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Effect of Type of Flour and Microalgae (*Chlorella vulgaris*) on the Rheological, Microstructural, Textural, and Sensory Properties of Vegan Muffins

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Abstract: The aim of this study was to develop a recipe for vegan muffins using wheat flour (100%) and a blend of whole-grain spelt flour (50%) and wheat flour (50%) enriched with microalgae (0, 0.5, 1.0, and 1.5% (g/100 g flour)). Replacing wheat flour with whole-grain spelt flour and adding microalgae while eliminating egg white from a recipe can affect the rheological properties of the dough and also the microstructure and texture of the muffins. The study analyzed the effects of the type of flour and the addition of microalgae on the rheological properties of the raw dough, measured through the oscillatory method, as well as the texture and microstructure, determined via X-ray microtomography. Additionally, the sensorial quality of the muffins was analyzed. The use of spelt flour in the formulation of muffins affected the rheological properties of the dough irrespective of the addition of microalgae. The dough made with spelt flour exhibited higher viscosity (consistency coefficient (K) was 74.7 $Pa \cdot s^n$), but it was more cohesive and less springy compared to the dough made with wheat flour alone, which had a K = 58.3 $Pa \cdot s^n$. Incorporating a mixture of spelt and wheat flour along with a 1.5% addition of microalgae made the dough more viscous (K = 118.6 $Pa \cdot s^n$), leading to a fine, porous microstructure (porosity was 69.7%) and a crumbly texture (hardness was 52.2 N) in the muffins. On the other hand, the wheat flour dough with 1.5% microalgae had a consistency coefficient of 69.3 $Pa \cdot s^n$, while the muffin porosity was 67.1% and the hardness 61.8 N. The microstructure had a strong effect on the texture of the muffin crumb. The new wheat flour products with microalgae exhibited a higher proportion of closed pores in their microstructure, whereas samples containing spelt flour and microalgae showed the opposite trend, with more open pores. The greatest difference in closed pores was observed with the addition of 1.5% of microalgae (33.4% in wheat muffins and 26.9% in spelled muffins). The presence of closed porosity contributed to the harder and less consistent texture observed in the muffins. However, despite the instrumental evaluation results, all the new products were accepted by consumers in terms of appearance, taste, and overall quality.

Keywords: microalgae; texture; color; microtomography; spelt flour; wheat flour

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1. Introduction

By 2050, according to the UN, the world's population is projected to have risen from 7 to 9.7 billion. To sustain this growing population, food production must be increased by 60% while also shaping awareness about nutrition, promoting balanced and healthy diets. Scientists are actively seeking alternatives to full-fledged animal protein that can be produced efficiently, quickly, and cost-effectively while placing a low burden on the planet [1].

Microalgae have gained recognition as a "superfood" and hold the potential to become a valuable source of supermaterials in the future, enhancing both the nutritional and functional quality of food [2–5]. Gong et al. [5], based on Spirulina cells, developed a hybrid forming strategy for mass production of drugs and explored their therapeutic efficacy for cancer cells. The commonly used microalgae varieties are Spirulina and Chlorella. The annual production is about 7500 tons of dry biomass of Spirulina and Chlorella microalgae (5000 and 2500 tons, respectively) [5]. Both Spirulina (arthrospira) and Chlorella (vulgaris) exhibit high contents of complete protein (more than 50% dry weight), encompassing all essential amino acids, including endogenous and exogenous ones, pigments, long chain polyunsaturated fatty acids, sterols, and other compounds [6]. They also contain vitamins A, E, C, and B-group vitamins. This protein is characterized by a high biological value [3,6]. *Chlorella vulgaris* green is characterized by a relatively low fat content (5%) and a high total mineral content of 24% and is rich in calcium (4.7%) as well as in manganese and iron [2]. The literature describes the effects of adding microalgae biomass, such as Spirulina arthrospira, *Chlorella vulgaris*, *Tetraselmis suecica*, and *Phaeodactylum tricornutum*, at 2 and 6% (g/100 g) on the physicochemical and sensory properties and antioxidant activity of cakes and the in vitro digestibility of microalgae biomass and cakes [7,8]. Microalgae biomass has also been used as an alternative ingredient in snacks [9,10], pasta [11], and bread [12].

Since microalgae belong to the plant kingdom, they can be consumed by people following a vegan diet. Proteins are an important nutrient, and in a vegan diet, it is necessary to ensure the supply of complete proteins and B vitamins, especially B_{12} [13]. Scientific articles previously claimed that microalgae are the only plant-based source of vitamin B_{12}. However, about 64% of the vitamin B_{12} in Spirulina arthrospira algae biomass consists of inactive analogs that cannot be absorbed by humans. In contrast, research by Merchant et al. [13] suggests that *Chlorella* sp. algae contain a form of vitamin B_{12} that is assimilable by humans, making them potentially the only plant source. Furthermore, bioactive constituents found in algae exhibit antibacterial, antiviral, anticancer, anti-inflammatory, analgesic, and antioxidant properties [6].

Muffins are highly favored by consumers due to their versatility and the ease with which they can be modified to create a wide variety of muffin flavors, from sweet to savory. They can also be a very good carrier of nutritional or health-promoting ingredients. Unlike many other snack products, muffins possess a notable nutritional value, which contributes to their popularity. However, it is important to remember that muffin dough is a complex mixture comprising interacting ingredients such as sugar, fat, flour, eggs, and baking powder. Additional common ingredients include emulsifiers, preservatives, and milk powder [14–17]. Typically, muffins exhibit a porous structure and significant volume. This structure is achieved by incorporating foam stabilizers, such as eggs, egg whites, and—to a lesser extent—milk proteins, which slow down the coalescence of air bubbles. Fats and oils are used to create a moist texture and prevent a dry mouthfeel [14,15]. When preparing vegan muffins, ingredients like flour, sugar, oil, vegetable beverage, raising agents, and salt can be used as substitutes [17].

Wheat (*Triticum aestivum* L.) grains are rich in valuable nutrients of carbohydrates, protein, dietary fiber, and fat as well as minerals (including P, K, Ca, and Mg), B vitamins, vitamin E, and several antioxidant compounds (such as phenolic acids and carotenoids) [18].

Replacing wheat flour in baked goods poses a significant technological challenge due to the essential role of gluten, a structural protein that contributes to the appearance and crumb structure of many baked goods. The gluten matrix also plays a crucial role in determining the rheological characteristics of dough [19].

Spelt (*Triticum aestivum* var. *spelta*) is an ancient subspecies of common wheat that possesses slightly different technological properties compared to common wheat. Spelt is characterized by a higher nutrient content. It contains more protein with a more favorable amino acid composition than common wheat, including increased levels of essential amino acids, such as lysine, leucine, and isoleucine. Spelt also exhibits higher digestibility. Additionally, it contains a higher proportion of total fat, including unsaturated fatty acids such as oleic and linoleic acid. Spelt grain has a higher concentration of fat-soluble and B vitamins compared to common wheat. The abundant presence of total ash in spelt grain indicates a rich mineral content. Iron, zinc, copper, magnesium, potassium, selenium, and other minerals have been found to be more abundant in spelt grain compared to common wheat [20,21]. Wheat and cereals, including spelt, are the most important sources of dietary fiber in the human diet. The main components of dietary fiber in spelt is arabinoxylan [21]. Sinkovič et al. [21] showed that the total arabinoxylan content in spelt flour ranged from 11.4–16.0%, and that in whole-meal spelt flour ranged from 29.9–37.3%. The higher fiber content of spelt is expected to have an adverse effect on dough rheology. The compounds that make up dietary fiber have different binding capacities for water [22]. From a technological standpoint, processing spelt dough can be challenging due to its softness and stickiness after kneading [19].

Rheological, structural, and textural problems are commonly observed in bakery products that do not contain animal-derived raw materials, such as eggs and milk. Therefore, there is a continuous search for additives that can enhance the nutritional value of vegan products while providing them with the right structure, texture, and sensory characteristics. Enriching raw materials often have to be limited, as they significantly degrade the quality of the dough and the microstructure and texture of the final product. Our study may be important in expanding knowledge regarding dough rheological properties, color, microstructure, and texture of vegan muffins. To the best of our knowledge, the 3D microstructure of such products and its impact on texture is not yet described in the literature. The aim of this study was to evaluate the effect of the type of flour and the addition of microalgae on the rheologic properties of the dough, as well as on the microstructure, texture, and sensorial evaluation of muffins made without animal raw materials.

2. Materials and Methods

2.1. Materials

The study material included raw doughs of vegan muffins prepared using wheat flour type 450 (100%—referred to as sample CW) and a blend of whole-grain spelt flour type 2000 (50%) and wheat type 450 (50%) (referred to as sample SW). Microalgae C. vulgaris powder (MyVita, Proness, Legnica, Poland) was added to the dough at three different concentrations: 0.5, 1.0, and 1.5% (g/100 g flour). In samples marked CW, CWa0.5, CWa1.0, and CWa1.5, the base was wheat flour, while in SW, SWa0.5, SWa1.0, and SWa1.5, the base was a blend of whole-grain spelt flour (50%) and wheat flour (50%). The amounts of all ingredients in the dough recipe were expressed in % (g/100 g flour) and were the same in all samples: 93.3% of soy beverage (OraSi, Unigra S. r. I., Poland), 44.4% of sucrose (White Sugar, Sudzucker, Wrocław, Poland), 28.9% of rapeseed oil (Wielkopolski, EOL Poland), 1.8% of baking powder (sodium bicarbonate, Dr. Oetker, Bielefeld, Germany), 0.5% of soy lecithin (Młyn Oliwski, Gdańsk, Poland), and 0.4% iodized table salt (Cenos Sp. zo.o., Września, Poland). For the preparation of muffin dough, flours from domestic manufacturers were used: wheat flour type 450 (Lubella, Lublin, Poland) and whole-grain spelt flour type 2000 (Młyn Niedźwiady, Niedźwiady, Poland).

The wheat flour type 450 provided by the manufacturer contained 74 g/100 g of carbohydrates, 10 g/100 g of protein, 3.1 g/100 g of fiber, 1.1 g/100 g of fat, and 0.03 g/100 g of salt.

The whole-grain spelt flour type 2000 contained 59 g/100 g of carbohydrates, 13 g/100 g of protein, 13 g/100 g of fiber, 2.3 g/100 g of fat, and <0.01 g/100 g of salt.

Wheat flour type 450 contained water 14 g/100 g and was re-dried to have the same water content as whole-grain spelt flour. The water content was determined gravimetrically by drying the flour at 105 °C. Water contents of the wheat and whole-grain spelt flours were 10 $\pm$ 1.1 g/100 g and 11 $\pm$ 0.6 g/100 g, respectively, and were not statistically significantly different.

2.2. Dough Preparation and Baking

The dough was prepared using a Kitchen Aid Artisan 5 robot (USA) with the mixing speed set to "1" as indicated on the machine's scale. Precise measurements were taken using an analytical balance model PS 600/C/2 (Radwag, Poland).

The first step involved mixing the dry ingredients flour, sugar, baking powder, soy lecithin, salt, and microalgae for 1.5 min. Next, the liquid ingredients, namely soy beverage and rapeseed oil, were mixed for an additional 1.5 min. Then, the liquid ingredients were combined with the solid ingredients and mixed for another 1.5 min. Throughout the dough preparation process, the laboratory maintained a temperature of 21 °C. The dough was divided into 50 g portions and placed in paper molds (La Cucina, Wan Chai, Hong Kong), which were then arranged in a metal mold designed for baking muffins. Baking took place in an electric oven (Amica, Poland) at a temperature of 180 °C for 25 min. After baking, the muffins were cooled to room temperature, wrapped in polyethylene film, and stored at 21 °C for 24 h to allow the moisture content to equalize.

2.3. Rheological Properties of Dough

The rheological properties of the dough were tested using a Haake Mars 40 oscillating rheometer (Thermo Scientific, Karlsruhe, Germany). A plate-to-plate measuring system with a diameter of 35 mm (equipped with serrations to prevent spillage) and a measuring gap of 1 mm were employed. Dough samples were tested immediately after preparation to minimize changes in the dough's characteristics. Duplicate tests were conducted for each type of dough.

Constant shear tests were carried out in controlled rate mode, with a linearly increasing shear rate ranging from 1 to 100/s. The resulting experimental flow curves, depicting shear stress vs. shear rate, were compared using the Ostwald–de Waele equation: $\eta = K\gamma^{n-1}$ (where η represents the apparent viscosity, K is the consistency coefficient ($Pa \cdot s^n$), γ denotes the shear rate (s^{-1}), and n signifies the flow rate. For shear-thinning fluids, n < 1, while for Newtonian fluids, n = 1 [14,15].

Two different dynamic rheological oscillation tests were conducted. The first was an amplitude sweep, in which the strain was varied from 0.1 to 100% to identify the linear viscoelastic region of the dough. The second test was a frequency sweep, which involved varying the frequency from 0.1 to 10 Hz while maintaining a constant strain of 1% at a temperature of 20 °C.

The mechanical spectra of the tested doughs were determined, and the changes in elastic moduli (G'), viscous moduli (G''), and loss angle tan(δ) were determined. These values were evaluated across an oscillation frequency range spanning from 0.1 to 100 Hz.

2.4. Water Content and Activity, Muffin Crumb Color

The muffins were cut in half, and from the center, pieces of crumb were taken to determine the water content and water activity.

The water content of the muffin samples was determined by measuring the weight loss during drying at 105 °C for 3 h until a constant weight was achieved.

The water activity of the muffins was tested using an Acqualab instrument (DECAGON DEVICES. Inc., Pullman, WA, USA).

To evaluate the crumb color of the muffins, they were cut transversely at a height of 2.5 cm. The color measurements were conducted using a CR-300 colorimeter (Konica Minolta, Japan) based on the CIELab color system. The color parameters were determined by averaging the results from 10 replicates. The obtained color parameters were then used to calculate the color chroma, $C^* = ((a^*)^2 + (b^*)^2)^{0.5}$, and hue angle, $h_{ab} = \arctan(b^*/a^*)$, where C* is the chroma, a* is the contribution of red color, b* is the contribution of yellow color, and h_{ab} is the hue.

2.5. 2D and 3D Microstructure Examination of Muffin Crumb

The microstructure of the muffin was measured using a SkyScan 1272 microtomograph, specifically a micro-CT system (Brucker MicroCT, Kontich, Belgium). The scan was performed on specimens measuring 25 × 25 × 25 mm, and the scanning parameters and processing methods described in the literature [23] were followed. The resulting images had a pixel size of 20 μm.

2.6. Muffin Crumb Texture Examination

The textural properties of the muffins were tested using a TA-T2i Texture Analyser (Stable Micro System, Godalming, UK). The muffins were cut transversely at a height of 25 mm, and a profile texture analysis (TPA) test was conducted.

During the TPA test, the samples were compressed until they reached 50% deformation at a speed of 1 mm/s. There was a 5 s interval between the first and second compression. A piston with a diameter of 75 mm was used for the analysis. A total of ten samples for each type of muffin were tested to ensure reliable and representative results.

2.7. Sensorial Evaluation

For the evaluation, a group of 20 individuals who had undergone prior training participated. Among the respondents, 85% were women and 15% were men. The age distribution of the respondents was as follows: 95% were in the 18–25 age range, while 5% were in the 26–30 age range.

The participants were asked to rate various quality characteristics on a scale ranging from 1 (undesirable trait) to 5 (highly desirable trait). The quality characteristics assessed were as follows: external appearance, taste, smell, crumb structure and texture, and overall desirability.

2.8. Statistical Analysis

The obtained results were analyzed using Statistica 13.1 (StatSoft, Krakow, Poland). Analysis of variance was employed to evaluate the effect of both the type of flour and the addition of microalgae as well as to examine the differences between the samples. To determine significant differences between groups, the least significant differences were calculated using Duncan's test. The level of significance was set at $\alpha < 0.05$.

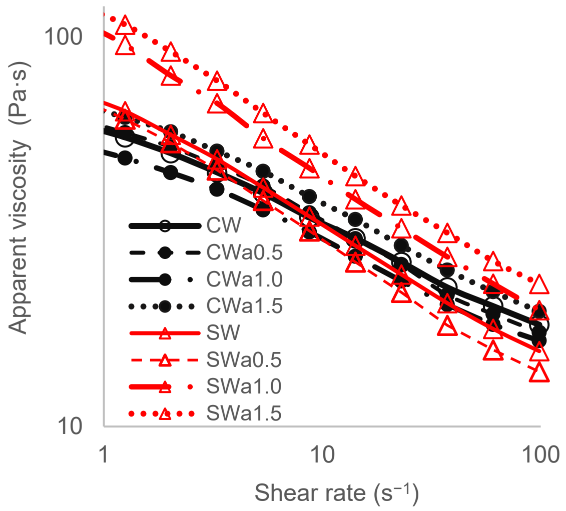
3. Results

3.1. Rheological Properties of Dough

The values of apparent viscosity as a function of shear rate, as well as the consistency coefficient and flow index, are shown in Figure 1. All of the tested raw doughs for vegan muffins exhibited shear thinning behavior, characterized by high viscosity. This finding aligns with previous studies by many authors [14–17].

High dough viscosity can favorably influence baking porosity and texture. Viscosity is a factor that controls the final porosity of the baked product due to its effect on the incorporation and movement of gas bubbles. Our research shows that the higher the viscosity of the dough (Figure 1a), the more pores and larger porosity the muffins had (Table 3). On the other hand, the pores had a smaller structure thickness, and the muffin texture was very soft and delicate (Table 4). The rate at which gas bubbles rise under buoyant force is inversely proportional to viscosity. Increased dough viscosity helps retain gas bubbles within the dough, contributing to greater stability throughout the baking process [14]. Our observations, however, show that too high a dough viscosity is not desirable; it creates a large number of pores but lower structure thickness, and this does not favor the texture.

Within the studied range of shear rates (1–100 s^{-1}), the Ostwald–de Waele power equation provided a good fit (r^2 ranging from 0.996 to 0.999) to describe the relationship. The consistency coefficient (K) of the dough made with wheat flour (CW) was 58.3 $Pa \cdot s^n$, significantly lower than that of the dough made with a mixture of spelt and wheat flour (SW), which had a K value of 74.7 $Pa \cdot s^n$ (Figure 1). The addition of 1.5% microalgae (CWa1.5) resulted in an approximately 16% increase in the consistency coefficient compared to the CW sample. Similarly, the doughs based on the mixture of spelt and wheat flours with 1 and 1.5% microalgae additions (SWa1.0 and SWa1.5) exhibited significantly higher K values compared to the microalgae-free dough (SW), with increases of 25 and 37%, respectively. The flow index (n) was lower in the dough containing spelt flour, indicating higher viscosity compared to the dough made with wheat flour (Figure 1). Several studies have demonstrated that dough viscosity is influenced by the type and quantity of ingredients used [14,15]. The higher fiber content in spelt flour may have contributed to increased water binding and subsequently led to a higher consistency coefficient and a lower flow index. Additionally, the microalgae, known for their gelling properties [2,6], likely enhanced the dough structure when combined with spelt flour, resulting in higher K values and lower n values.



(**a**)

Dought type	K (Pa·s^n)	n (-)
CW	58.34 ± 1.34 ^{a}	0.746 ± 0.000 ^{c}
CWa0.5	59.12 ± 3.68 ^{a}	0.738 ± 0.008 ^{c}
CWa1.0	52.34 ± 0.56 ^{a}	0.751 ± 0.001 ^{c}
CWa1.5	69.73 ± 3.70 ^{bc}	0.740 ± 0.000 ^{c}
SW	74.69 ± 6.51 ^{c}	0.662 ± 0.014 ^{b}
SWa0.5	61.95 ± 7.45 ^{ab}	0.665 ± 0.018 ^{b}
SWa1.0	99.75 ± 0.11 ^{d}	0.666 ± 0.008 ^{b}
SWa1.5	118.60 ± 2.26 ^{e}	0.629 ± 0.005 ^{a}

(**b**)

Figure 1. (**a**) Flow curves of raw doughs. (**b**) The average value of consistency coefficient (K) and flow index (n) of raw dough. Explanations: CW—wheat flour (100%), SW—whole-grain spelt flour (50%) and wheat flour (50%), microalgae addition: a0.5, a1.0, and a1.5%. a, b, c, d, e—homogeneous groups, $p < 0.05$.

The rheological studies conducted on raw doughs provide valuable insights into the relationship between rheological parameters and dough composition and structure. Determining the ratio between elastic and viscous characteristics of the dough allows pastry and baking technologists to effectively model dough properties and achieve optimal results during reformulation and new product development [14,15,17].

Figure 2 presents the mechanical spectra of the raw doughs. All tested doughs exhibited elastic characteristics, with an increase in the values of the elastic modulus (G′) and viscous modulus (G″) as the sweep frequency increased. They displayed the typical behavior of soft gels, with G′ values higher than G″. The tgδ values, representing the loss angle, were lower than unity for all samples, indicating the weakly fluid nature of the doughs tested.

When formulating the dough, the inclusion of 50% whole-grain spelt flour (sample SW) resulted in a decrease in both G′ and G″ compared to the dough based on wheat flour (CW). This outcome aligns with expectations based on the available literature. Spelt flour contains significantly more fiber, which may lead to damage to the gluten network [17]. Although spelt flour has a higher protein content, it contains less gluten compared to wheat flour, resulting in a stronger and more elastic dough with better baking properties [20]. Pruska-Kędzior et al. [19] suggested that gluten proteins in spelt are less elastic and stretchier compared to those in common wheat proteins. The addition of microalgae influenced the viscoelastic behavior of the dough. Doughs made from a mixture of whole-grain spelt and wheat flour with microalgae exhibited higher G′ and G″ compared to doughs made solely from wheat flour (Figure 2). The most significant changes in rheological properties were observed with 1 and 1.5% microalgae additions. Sanz et al. [15] argued that increased elasticity reflects greater structural complexity in the doughs. However, the alteration of dough rheological properties is not solely attributable to the effect of microalgae on gluten. The muffin dough consists of ingredients mixed in a way that causes aeration (adding bubbles to the mix). The formation of fine bubbles provides nucleation sites for the CO_2 generated by the baking powder after starch gelatinization and protein denaturation during baking. This causes a porous crumb to form. The number and size of the bubbles depend on the time of mixing and the viscosity of the dough. Too high a viscosity may cause poor bubble retention [22]. The present study expands our knowledge of the rheological properties of doughs made from wheat flour and a mixture of whole-grain spelt and wheat flour with microalgae. It demonstrated that the modulus values increase nonlinearly with increasing amounts of microalgae in the dough. Furthermore, the combination of microalgae with whole-grain spelt flour creates a dough with enhanced stability, elasticity, and extensibility compared to a wheat flour dough with microalgae (Figure 2b). This finding is not easily explained, as the rheological properties of spelt gluten are predominantly influenced by gliadins, whereas those of common wheat gluten are primarily affected by glutenins [19]. The higher fiber content in whole-grain flour also affects the acidity of the dough matrix as well as the water-binding and rheological characteristics of the dough. Interactions between proteins, fiber, fat, and sucrose contribute to the overall rheological properties of the dough.

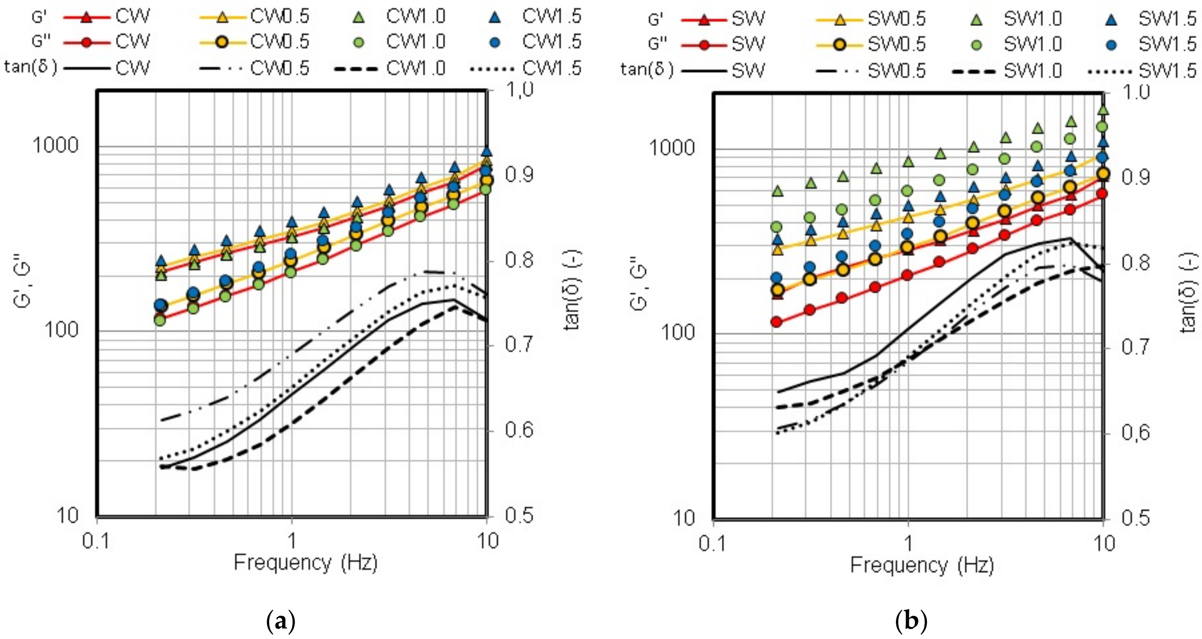


Figure 2. Mechanical spectra of raw doughs based on (**a**) wheat flour (CW) and (**b**) a mixture of whole-grain spelt flour (50%) and wheat flour (50%) (SW). Explanations: microalgae addition: a0.5, a1.0, and a1.5%, G′—preservation modulus, G″—loss modulus, and tgδ—tangent of phase lag angle.

3.2. Water Content, Water Activity (a_w), and Muffin Crumb Color

The replacement of 50% wheat flour with spelt flour did not significantly affect the moisture content of the muffins (p = 0.059) (Table 1). The addition of microalgae led to a significant reduction in moisture content across the samples (p < 0.001). Specifically, the addition of 0.5% microalgae resulted in a decrease in moisture content of about 1 p.p. (percentage point), and 1.5% microalgae reduced the moisture content by about 3 p.p. compared to samples without microalgae (Table 1). Similar results were observed in muffins enriched with brewer's spent grain flours, and grape pomace [17,24].

Table 1. Water content and activity (each value is presented as mean ± SD; a, b, c, d, e, f—homogenous group, $\alpha < 0.05$).

Muffin Type	Water Content (%)		Water Activity (-)	
CW	25.68 ± 0.93^{d}		0.889 ± 0.005^{cd}	
CWa0.5	24.40 ± 0.09^{c}		0.875 ± 0.013^{abc}	
CWa1.0	23.99 ± 0.21^{c}		0.860 ± 0.022^{ab}	
CWa1.5	22.51 ± 0.23^{a}		0.845 ± 0.013^{a}	
SW	25.48 ± 0.30^{d}		0.888 ± 0.003^{cd}	
SWa0.5	24.25 ± 0.19^{c}		0.895 ± 0.003^{de}	
SWa1.0	23.20 ± 0.26^{b}		0.908 ± 0.004^{e}	
SWa1.5	22.53 ± 0.35^{a}		0.923 ± 0.003^{f}	
ANOVA				
Factor	F	*p*	F	*p*
Flour type (X)	3.9	0.059	83.1	<0.001
Microalgae addition (Y)	81.8	<0.001	0.2	0.881
X × Y	1.5	0.231	19.0	<0.001

The water activity (a_w) of the muffins was influenced by the type of flour and the interaction between flour type and microalgae addition (Table 1). Muffins made with spelt flour and microalgae exhibited higher water activity compared to samples made with wheat flour and microalgae ($p < 0.001$). The inclusion of 1 and 1.5% microalgae had the effect of decreasing aw in wheat flour muffins, while it increased aw in samples containing both spelt and wheat flour (Table 1). The interactions between muffin ingredients can affect water binding and, consequently, contribute to variations in water activity. Despite similar water content, the differences in water activity observed may be attributed to the interactions between ingredients and the competition between biopolymers for water. Whole-grain flour, although containing more fiber, has fewer carbohydrates (starch) compared to wheat flour.

Color is an important parameter for muffins as it directly impacts the acceptability of the product and is influenced by the type of raw materials used. Table 2 presents various color parameters, including L* (brightness), a* (redness), b* (yellowing), C* (saturation), and hab (color tone). Both the use of whole spelt flour ($p < 0.001$) and the addition of microalgae ($p < 0.001$) significantly influence the L* value of the muffin (Table 2). Whole-grain spelt flour leads to a darker color of the muffin, as indicated by the lower L* value (Table 2). This dark color is attributed to the high concentration of relatively colorless polyphenolic compounds present in whole-grain spelt flour, such as flavan-3-ols, amino-phenolic compounds, benzoic and cinnamic acid derivatives, and proanthocyanidines [25]. In addition, the high fiber content of the spelt flour used may contribute to the darker color of the crumb, which aligns with findings from studies on muffins based on barnyard millet flour [26].

The addition of microalgae also leads to a darker color in the muffins. The green pigment present in microalgae significantly alters the a* and b* parameters, consequently affecting the C* (saturation) and hab (color tone) of the muffin. Color tone represents the dimension of color and describes the specific shade, ranging from red (0°) to yellow (90°), green (180°), blue (270°), and back to red (360°). A hab value above 90° indicates a yellower hue, while higher values suggest less yellow and more green [24]. Muffins made with wheat flour but without microalgae exhibited a yellow color, whereas the use of whole-grain spelt flour resulted in a change in color tone (259°), indicating a green–blue hue. Samples with added microalgae showed hab angles ranging from 95° to 109°, indicating a green color for the muffins (Table 2). Previous studies utilizing microalgae (A. platensis) in cakes have reported similar effects [7].

Table 2. Color parameters of vegan muffins with added microalgae (each value is presented as mean ± SD; a, b, c, d, e, f, g, h—homogenous group, $\alpha < 0.05$).

Muffin Type	L*	a*	b*	C*	h_{ab} (°)
CW	70.20 ± 1.74 ^{g}	−0.66 ± 0.22 ^{g}	19.03 ± 0.62 ^{e}	19.04 ± 0.62 ^{de}	91.95 ± 0.68 ^{a}
CW0.5	59.91 ± 1.90 ^{e}	−5.04 ± 0.35 ^{c}	18.73 ± 0.95 ^{de}	19.40 ± 0.91 ^{e}	105.07 ± 1.34 ^{e}
CW1.0	55.36 ± 0.93 ^{c}	−5.91 ± 0.33 ^{b}	18.13 ± 0.60 ^{bc}	19.07 ± 0.65 ^{de}	108.02 ± 0.69 ^{f}
CW1.5	51.46 ± 1.14 ^{b}	−6.22 ± 0.41 ^{a}	17.82 ± 0.62 ^{c}	18.88 ± 0.70 ^{d}	109.18 ± 0.78 ^{g}
SW	63.01 ± 1.75 ^{f}	3.54 ± 0.40 ^{h}	18.56 ± 0.57 ^{d}	18.90 ± 0.59 ^{d}	259.24 ± 1.11 ^{h}
SW0.5	57.26 ± 1.71 ^{d}	−1.60 ± 0.36 ^{f}	18.16 ± 0.44 ^{c}	18.24 ± 0.45 ^{c}	94.99 ± 1.09 ^{b}
SW1.0	52.13 ± 1.08 ^{b}	−2.98 ± 0.30 ^{e}	17.50 ± 0.30 ^{b}	17.75 ± 0.31 ^{b}	99.61 ± 0.94 ^{c}
SW1.5	49.57 ± 1.05 ^{a}	−3.99 ± 0.22 ^{d}	16.81 ± 0.70 ^{a}	17.28 ± 0.69 ^{a}	103.34 ± 0.81 ^{d}

ANOVA

Factor	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*
Flour type (X)	263.1	<0.001	3637.5	<0.001	45.5	<0.001	110.0	<0.001	55819	<0.001
Microalgae addition (Y)	919.5	<0.001	3131.0	<0.001	44.2	<0.001	16.2	<0.001	57274	<0.001
X × Y	26.2	<0.001	60.8	<0.001	1.5	0.227	10.0	<0.001	84054	<0.001

3.3. Two- and Three-Dimensional Microstructure of Muffin Crumb

High-quality muffins are characterized by a light, porous structure and high volume [26]. Figure 3 displays images obtained through X-ray microtomography, revealing the microstructure of the samples. All samples exhibited a highly porous microstructure. Wheat muffins exhibited a higher prevalence of closed pores, while open pores were more prominent in spelt muffins (Table 3). The average surface area of samples made with wheat flour was larger compared to those made with spelt flour. This variation in microstructure was likely influenced by the lower viscosity of the wheat dough.

The structure of muffins is not solely dependent on the number of pores but can also be influenced by the distribution of pore sizes [23]. The distribution of pore areas in the microstructure of wheat flour (CW) muffins exhibited the greatest variability (Figure 4). CW samples had 30% of pores with an area ranging from 0.1 to 0.2 mm^2, 54% in the range of 0.2 to 0.3 mm^2, and 15% >0.3 mm^2. In contrast, muffins made with spelt and wheat flour (SW) displayed finer pores in their microstructure: 16% with an area <0.1 mm^2, 54% in the range of 0.1–0.2 mm^2, and 27% in the range of 0.2–0.3 mm^2, with no pores >0.3 mm^2. The inclusion of 1% microalgae in wheat flour muffins (CWa1.0) resulted in the most uniform microstructure, with 70% of the pores falling within the 0.1–0.2 mm^2 range. Both wheat (CWa1.5) and spelt (SWa1.5) muffins with 1.5% microalgae exhibited approximately 50% of pores with an area of 0.1–0.2 mm^2, and 42 and 28% with an area of 0.2–0.3 mm^2, respectively. In our study, the addition of microalgae led to a more homogeneous microstructure, possibly due to the increased viscosity of the dough.

Table 3. The microstructure 2D and 3D vegan muffins (each value is presented as mean ± SD; a, b, c, d, e—homogenous group, $\alpha < 0.05$.

Muffin Type	2D Microstructure (n = 541)			3D Microstructure (n = 2)				
	Closed Porosity (%)	Open Porosity (%)	Average Pores Area (Mm^2)	Total Porosity (%)	Percent Object Volume (%)	Number of Pores	Structure Mode Index (-)	Structure Thickness (Mm)
CW	33.84 ± 6.59 ^{cd}	44.67 ± 10.24 ^{b}	0.24 ± 0.06 ^{g}	64.34 ^{b}	35.66 ^{b}	3036 ^{a}	−4.33	0.122 ^{c}
CWa0.5	37.17 ± 6.13 ^{e}	39.56 ± 8.46 ^{a}	0.16 ± 0.06 ^{d}	62.95 ^{a}	37.05 ^{b}	3955 ^{b}	−3.71	0.108 ^{a}
CWa1.0	37.48 ± 7.81 ^{e}	38.86 ± 10.04 ^{a}	0.13 ± 0.04 ^{b}	63.00 ^{a}	37.00 ^{b}	5234 ^{d}	−3.71	0.102 ^{a}
CWa1.5	33.41 ± 6.19 ^{c}	48.98 ± 11.42 ^{c}	0.20 ± 0.06 ^{f}	67.08 ^{c}	32.92 ^{a}	3376 ^{a}	−4.36	0.118 ^{c}
SW	28.14 ± 6.19 ^{b}	56.43 ± 11.04 ^{e}	0.17 ± 0.07 ^{e}	69.45 ^{d}	30.55 ^{a}	3407 ^{a}	−4.37	0.117 ^{c}
SWa0.5	34.34 ± 7.43 ^{d}	50.46 ± 9.80 ^{d}	0.11 ± 0.05 ^{a}	68.56 ^{d}	31.44 ^{a}	5896 ^{e}	−3.09	0.104 ^{a}
SWa1.0	36.80 ± 6.58 ^{e}	43.64 ± 9.22 ^{b}	0.14 ± 0.06 ^{c}	65.35 ^{b}	34.65 ^{b}	4309 ^{c}	−3.43	0.105 ^{a}
SWa1.5	26.88 ± 5.36 ^{a}	57.62 ± 5.43 ^{f}	0.16 ± 0.06 ^{d}	69.66 ^{d}	30.34 ^{a}	3691 ^{a}	−3.42	0.108 ^{a}

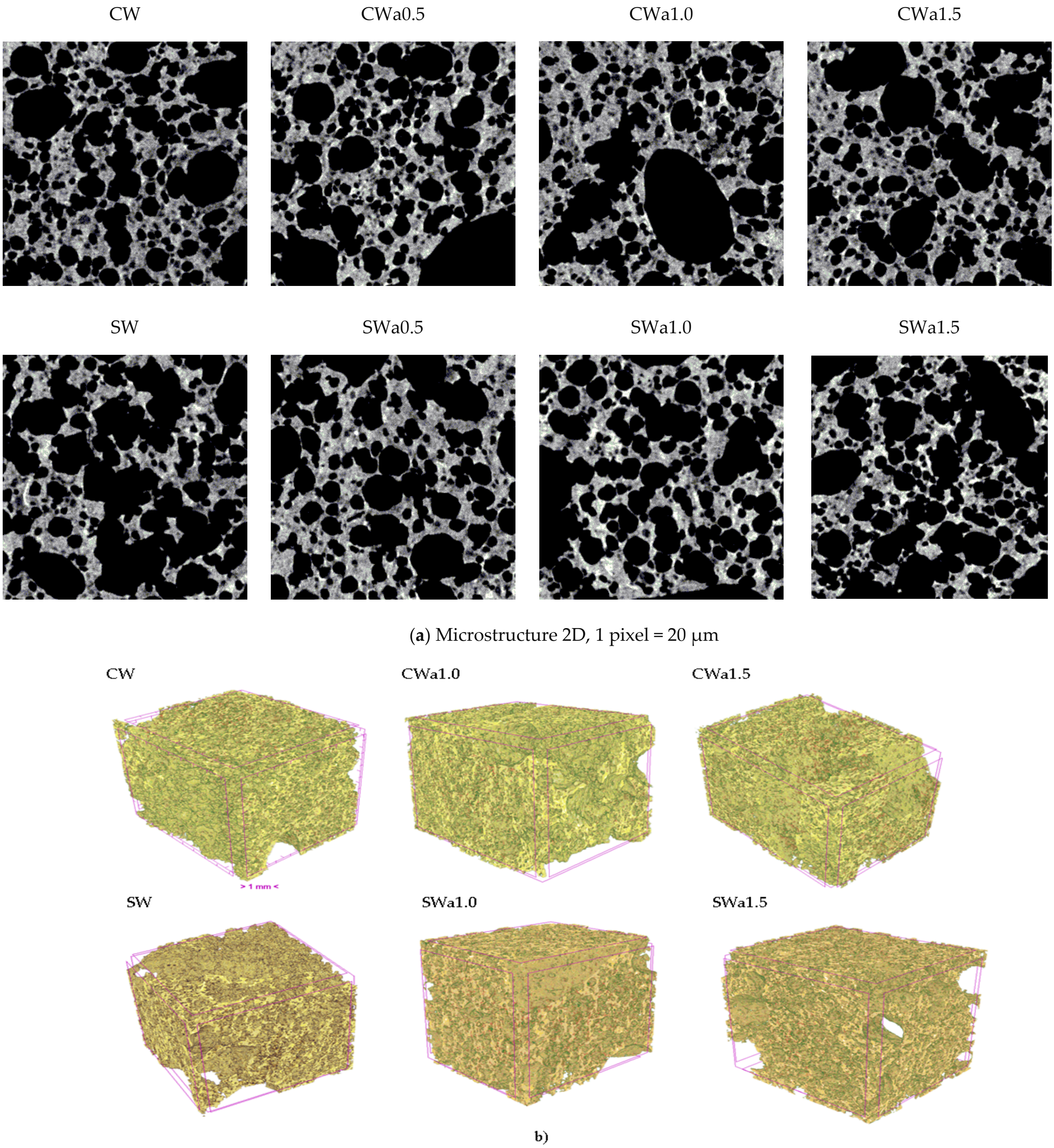


Figure 3. Microstructure X-ray CT images of vegan muffins: (**a**) 2D and (**b**) 3D. Explanations: CW—100% wheat flour, SW—whole-grain spelt flour (50%) and wheat flour (50%), microalgae addition: a0.5, a1.0, and a1.5%.

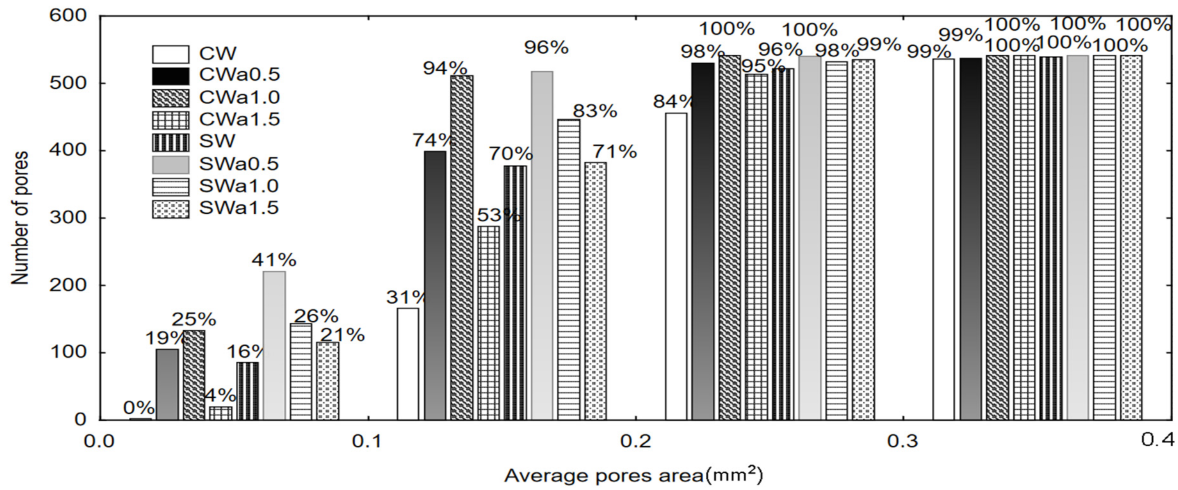


Figure 4. Cumulative distribution of the average pores area in vegan muffins. Explanations: CW—100% wheat flour, SW—whole spelt flour (50%), and wheat flour (50%), microalgae addition: a0.5, a1.0, a1.5%.

Spelt muffins exhibited a higher 3D total porosity (approximately 5 p.p.) compared to the corresponding wheat muffins in terms of microalgae content (Table 3). The addition of 0.5 and 1.0% microalgae resulted in a decrease in porosity, while the use of 1.5% microalgae resulted in porosity similar to that of samples without microalgae. Muffins made with wheat flour had a higher percentage of object volume, indicating a greater proportion of solids in the sample volume regardless of microalgae addition. Furthermore, they displayed a lower number of pores compared to samples made with spelt flour (Table 3). The examined muffin types exhibited similar pore shapes, as indicated by the structure model index values (Table 3). In our study, we proved that wheat flour alone affects the formation of closed pores to a greater extent than a blend of whole-grain spelt and wheat flour. The structure model index (SMI) indicates the relative prevalence of rods and plates in a 3D structure. The structure model index had a negative value. SMI involves the measurement of surface convexity. This parameter may be important in the sensory perception of porous food. An ideal plate, cylinder, and sphere have SMI values of 0, 3, and 4. Note that the concave surfaces of the closed pores represent negative convexity of the SMI, cause dilatation of the enclosed space decreased the surface area [27]. The thickness of the structure determines the thickness of the pore walls in the products. Regardless of the flour type, muffins without microalgae and wheat muffins with 1.5% microalgae had the thickest pore walls (0.122–0.117). In spelt muffins, a decrease in pore wall thickness was observed with increasing microalgae addition. Samples without microalgae (SW) had the highest structure thickness, and as the microalgae content increased, the structure thickness decreased, reaching its lowest point in muffins with 1.0 and 1.5% microalgae addition (SWa1.0 and SWa1.5) (Table 3). In the case of baked goods such as muffins, the structure of which is created by aerating the protein, it is extremely difficult to obtain the effect characteristic of traditional muffins containing structure-forming egg white [22]. The addition of dietary fiber may have a beneficial effect on the formation of the structure of the dough and the finished product [22,24], which is why the high content of fiber in spelt flour could have improved the structure.

3.4. *Muffin Crumb Texture*

The texture is a crucial characteristic that influences eating habits, serves as an indicator of food freshness and quality, and shapes consumer preferences. Muffins, being a sponge-fat dough product, should possess a soft, tender crumb, indicating minimal hardness and chewiness but high elasticity. These texture attributes are commonly associated with products containing sugars and fats [23]. Statistical analysis reveals that the hardness of muffins was influenced by the type of flour ($p < 0.001$) and the addition of microalgae ($p = 0.006$) (Table 4). Muffins made with wheat flour (CW) exhibited greater hardness compared to samples made with spelt flour (SW) (Table 4). The addition of 0.5% microalgae increased the hardness of the muffins, while higher microalgae concentrations did not significantly impact hardness in comparison to samples without microalgae. Elasticity, which demonstrates the product's ability to regain its shape after force application, and cohesion did not exhibit significant changes (Table 4). Chewiness, a parameter related to the ease or difficulty of chewing food and forming a bite before swallowing [26], was adversely affected by the type of flour ($p < 0.001$). Muffins made with a mixture of spelt and wheat flour displayed significantly lower chewiness (Table 4). Similarly, incorporating barnyard millet flour into muffin mixtures led to reduced hardness and cohesiveness of the products [26]. The authors explained these texture differences by the dilution of gluten. In our study, the variations in texture observed between muffins based on wheat flour and whole-grain spelt and wheat flour mixtures with the addition of microalgae may be attributed to the rheological properties of the different doughs, consequently leading to the formation of more pores (particularly open pores) and a more porous microstructure.

Table 4. Texture parameters of vegan muffins with added microalgae (each value is presented as mean ± SD; a, b, c, d—homogenous group, $\alpha < 0.05$).

Muffin Type	**Hardness (N)**		**Elasticity (-)**		**Cohesiveness (-)**		**Chewiness (-)**	
CW	62.55 ± 6.76 [c]		0.85 ± 0.03		0.49 ± 0.04		25.85 ± 3.63 [a]	
CWa0.5	64.65 ± 8.28 [d]		0.81 ± 0.04		0.46 ± 0.05		24.14 ± 2.71 [a]	
CWa1.0	62.17 ± 5.10 [c]		0.82 ± 0.02		0.46 ± 0.04		23.41 ± 1.83 [a]	
CWa1.5	61.75 ± 5.86 [c]		0.83 ± 0.02		0.46 ± 0.02		23.52 ± 2.08 [a]	
SW	52.75 ± 5.10 [a]		0.76 ± 0.06		0.42 ± 0.06		16.71 ± 2.77 [b]	
SWa0.5	57.41 ± 5.32 [b]		0.76 ± 0.03		0.42 ± 0.05		18.16 ± 1.52 [b]	
SWa1.0	52.80 ± 4.33 [a]		0.79 ± 0.03		0.45 ± 0.04		18.54 ± 1.33 [b]	
SWa1.5	52.25 ± 3.61 [a]		0.77 ± 0.05		0.43 ± 0.05		17.12 ± 2.66 [b]	
ANOVA								
Factor	F	p	F	p	F	p	F	p
Flour type (X)	100.97	<0.001	3.27	0.072	2.93	0.088	82.50	<0.001
Microalgae addition (Y)	4.28	0.006	1.48	0.221	0.61	0.609	1.02	0.387
X × Y	0.74	0.531	0.55	0.652	0.99	0.399	0.81	0.492

3.5. Sensorial Evaluation of Vegan Muffins

To assess the acceptability of the muffins, a sensory analysis was conducted. Figure 5 presents a summary of the results from the evaluation of sensory characteristics, providing the average values for all attributes assessed. It was observed that despite differences in microstructure and texture, there were no significant variations ($p > 0.05$) in the sensorial evaluation results for all attributes. However, it is worth noting that the muffins made with spelt flour and added microalgae exhibited a darker color and an intense "grassy" aroma. These findings demonstrate that microalgae can be successfully utilized in the production of vegan muffins. Similarly, Batista et al. [7] demonstrated that muffins containing 2 and 6% Chlorella, as well as Spirulina, were acceptable to the panelists. Furthermore, Lucas et al. [10] confirmed the satisfactory sensory quality of Spirulina bars, which were well-received by school-aged children despite variations in texture.

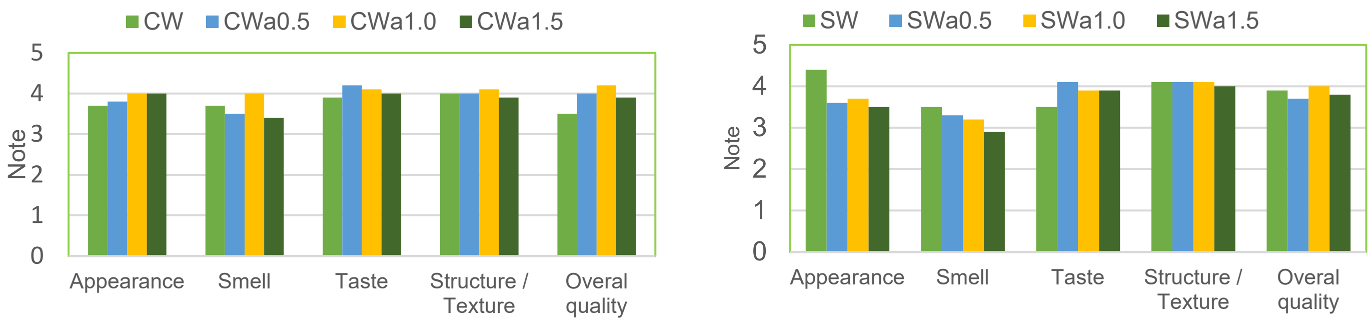


Figure 5. Sensorial evaluation of vegan muffins. Explanations: CW—100% wheat flour, SW—whole spelt flour (50%), and wheat flour (50%), microalgae addition: a0.5, a1.0, and a1.5%.

4. Conclusions

The primary objective of this study was to evaluate the impact of wheat flour and a combination of whole-grain spelt and wheat flours, along with the addition of microalgae as a functional ingredient at different levels (0.5, 1.0, and 1.5%), on the quality of vegan muffins. The study investigated the effects of flour type and microalgae addition on the rheological properties of the dough, as well as the color, microstructure, texture, and sensory properties of the muffins. Doughs containing spelt flour and microalgae exhibited a higher consistency coefficient index and lower flow index compared to doughs with wheat flour. The doughs with spelt flour and 1.0 and 1.5% microalgae additions demonstrated the highest consistency coefficients (99.7 Pa·s^n and 118.6 Pa·s^n, respectively), resulting in muffins with approximately 69% total porosity and a hardness of 52 N. In contrast, muffins made with wheat flour with 1.0 and 1.5% microalgae additions displayed low consistency coefficients (52.3 Pa·s^n and 69.7 Pa·s^n, respectively), resulting in greater total porosities (63% and 67%, respectively), more closed porosities, and larger average pores area in their microstructures. The taste and color of all samples were acceptable, but the addition of microalgae imparted a strong aroma. An important finding of this study is the successful development of vegan muffins enriched with microalgae using a blend of whole-grain spelt and wheat flour. With the addition of 1.5% microalgae, viscosity increased and structure and texture improved. The limitation above all is the grassy aroma. Our research shows that the addition of 1% microalgae is optimal for structure, texture, and sensory properties. However, more research is needed to confirm the nutritional value of the newly developed vegan muffins.

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Abbreviations

CW	Common wheat flour
SW	Whole spelt flour (50%), and wheat flour (50%), Microalgae addition: a0.5, a1.0, and a1.5%
G'	Elastic modulus
G''	Viscous modulus
tgδ	Loss angle
a_w	Water activity
τ	Shear stress (Pa)
γ	Shear rate (s^{-1})
K	Consistency coefficient (Pa·s^n)
n	Flow behavior index
r^2	Determination coefficient
L*	Lightness
a*	Redness
b*	Yellowness
C*	Chroma
h_{ab}	Hue

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