microalgae. Air drying, the method used by the Etika spirulina, produces fewer or no compounds linked to Maillard and subsequent Strecker reactions compared to oven drying and spray drying (Ughetti et al., 2024). Nevertheless, this method can cause oxidative stress for carotenoids and lipids due to significant exposure to air and produce undesirable molecules such as ketones, alcohol and linear aldehydes, which correspond to most of the molecules found in this study. Based on these semi-quantitative results (relative proportions), fresh spirulina can be considered more neutral in terms of odour and could therefore be more suitable for significant protein enrichment of food products.

### 3.2.Cooking quality parameters

For pasta, the incorporation of fresh biomass instead of dried biomass results in a greater increase in weight after cooking, although this increase is not proportional to the amount of biomass added (Table 5). Similar findings were reported by Özyurt et al. (2015). Fresh biomass results in a greater increase in weight than the control. These results contrast with that of Fradique et al. (2010) who observed an increase in weight with the use of dried spirulina in fresh pasta compared to the control formulation. As shown in Table 3, about viscosity, it appears that the interactions between dried and fresh spirulina with water are different.

Cooking losses in pasta are significantly higher when fresh biomass is incorporated compared to dried biomass, with losses increasing proportionally to the biomass incorporated. However, regardless of the form of biomass, cooking losses range between 2.77% and 5.02%, which remains below the acceptable threshold established by J.w and V.l (1988). Özyurt et al. (2015) similarly reported values below this technological limit but concluded that the addition of spirulina had no impact on cooking losses. In contrast, Zouari et al. (2011) observed a decrease in cooking losses with dried spirulina. Koli et al. (2021) observed no negative effects on cooking losses when fresh biomass was incorporated into dried pasta. These variations highlight the variability of results depending on the study, the type of pasta and the type of biomass. However, all reported cooking losses are within the acceptable range, which is promising for spirulina enrichment.

As noted by Özyurt et al. (2015), the incorporation of fresh biomass at 6% and 10% led to an increase in the swelling index, meaning that a greater amount of water is absorbed, which is obviously correlated to the increase in weight. Fradique et al. (2010) , Rodríguez De Marco et al. (2014) and Zouari et al. (2011) observed an increase in this parameter when incorporating dried biomass, which is not the case here, as products containing 6% and 10% of dried biomass have a lower SI than the control. Some proteins can retain water, and the drying process may have altered this ability and led to lower absorption.

Another hypothesis for higher cooking losses and higher weight gain for pasta made with fresh spirulina could be a weaker gluten network, which can ease the diffusion of water during cooking and decrease time required for starch gelatinization (Rodríguez De Marco et al., 2014). The presence of non-lysed cells, due to their size and different interactions with water than dried spirulina biomass could potentially interfere with the gluten network formation during mixing stage. Perform some texture measurement on the dough could help to determine if gluten network is the same across the different formulations.

Table 5. Cooking quality characteristics of pasta formulations enriched with spirulina.

| Cooking characteristics | Control 0% | SP 2% | | SP 6% | | SP 10% | |
|---|---|---|---|---|---|---|---|
| | | Dried | Fresh | Dried | Fresh | Dried | Fresh |
| Weight increase (%) | 53.29 ± 2.15$^b$ | 51.42 ± 1.3$^b$ | 65.76 ± 5.32$^a$ | 48.64 ± 1.14$^b$ | 63.99 ± 1.77$^a$ | 50.41 ± 1.14$^b$ | 65.93 ± 5.56$^a$ |
| Swelling Index (g/g dry matter) | 1.45 ± 0.02$^b$ | 1.4 ± 0.05$^b$ | 1.46 ± 0.04$^b$ | 1.33 ± 0.04$^c$ | 1.53 ± 0.04$^a$ | 1.35 ± 0.03$^c$ | 1.59 ± 0.07$^a$ |
| Cooking losses (%) | *ND* | 2.77 ± 0.19$^b$ | 2.92 ± 0.63$^b$ | 2.78 ± 0.09$^b$ | 4.08 ± 0.37$^a$ | 2.89 ± 0.22$^b$ | 5.02 ± 0.7$^a$ |

SP: spirulina. Data are expressed as mean ± standard deviation. For each row, a different letter indicates a significant difference according to Tukey's HSD post hoc test on the one-way Anova ($p$-value $< 0.05$).

For boiled foods such as pasta, consumers generally expect high-quality products to retain their colour after the cooking process (Nip, 2007). To evaluate this parameter, colour analyses were performed on the cooking water (Table 6). Indeed, significant colouration of the water may be perceived negatively by consumers (Pagani et al., 2007). Additionally, the bright green colour of pasta enriched with fresh spirulina may be attractive to consumer (Koli et al., 2021). High colour retention could therefore be a positive characteristic of spirulina-enriched products, such as pasta.

For the *b** axis (yellowness/blueness), the most variable parameter in the formulations, pasta made from fresh biomass induce a significantly more yellow and less blue coloration of the cooking water than equivalent products made with dried biomass. This result is consistent with the cooking losses results, which show greater losses for products enriched with fresh biomass, and could be explained by the higher initial content of phycocyanin, a soluble blue pigment present in fresh biomass. Indeed, phycocyanin is sensitive to desiccation, even at low temperature (Ma et al., 2019).

Table 6. Cooking water colour measurements of pasta formulations enriched with spirulina.

| | *L** | | *a** | | *b** | |
|---|---|---|---|---|---|---|
| Biomass content (% of DM) | Dried | Fresh | Dried | Fresh | Dried | Fresh |
| Control 0% | 12.13 ± 0.89$^{b}$ | | -1.02 ± 0.07$^{d}$ | | -2.48 ± 0.22$^{c}$ | |
| SP 2% | 11.99 ± 0.66$^{cy}$ | 13.68 ± 0.46$^{ax}$ | -2.04 ± 0.23$^{ax}$ | -3.12 ± 0.5$^{ay}$ | -1.79 ± 0.81$^{by}$ | 3.58 ± 1.87$^{bx}$ |
| SP 6% | 11.62 ± 0.48$^{cy}$ | 14.14 ± 1.19$^{ax}$ | -3.24 ± 0.27$^{bx}$ | -4.23 ± 0.26$^{by}$ | -1.28 ± 1.05$^{by}$ | 9.08 ± 2.29$^{ax}$ |
| SP 10% | 16.24 ± 0.71$^{ax}$ | 14.25 ± 1.49$^{ay}$ | -4.53 ± 0.3$^{cx}$ | -4.05 ± 0.08$^{by}$ | -0.03 ± 0.71$^{ay}$ | 8.1 ± 3.23$^{ax}$ |

SP: spirulina; DM: dry matter. Values are expressed as mean ± standard deviation (n = 9). Different letters (a-c) within the same column indicate significant differences, while different letters (x-y) within the same row highlight significant differences according to Tukey's HSD post hoc test on the one-way Anova (p-value < 0.05) between the *L**, *a**, or *b**.

For meat analogues, the type of biomass used and the degree of enrichment have no effect on the moisture loss and the baking yield when products are cooked 12 minutes at 180°C in the oven (Table 7). These two cooking quality parameters are clearly strongly correlated and must be considered for sensory and economic reasons. Cooking losses mainly concern water, but can also concern fats and minerals (Vu et al., 2022). The baking yield is slightly lower for the control than for the five formulations containing spirulina, which means that spirulina could potentially contribute to a slight reduction in cooking losses. The incorporation of fresh spirulina, even at a high percentage, does not affect the baking properties.

Table 7. Baking properties and quality parameters of raw, baked burgers with fresh and dried spirulina.

| | **SP 7.5%** | | **SP 12.5%** | | **SP 17.5%** | | **Control 0%** |
|---|---|---|---|---|---|---|---|
| | Fresh | Dried | Fresh | Dried | Fresh | Dried | |
| **Raw burgers** | | | | | | | |
| **Weight (g)** | 79.12 ± 2.28$^{a}$ | 82.61 ± 6.5$^{a}$ | 78.7 ± 3.49$^{a}$ | 79.18±5.24$^{a}$ | 76.66 ± 2.77$^{a}$ | 76.69 ± 3.44$^{a}$ | 78.71 ± 2.43$^{a}$ |
| **Height (cm)** | 1.57 ± 0.06$^{a}$ | 1.73 ± 0.06$^{a}$ | 1.7 ± 0.1$^{a}$ | 1.67 ± 0.06$^{a}$ | 1.57 ± 0.06$^{a}$ | 1.57 ± 0.06$^{a}$ | 1.6 ± 0$^{a}$ |
| **Diameter (cm)** | 8.93 ± 0.06$^{ab}$ | 8.97 ± 0.06$^{ab}$ | 8.93 ± 0.06$^{ab}$ | 9.13 ± 0.15$^{a}$ | 9 ± 0$^{ab}$ | 9 ± 0.17$^{ab}$ | 8.83 ± 0.06$^{b}$ |
| **Moisture (% w/w)** | 54.07 ± 0.35$^{bc}$ | 54.37 ± 0.54$^{bc}$ | 53.88 ± 0.54$^{c}$ | 53.89 ± 0.74$^{c}$ | 54.93 ± 0.48$^{b}$ | 55.48 ± 0.40$^{a}$ | 55.31 ± 0.28$^{a}$ |
| **Baked burgers** | | | | | | | |
| **Weight (g)** | 74.11 ± 2.24$^{a}$ | 77.14 ± 6.32 $^{a}$ | 72.66 ± 3.36$^{a}$ | 74.05 ± 4.92$^{a}$ | 71.63 ± 2.7$^{a}$ | 71.54 ± 3.36$^{a}$ | 72.68 ± 2.35$^{a}$ |
| **Height (cm)** | 1.63 ± 0.06$^{b}$ | 1.73 ± 0.06$^{ab}$ | 1.87 ± 0.12$^{a}$ | 1.7 ± 0.1 $^{ab}$ | 1.57 ± 0.06$^{b}$ | 1.57 ± 0.06$^{b}$ | 1.6 ± 0$^{b}$ |
| **Diameter (cm)** | 8.77 ± 0.15$^{ab}$ | 8.8 ± 0.1$^{ab}$ | 8.77 ± 0.12$^{ab}$ | 8.87 ± 0.12$^{a}$ | 8.6 ± 0.1$^{ab}$ | 8.67 ± 0.06$^{ab}$ | 8.57 ± 0.06 $^{b}$ |
| **Moisture (%)** | 49 ± 0.70$^{ab}$ | 49.09 ± | 47.67 ± | 49.53 ± | 50 ± 1.49$^{ab}$ | 50.11 ± | 49.19 ± |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1.26$^{ab}$ | 1.17$^{b}$ | 1.79$^{ab}$ | | 1.21$^{a}$ | 1.64$^{a}$ |
| **Baking properties** | | | | | | | |
| **Moisture loss (% w/w)** | 5.07 ± 0.13$^{a}$ | 5.28 ± 0.33$^{a}$ | 6.21 ± 0.81$^{a}$ | 4.36 ± 1.18$^{a}$ | 4.93 ± 0.62$^{a}$ | 5.37 ± 1.13$^{a}$ | 5.82 ± 0.48$^{a}$ |
| **Baking yield (% w/w)** | 93.66 ± 0.23$^{a}$ | 93.363 ± 0.32$^{a}$ | 92.317 ± 0.29$^{b}$ | 93.527 ± 0.06$^{a}$ | 93.443 ± 0.20$^{a}$ | 93.28 ± 0.42$^{a}$ | 92.34 ± 0.27$^{b}$ |

SP: spirulina; ND: not determined. Values are expressed as mean ± standard deviation (n = 9). For each row, different letters indicate significant differences according to Tukey's HSD post hoc test on the one-way Anova ($p < 0.05$).

When fresh spirulina is incorporated instead of rehydrated dried biomass into pasta and meat analogues, the results vary depending on the food model considered. In pasta enriched with 6% and 10%, more water is absorbed during the cooking process (leading to an increase in weight), while more solids, probably pigments, are lost to the cooking water. This results in greater colouring of the cooking medium, which is undesirable for consumers. For meat analogues, the cooking properties remain unchanged, whether fresh or rehydrated dried spirulina is used, regardless of the level of enrichment. Although the two food models differ in nature, particularly due to the presence of textured wheat protein in meat analogues, the applied cooking method, i.e.  boiling in water for pasta versus baking in the oven for meat analogues, could influence these results, as the respective heat and mass transfer mechanisms are completely different. A potential counter-experiment could involve boiling burgers in water to examine the differences in cooking properties between products enriched with fresh or dried spirulina. However, this approach would deviate from the initial burger model selected.

### 3.3.Texture profile analysis

For meat analogues, the incorporation of spirulina, whether fresh or dried and across a range of incorporation levels, does not significantly affect the texture under the conditions applied for meat analogue preparation and TPA analysis (Fig.  1). Products containing spirulina exhibit no detectable textural differences compared to the control, indicating that the microalgae does not impair the ability of textured wheat protein to hydrate and provide minced-meat texture. Indeed, spirulina proteins may confer additional textural advantages owing to their emulsifying capacity, water-holding ability, and foaming properties, which are comparable to those of whey or soy proteins (Fu et al., 2021). Moreover, spirulina proteins supplementation may enhance the chewiness of meat analogues (Bakhsh et al., 2023). At 12.5% inclusion, some differences become apparent: fresh spirulina slightly increases chewiness, springiness, resilience and cohesiveness relative to the control, whereas dried spirulina yields a firmer texture, a trend not observed at other enrichment levels. Even at high inclusion rate, replacing dried spirulina with fresh spirulina does not adversely affect texture. Reproducing the texture of meat remains a persistent challenge. Although high-moisture extrusion of soy or wheat gluten is a common industrial approach for producing meat analogues applying this process to spirulina protein has so far achieved only limited success, highlighting the need for further investigation (Zhu et al., 2024).

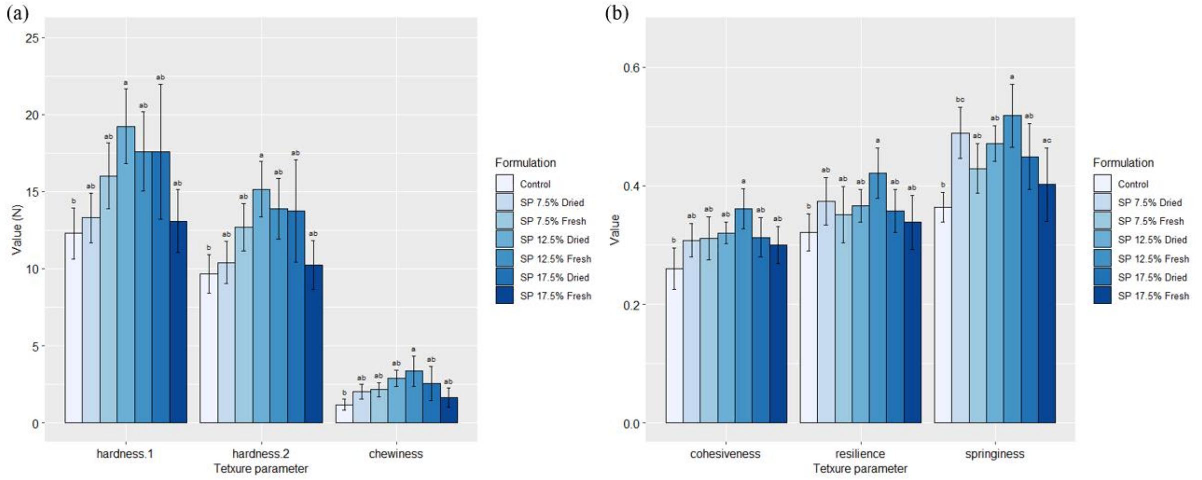


Fig. 1. Hardness bite 1, hardness bite 2 and chewiness (a) and cohesiveness, resilience and springiness (b) according to burger formulations enriched with fresh or dried spirulina (% w/w). SP: spirulina. Data are expressed as mean ± standard deviation. For each parameter, different letters indicate a significant difference according to Tukey's HSD post hoc test on the one-way Anova on the estimated marginal means from the linear mixed model ($p$-value < 0.05).

The incorporation of fresh spirulina biomass into pasta had no measurable effect on firmness or chewiness, in contrast to dried biomass (Fig. 2) when compared with the control. Pasta enriched with 6% and 10% dried biomass exhibited significantly greater chewiness (Fig. 2b) and only the 6% formulation showed a firmness different from that of the equivalent fresh biomass product (Fig. 2a). Thus, the incorporation of fresh spirulina does not alter the texture of fresh pasta, whereas dried spirulina does. Because pasta enriched with fresh spirulina (6% and 10%) loses more solids to the cooking water and absorbs more water (as reflected by higher swelling index and weight), its texture is likely softer, i.e. less firm.

These observations are consistent with previous findings: Zouari et al. (2011), reported that pasta containing 1% to 3% dried spirulina exhibited greater firmness than the control and Rodríguez De Marco et al. (2014) likewise found that substituting flour with dried spirulina increased firmness and chewiness. In contrast, Fradique et al. (2010) observed no significant difference in firmness between control pasta and pasta enriched with dried biomass. Koli et al. (2021) reported that dried pasta enriched with fresh spirulina at 10%, 15% and 20% showed lower firmness than the control.

As with cooking quality parameters, the effect of incorporating fresh spirulina versus rehydrated dried spirulina depends on the food matrix. In meat analogues, no texture differences are observed, likely due to the strong functional contribution of textured wheat protein, which may mask potential effects from other ingredients. In pasta, however, dried spirulina at 6% and 10% alters texture, primarily by increasing chewiness, making it distinct from the control formulation. In contrast, fresh spirulina, regardless of inclusion levels, does not produce any measurable texture change in either food model.

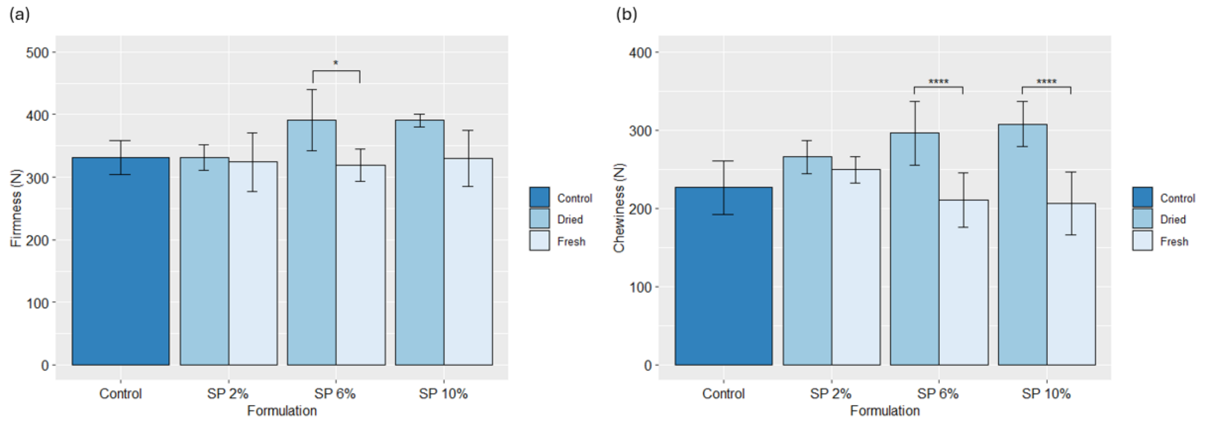


Fig. 2. Pasta's firmness (a) and chewiness (b) according to formulations. SP: spirulina. Data are expressed as mean ± standard deviation. For each enrichment percentage (% w/w), a significant difference, according to Tukey's HSD post hoc test on the one-way Anova, is expressed as: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

### 3.4.Flash profile sensory analysis

For both food models, the panel perceived the formulation differently. The combination of the pre-established and the panel-generated descriptors (attributes) allowed clear discrimination among formulations (). Only a few descriptors were non-discriminatory namely, "sticky" and "chocolate" for pasta, and "elasticity" and "boiled egg" for burgers, indicating that the remaining descriptors were well-suited to the study.

Table 8). Only a few descriptors were non-discriminatory namely, "sticky" and "chocolate" for pasta, and "elasticity" and "boiled egg" for burgers, indicating that the remaining descriptors were well-suited to the study.

Table 8. Attributes discriminatory potential on products.

| Attributes | **Discrimination effect on pasta** | **Discrimination effect on burger** |
|---|---|---|
| **Cereals** | <0.001 | 0.001 |
| **Hard-boiled egg** | 0.004 | 0.143 |
| **Saline** | 0.002 | <0.001 |
| **Pasta** | <0.001 | NA |
| **Earthy** | <0.001 | <0.001 |
| **Firm texture** | <0.001 | NA |
| **Sticky** | 0.145 | NA |
| **Fungal** | <0.001 | 0.002 |
| **Fishy** | <0.001 | <0.001 |
| **Chocolate** | 0.079 | 0.04 |
| **Bitter** | 0.001 | <0.001 |
| **Astringent** | 0.007 | 0.038 |
| **Roast chicken** | NA | 0.01 |
| **Minced meat texture** | NA | 0.018 |
| **Elasticity** | NA | 0.224 |
| **Juicy** | NA | 0.016 |

p-value < 0.05 means the attribute can be used to discriminate between products. NA: Not available.

For each food product, two principal components (F1 and F2) were retained following principal component analysis (PCA) of the ranking results for each descriptor. In burgers, the first two components explained over 67% of the variability (Fig.  3a). F1 primarily discriminated samples according to enrichment level, while F2 tended to separate them based on biomass form, particularly at 17.5% enrichment level. These results suggest that sensory properties are more strongly influenced by the enrichment level than by biomass type. Notably, the enrichment levels achieved in this study were substantially higher than those typically studied. Grahl et al. (2018) similarly found that incorporating spirulina powder into extruded meat analogues significantly affected sensory properties, a trend consistent with the present findings. In pasta (Fig.  3b), F1 accounted for 71.63% of the total variability, substantially exceeding that observed for meat analogues. As shown in **Error! Reference source not found.**b, formulations containing dried biomass were closely grouped and clearly separated from those containing fresh biomass, which were more closely associated with the control. This pattern is consistent with the findings of Koli et al. (2021) who reported that dehydrated pasta enriched with 20% fresh spirulina was nearly as acceptable to consumers as pasta without spirulina. Pasta enriched with fresh biomass is then more "neutral", sharing sensory characteristics with plain pasta. This interpretation aligns with the VOCs analysis, which likewise indicated a more neutral profile for fresh biomass.

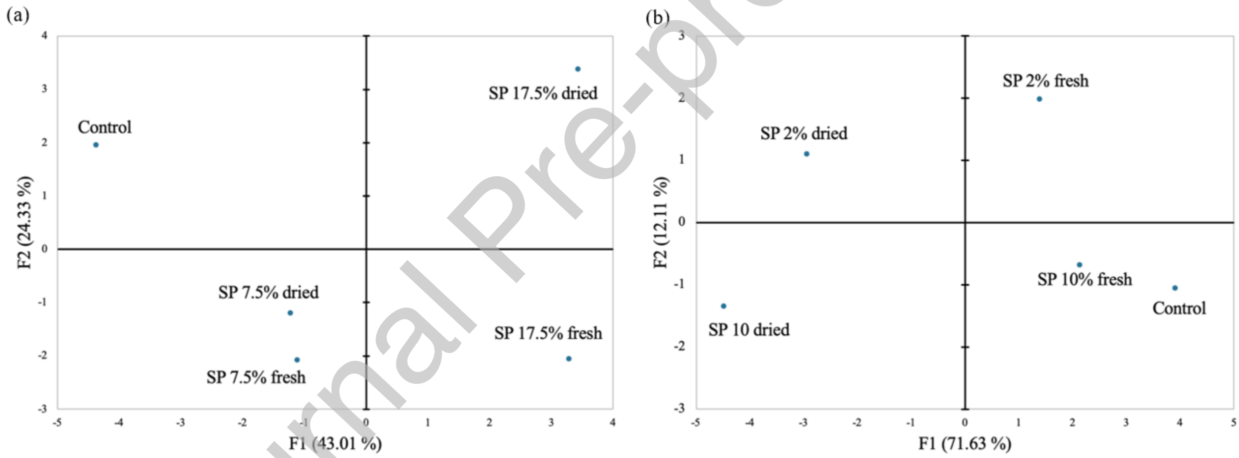


Fig.  3. Retained principal component F1 and F2 from the principal component analysis (PCA) on the flash profile results for the 5 formulations of meat analogues (a) and pasta (b) formulations enriched at different levels (% w/w) of spirulina biomass. SP: spirulina.

PCA identified six key sensory attributes. For pasta these were "firm texture", "cereal", "earthy", "fishy", "saline" and "plain pasta", and for meat analogues they were "cereal", "saline", "roast chicken", "minced meat texture", "fungal" and "fishy". Fig.  4 illustrates their relative distribution across tested formulations. Pasta made with dried biomass, particularly at higher enrichment levels, was strongly associated with "fishy", "earthy" and "saline" descriptors. In contrast, formulations containing fresh biomass were more closely aligned with the control pasta, sharing attributes such as "plain pasta", "firm texture" and "cereal". Consistent with this, instrumental texture measurements (Fig.  2b) showed that pasta enriched with 10% dried spirulina differed significantly in chewiness from both pasta enriched with 10% of fresh spirulina and the control, reflecting the sensory attribute "firm texture" highlighted in Fig.  4.

For meat analogues, products containing 17.5% spirulina, regardless of the biomass form, were characterized by fishy, fungal and saline notes, which are generally undesirable in this product category. The fungal note is a distinctive feature of spirulina-enriched products compared with those enriched with other algae (Rabitti et al., 2024). Moreover, products enriched with 7.5% displayed stronger meaty notes than those with 17.5%, indicating that, unlike in pasta, enrichment level plays a greater role than biomass form in shaping sensory properties. Nevertheless, at high inclusion levels, fresh biomass appears preferable to dried biomass as the intensity of the undesirable "fishy" note was lower. As expected, the control product was predominantly associated with cereal notes.

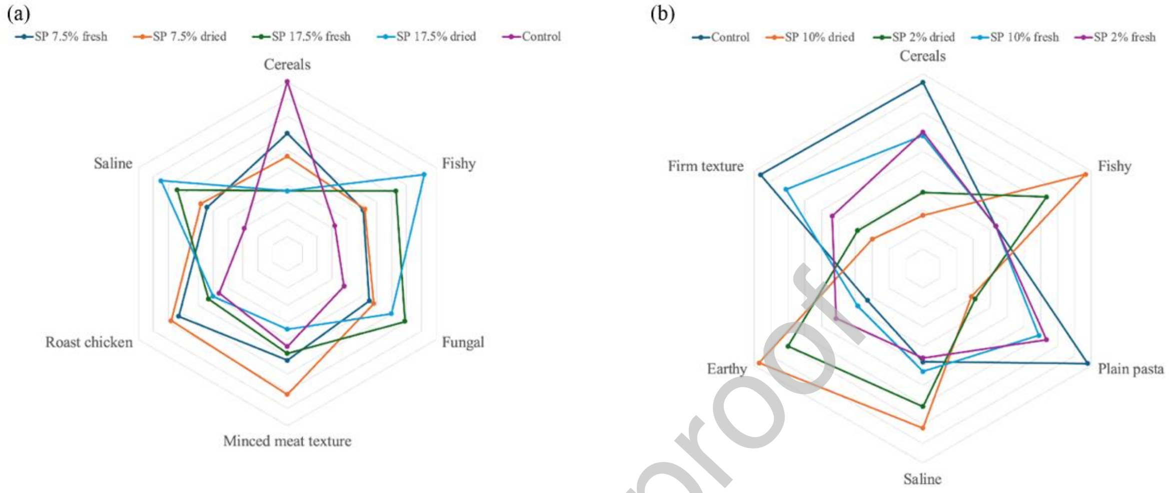


Fig. 4. Radar chart (relative proportions) of the 6 main key discriminative sensory attributes of burgers (a) and pasta (b) from the flash profile. SP: spirulina.

When spirulina is used for food enrichment, both its physical form and the chosen food matrix, along with the cooking process applied, can influence the overall sensory perception. For meat analogues, sensory notes are primarily driven by enrichment levels, whereas for pasta, they are more strongly affected by the physical form of biomass. This indicates that even when the raw material has a more neutral flavour profile, the food matrix and the cooking method must be considered to optimise sensory acceptance. In the present study, meat analogues are oven cooked, a process that can promote the formation of Strecker aldehydes and other undesirable volatile compounds (Ughetti et al., 2024). This may account for the smaller sensory differences observed between products enriched with fresh versus dried biomass, compared with boiled products such as pasta. In addition, during boiling, pasta absorbs substantial amounts of water which dilutes the initial biomass concentration and may attenuate off-flavours. A gentler cooking method, such as a lower cooking temperature, could be explored for meat analogues to reduce Maillard reactions and subsequent Strecker degradation, thereby limiting the formation of off-flavours even when using otherwise neutral fresh spirulina.

## 4. Conclusion

Spirulina is renowned for its exceptionally high protein content, which can reach up to 80% of its dry matter. For practical and industrial purposes, the dried form is most commonly used and studied. However, the incorporation of dried spirulina into food products is often quantitatively limited due to the presence of undesirable off-flavours.

The use of the fresh form could allow for higher incorporation levels in food products, primarily for sensory reasons, as its aroma is more neutral and lacks the off-flavours characteristic of the dried form. In widely consumed products such as pasta, inclusion rates as high as 10% (dry equivalent) can be achieved without compromising taste or affecting texture, unlike with dried spirulina. Nevertheless, the higher cooking losses and more intense coloration of the cooking water remain drawbacks that warrant further investigation. In contrast, for meat analogue products such as burger, the percentage of incorporation must be considered carefully, as flavour can be affected when products are baked at 180°C. However, no adverse effects on texture or cooking quality have been reported with respect to the form of biomass used.

The present work examined only one drying method (air-drying) and frozen fresh spirulina. Future studies should investigate alternative drying techniques such as spray-dried, freeze-dried as well as non-frozen fresh spirulina, to determine whether similar results are obtained.

From a food product development perspective, other uncooked products with semi-liquid textures, such as spreads, may be suitable candidates for fresh spirulina incorporation. Given that fresh spirulina contains approximatively 23% dry matter, the high-water content could limit its application in solid products.

Furthermore, the development of palatable, nutritious and safe food products using fresh biomass without a prior freezing step will require the establishment of new industrial value chains, including efficient post-harvest handling, stabilisation and preservation methods.

## Declaration of competing interest

There is no conflict of interest between the authors and organisations.

## Data availability

Data are available on request.

## Funding acknowledgements

This research is part of the convention FoodWal 2210182 and has been financed by SPW Research (Win4Excellence program).

## CRediT authorship contribution statement

**De Rijdt Mathieu:** Writing – original draft, Investigation, Methodology, Conceptualization **Verhagen Jeanne:** Writing – original draft, Investigation, Methodology, Conceptualization. **Brostaux Yves:** Software **Goffin Dorothée:** Funding acquisition, Project administration, Methodology, Conceptualization, Validation. **Haubruge Eric**: Funding acquisition, Project administration, Validation, Conceptualization.

## References

Acree, T., & Arn, H. (2004). *Flavornet and human odor space*. https://www.flavornet.org/flavornet.html

Afyounizadeh Esfahani, D., & Goli, M. (2022). The effect of Stevioside-Isomalt, whey protein concentrate, and Bacillus coagulans on the physicochemical and sensory properties of Iranian probiotic Masghati sweet. *Journal of Food Processing and Preservation*, *46*(10), e16028. https://doi.org/10.1111/jfpp.16028

Bakhsh, A., Park, J., Baritugo, K. A., Kim, B., Sil Moon, S., Rahman, A., & Park, S. (2023). A holistic approach toward development of plant-based meat alternatives through incorporation of novel microalgae-based ingredients. *Frontiers in Nutrition*, *10*, 1110613. https://doi.org/10.3389/fnut.2023.1110613

Batista, A. P., Niccolai, A., Fradinho, P., Fragoso, S., Bursic, I., Rodolfi, L., Biondi, N., Tredici, M. R., Sousa, I., & Raymundo, A. (2017). Microalgae biomass as an alternative ingredient in cookies : Sensory, physical and chemical properties, antioxidant activity and in vitro digestibility. *Algal Research*, *26*, 161-171. https://doi.org/10.1016/j.algal.2017.07.017

Bchir, B., Felfoul, I., Bouaziz, M. A., Gharred, T., Yaich, H., Noumi, E., Snoussi, M., Bejaoui, H., Kenzali, Y., Blecker, C., & Attia, H. (2019). *Investigation of physicochemical, nutritional, textural, and sensory properties of yoghurt fortified with fresh and dried Spirulina (Arthrospira platensis)*. *26*(5), 1565-1576. https://www.proquest.com/docview/2335661584/citation/A662E3C84386460CPQ/1

Becker, E. W. (2007). Micro-algae as a source of protein. *Biotechnology Advances*, *25*(2), 207-210. https://doi.org/10.1016/j.biotechadv.2006.11.002

Ben Atitallah, A., Hentati, F., Dammak, M., Hadrich, B., Fendri, I., Ayadi, M.-A., Michaud, P., Abdelkafi, S., & Barkallah, M. (2019). Effect of Microalgae Incorporation on Quality Characteristics and Functional and Antioxidant Capacities of Ready-to-Eat Fish Burgers Made from Common Carp (Cyprinus carpio). *Applied Sciences*, *9*(9), Article 9. https://doi.org/10.3390/app9091830

Boyanova, P., Gradinarska, D., Dobreva, V., Panayotov, P., Momchilova, M., & Zsivanovits, G. (2022). Effect of *Spirulina platensis* on the quality and antioxidants characteristics of ice cream. *BIO Web of Conferences*, *45*, 01009. https://doi.org/10.1051/bioconf/20224501009

Cho, Y., Bae, J., & Choi, M.-J. (2023). Physicochemical Characteristics of Meat Analogs Supplemented with Vegetable Oils. *Foods*, *12*(2), 312. https://doi.org/10.3390/foods12020312

Czerny, M., Christlbauer, M., Christlbauer, M., Fischer, A., Granvogl, M., Hammer, M., Hartl, C., Hernandez, N. M., & Schieberle, P. (2008). Re-investigation on odour thresholds of key food aroma compounds and development of an aroma language based on odour qualities of defined aqueous odorant solutions. *European Food Research and Technology*, *228*(2), 265-273. https://doi.org/10.1007/s00217-008-0931-x

Dairou, V., & Sieffermann, J.-M. (2002). A Comparison of 14 Jams Characterized by Conventional Profile and a Quick Original Method, the Flash Profile. *Journal of Food Science*, *67*(2), 826-834. https://doi.org/10.1111/j.1365-2621.2002.tb10685.x

De Marco Castro, E., Shannon, E., & Abu-Ghannam, N. (2019). Effect of Fermentation on Enhancing the Nutraceutical Properties of Arthrospira platensis (Spirulina). *Fermentation*, *5*(1), 28. https://doi.org/10.3390/fermentation5010028

Delarue, J., & Sieffermann, J.-M. (2004). Sensory mapping using Flash profile. Comparison with a conventional descriptive method for the evaluation of the flavour of fruit dairy products. *Food Quality and Preference*, *15*(4), 383-392. https://doi.org/10.1016/S0950-3293(03)00085-5

FAO. (2021). *La situation mondiale de l'alimentation et de l'agriculture 2021 : Rendre les systèmes agroalimentaires plus résilients face aux chocs et aux situations de stress*. FAO. https://doi.org/10.4060/cb4476fr

Feiner, G. (2006). 4—Definitions of terms used in meat science and technology. In G. Feiner (Éd.), *Meat Products Handbook* (p. 46-71). Woodhead Publishing. https://doi.org/10.1533/9781845691721.1.46

Fradinho, P., Niccolai, A., Soares, R., Rodolfi, L., Biondi, N., Tredici, M. R., Sousa, I., & Raymundo, A. (2020a). Effect of Arthrospira platensis (spirulina) incorporation on the rheological and bioactive properties of gluten-free fresh pasta. *Algal Research*, *45*, 101743. https://doi.org/10.1016/j.algal.2019.101743

Fradinho, P., Niccolai, A., Soares, R., Rodolfi, L., Biondi, N., Tredici, M. R., Sousa, I., & Raymundo, A. (2020b). Effect of Arthrospira platensis (spirulina) incorporation on the rheological and bioactive properties of gluten-free fresh pasta. *Algal Research*, *45*, 101743. https://doi.org/10.1016/j.algal.2019.101743

Fradique, Ḿonica, Batista, A. P., Nunes, M. C., Gouveia, L., Bandarra, N. M., & Raymundo, A. (2010). Incorporation of Chlorella vulgaris and Spirulina maxima biomass in pasta products. Part 1 : Preparation and evaluation. *Journal of the Science of Food and Agriculture*, *90*(10), 1656-1664. https://doi.org/10.1002/JSFA.3999

Fu, Y., Chen, T., Chen, S. H. Y., Liu, B., Sun, P., Sun, H., & Chen, F. (2021). The potentials and challenges of using microalgae as an ingredient to produce meat analogues. *Trends in Food Science & Technology*, *112*, 188-200. https://doi.org/10.1016/j.tifs.2021.03.050

Grahl, S., Palanisamy, M., Strack, M., Meier-Dinkel, L., Toepfl, S., & Mörlein, D. (2018). Towards more sustainable meat alternatives : How technical parameters affect the sensory properties of extrusion products derived from soy and algae. *Journal of Cleaner Production*, *198*, 962-971. https://doi.org/10.1016/j.jclepro.2018.07.041

Ismaiel, M. M. S., El-Ayouty, Y. M., & Piercey-Normore, M. (2016). Role of pH on antioxidants production by Spirulina (Arthrospira) platensis. *Brazilian Journal of Microbiology*, *47*(2), 298-304. https://doi.org/10.1016/j.bjm.2016.01.003

Jia, X., Cui, H., Qin, S., Ren, J., Zhang, Z., An, Q., Zhang, N., Yang, J., Yang, Y., Fan, G., & Pan, S. (2024). Characterizing and decoding the key odor compounds of *Spirulina platensis* at different processing stages by sensomics. *Food Chemistry*, *461*, 140944. https://doi.org/10.1016/j.foodchem.2024.140944

J.w, D., & V.l, Y. (1988). *Evaluation of durum wheat, semolina, and pasta in the United States.* https://agris.fao.org/search/en/providers/123819/records/64735a67e17b74d222515f15

Koli, D. K., Rudra, S. G., Pabbi, S., & Kalwani, M. (2021). An assessment of the nutritional, physicochemical and sensory attributes of pasta enriched with fresh Spirulina wet biomass. *Journal of Pharmacognosy and Phytochemistry*, *10*(1), 1174-1179. https://www.phytojournal.com/archives/2021.v10.i1.13499/an-assessment-of-the-nutritional-physicochemical-and-sensory-attributes-of-pasta-enriched-with-fresh-spirulina-wet-biomass

Lemes, A. C., Takeuchi, K. P., Carvalho, J. C. M. D., & Danesi, E. D. G. (2012). Fresh pasta production enriched with Spirulina platensis biomass. *Brazilian Archives of Biology and Technology*, *55*(5), 741-750. https://doi.org/10.1590/S1516-89132012000500014

Ma, Z., Ahmed, F., Yuan, B., & Zhang, W. (2019). Fresh living Arthrospira as dietary supplements : Current status and challenges. *Trends in Food Science & Technology*, *88*, 439-444. https://doi.org/10.1016/j.tifs.2019.04.010

Markwell, M. A. K., Haas, S. M., Bieber, L. L., & Tolbert, N. E. (1978). A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. *Analytical Biochemistry*, *87*(1), 206-210. https://doi.org/10.1016/0003-2697(78)90586-9

Martelli, F., Cirlini, M., Lazzi, C., Neviani, E., & Bernini, V. (2021). Solid-State Fermentation of Arthrospira platensis to Implement New Food Products : Evaluation of Stabilization Treatments and Bacterial Growth on the Volatile Fraction. *Foods*, *10*(1), Article 1. https://doi.org/10.3390/foods10010067

Mirani, A., & Goli, M. (2021). Production of the eggplant-fiber incorporated cupcake and evaluating its chemical, textural and colorimetric properties over a ten-day storage time. *Journal of Food Processing and Preservation*, *45*(4), e15311. https://doi.org/10.1111/jfpp.15311

Moll, P., Salminen, H., Schmitt, C., & Weiss, J. (2023). Pea protein–sugar beet pectin binders can provide cohesiveness in burger type meat analogues. *European Food Research and Technology*, *249*(4), 1089-1096. https://doi.org/10.1007/s00217-022-04199-1

Moran, L., Bou, G., Aldai, N., Ciardi, M., Morillas-España, A., Sánchez-Zurano, A., Barron, L. J. R., & Lafarga, T. (2022). Characterisation of the volatile profile of microalgae and cyanobacteria using solid-phase microextraction followed by gas chromatography coupled to mass spectrometry. *Scientific Reports*, *12*(1), 3661-3661. https://doi.org/10.1038/s41598-022-07677-4

Nip, W.-K. (2007). Fundamentals of Food Manufacturing. In *Handbook of Food Products Manufacturing* (p. 1-44). John Wiley & Sons, Ltd. https://doi.org/10.1002/9780470113554.ch1

Oliviero, T., & Fogliano, V. (2016). Food design strategies to increase vegetable intake : The case of vegetable enriched pasta. *Trends in Food Science & Technology*, *51*, 58-64. https://doi.org/10.1016/j.tifs.2016.03.008

Özyurt, G., Uslu, L., Yuvka, I., Gökdoğan, S., Atci, G., Ak, B., & Işik, O. (2015a). Evaluation of the Cooking Quality Characteristics of Pasta Enriched with *Spirulina Platensis*. *Journal of Food Quality*, *38*(4), 268-272. https://doi.org/10.1111/jfq.12142

Özyurt, G., Uslu, L., Yuvka, I., Gökdoğan, S., Atci, G., Ak, B., & Işik, O. (2015b). Evaluation of the Cooking Quality Characteristics of Pasta Enriched with Spirulina Platensis. *Journal of Food Quality*, *38*(4), 268-272. https://doi.org/10.1111/jfq.12142

Pagani, M. A., Lucisano, M., & Mariotti, M. (2007). Traditional Italian Products from Wheat and Other Starchy Flours. In *Handbook of Food Products Manufacturing* (p. 327-388). John Wiley & Sons, Ltd. https://doi.org/10.1002/9780470113554.ch17

Palanisamy, M., Töpfl, S., Berger, R. G., & Hertel, C. (2019). *Physico-chemical and nutritional properties of meat analogues based on Spirulina/lupin protein mixtures*. *245*(9), 1889-1898. https://doi.org/10.1007/s00217-019-03298-w

Paraskevopoulou, A., Kaloudis, T., Hiskia, A., Steinhaus, M., Dimotikali, D., & Triantis, T. M. (2024). Volatile Profiling of Spirulina Food Supplements. *Foods*, *13*(8), Article 8. https://doi.org/10.3390/foods13081257

Prasetiyo Himawan, Purwaningsih Sri, Setyaningsih Iriani, Nurilmala Mala, Uju Uju, & Tarman Kustiyariah. (2024). Off-odour Identification from Volatile Organic Compounds (VOCs) of Spirulina. *BIO Web of Conferences*, *92*, 02006-. https://doi.org/10.1051/bioconf/20249202006

Rabitti, N. S., Bayudan, S. R., Laureati, M., Neugart, S., Schouteten, J., Apelman, L., Dahlstedt, S., & Sandvik, P. (2024). Snacks from the sea : A cross-national comparison of consumer acceptance for crackers added with algae. *EUROPEAN FOOD RESEARCH AND TECHNOLOGY*, *250*(8), Article 8. https://doi.org/10.1007/s00217-024-04530-y

Raczyk, M., Polanowska, K., Kruszewski, B., Grygier, A., & Michałowska, D. (2022a). Effect of Spirulina (Arthrospira platensis) Supplementation on Physical and Chemical Properties of Semolina (Triticum durum) Based Fresh Pasta. *Molecules*, *27*(2), 355. https://doi.org/10.3390/molecules27020355

Raczyk, M., Polanowska, K., Kruszewski, B., Grygier, A., & Michałowska, D. (2022b). Effect of Spirulina (Arthrospira platensis) Supplementation on Physical and Chemical Properties of Semolina (Triticum durum) Based Fresh Pasta. *Molecules*, *27*(2), Article 2. https://doi.org/10.3390/molecules27020355

Ramírez-Rodrigues, M. M., Estrada-Beristain, C., Metri-Ojeda, J., Pérez-Alva, A., & Baigts-Allende, D. K. (2021). Spirulina platensis Protein as Sustainable Ingredient for Nutritional Food Products Development. *Sustainability*, *13*(12), 6849-. https://doi.org/10.3390/su13126849

Rezvani, Z., Mortezapour, H., Ameri, M., Akhavan, H.-R., & Arslan, S. (2022). Drying of Spirulina with a continuous infrared-assisted refractance window$^{TM}$ dryer equipped with a photovoltaic-thermal solar collector. *Heat and Mass Transfer*, *58*(10), 1739-1755. https://doi.org/10.1007/s00231-022-03210-5

Rodríguez De Marco, E., Steffolani, M. E., Martínez, C. S., & León, A. E. (2014). Effects of spirulina biomass on the technological and nutritional quality of bread wheat pasta. *LWT - Food Science and Technology*, *58*(1), 102-108. https://doi.org/10.1016/j.lwt.2014.02.054

Smith, K., Watson, A. W., Lonnie, M., Peeters, W. M., Oonincx, D., Tsoutsoura, N., Simon-Miquel, G., Szepe, K., Cochetel, N., Pearson, A. G., Witard, O. C., Salter, A. M., Bennett, M., & Corfe, B. M. (2024). Meeting the global protein supply requirements of a growing and ageing population. *European Journal of Nutrition*, *63*(5), 1425-1433. https://doi.org/10.1007/s00394-024-03358-2

Spanoghe, J., Vermeir, P., & Vlaeminck, S. E. (2021). Microbial food from light, carbon dioxide and hydrogen gas : Kinetic, stoichiometric and nutritional potential of three purple bacteria. *Bioresource Technology*, *337*, 125364. https://doi.org/10.1016/j.biortech.2021.125364

Stunda-Zujeva, A., & Berele, M. (2024). Algae as a Functional Food : A Case Study on Spirulina. In A. Abomohra & S. Ende (Éds.), *Value-added Products from Algae : Phycochemical Production and Applications* (p. 563-594). Springer International Publishing. https://doi.org/10.1007/978-3-031-42026-9_17

Twarogowska, A., Van Droogenbroeck, B., & Fraeye, I. (s. d.). *Application of Belgian endive (Cichorium intybus var. Foliosum) dietary fiber concentrate to improve nutritional value and functional properties of plant-based burgers*. https://doi.org/10.1016/j.fbio.2022.101825

Ughetti, A., D'Eusanio, V., Strani, L., Russo, A. L., & Roncaglia, F. (2024). Influence of Drying and Storage Conditions on the Volatile Organic Compounds Profile of Spirulina Platensis. *Separations*, *11*(6), Article 6. https://doi.org/10.3390/separations11060180

Vu, G., Zhou, H., & McClements, D. J. (2022). Impact of cooking method on properties of beef and plant-based burgers : Appearance, texture, thermal properties, and shrinkage. *Journal of Agriculture and Food Research*, *9*, 100355. https://doi.org/10.1016/j.jafr.2022.100355

Wang, M., Yin, Z., Sun, W., Zhong, Q., Zhang, Y., & Zeng, M. (2023). Microalgae play a structuring role in food : Effect of *spirulina platensis* on the rheological, gelling characteristics, and mechanical properties of soy protein isolate hydrogel. *Food Hydrocolloids*, *136*, 108244. https://doi.org/10.1016/j.foodhyd.2022.108244

Zhao, Y., Li, K., Zhang, X., Zhang, T., Zhao, J., Jiang, L., & Sui, X. (2024). Protein blend extrusion : Crafting meat analogues with varied textural structures and characteristics. *Food Chemistry*, *460*, 140709. https://doi.org/10.1016/j.foodchem.2024.140709

Zhu, J., Xiao, X., Du, W., Cai, Y., Yang, Z., Yin, Y., Wakisaka, M., Wang, J., Zhou, Z., Liu, D., & Fang, W. (2024). Leveraging microalgae as a sustainable ingredient for meat analogues. *Food Chemistry*, *450*, 139360. https://doi.org/10.1016/j.foodchem.2024.139360

Zouari, N., Abid, M., Fakhfakh, N., Ayadi, M. A., Zorgui, L., Ayadi, M., & Attia, H. (2011). Blue-green algae ( *Arthrospira platensis* ) as an ingredient in pasta : Free radical scavenging activity, sensory and cooking characteristics evaluation. *International Journal of Food Sciences and Nutrition*, *62*(8), 811-813. https://doi.org/10.3109/09637486.2011.582461

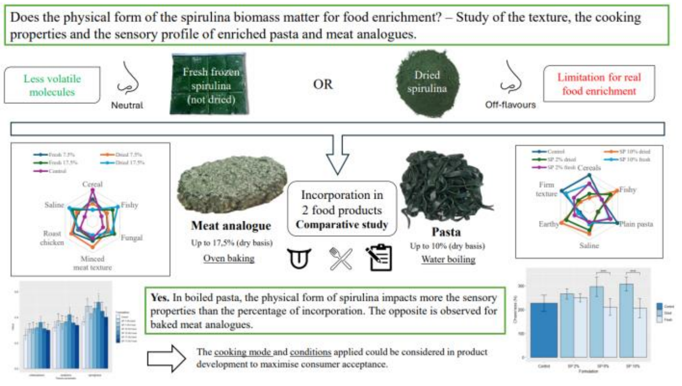
# Ethical statement

- This study does not involve animal study.

- A few humans have eaten products containing spirulina (sensory description of food products).

- Appropriate protocols for protecting the rights and privacy of all participants were utilized during the execution of the research.
- Full disclosure of study requirements and risks have been done verbally.
- All participants were consents (on a voluntary basis).
- No release of participant data has been made without their agreement.
- Data were treated anonymously.
- Ability to withdraw from the study at any time was respected.

## Graphical abstract



## Declaration of interests

☒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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: