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LOW-FAT COOKIES WITH *CHLORELLA VULGARIS*: EFFECTS ON DOUGH RHEOLOGY, PHYSICAL, TEXTURAL AND SENSORY PROPERTIES OF COOKIES

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ABSTRACT
Microalgae are an enormous biomass used for fortification in foods that represent a promising source of protein. Enrichment of baked foods with microalgae is a challenge for its textural and structural impacts. In this study, dough rheology, physical, textural and sensorial properties of low-fat cookies with *Chlorella vulgaris* at various concentration (0.5, 1.5 and 3.0 %) were evaluated. Dry matter (93.20 to 94.89, %), protein (5.55 to 7.08, %), ash (0.47 to 0.55, %) and fat (12.35 to 13.37, %) contents of cookies were significantly increased with increasing microalgae concentration, whereas carbohydrate amount did not change significantly. The creep parameters, recovery behavior and viscoelasticity were increased with the addition of Chlorella biomass where results clearly showed more stiff dough structure. As the microalgae concentration increased, a significant color change (33.25, ΔE) with high hardness (198.69 up to 330.86, N) and fracturability (88.66 up to 165.18, N) properties were observed.
Keywords: Low-fat cookie, *Chlorella vulgaris*, microalgae, rheology, textural properties

CHLORELLA VULGARIS İÇEREN AZ YAĞLI KURABİYELER: HAMUR REOLOJİSİ, FİZİKSEL, DOKUSAL VE DUYUSAL ÖZELLİKLER ÜZERİNE ETKİLERİ

ÖZ
Mikroalgler, umut verici bir protein kaynağını temsil eden gıdalarda zenginleştirme için kullanılan muazzam bir biyokütledir. Fırınlanmış gıdaların mikroalglerle zenginleştirilmesi, dokusal ve yapısal etkileri nedeniyle zorlu bir iştir. Bu çalışmada, çeşitli konsantrasyonlarda (%0.5, 1.5 ve 3.0) *Chlorella vulgaris* içeren az yağlı kurabiyelerin hamur reolojisi, fiziksel, dokusal ve duyusal özellikleri değerlendirilmiştir. Kurabiyelerin kuru madde (%93,20 ila 94,89), protein (%5,55 ila 7,08), kül (%0,47 ila 0,55) ve yağ (%12,35 ila 13,37) içerikleri artan mikroalg konsantrasyonu ile önemli ölçüde artarken,

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karbonhidrat miktarı önemli ölçüde değişmemiştir. Sürünme parametreleri, toparlanma davranışı ve viskoelastisite, sonuçların daha sert hamur yapısını açıkça gösterdiği Chlorella biyokütlesinin eklenmesiyle artmıştır. Mikroalg konsantrasyonu arttıkça, yüksek sertlik (198.69 ila 330.86, N) ve kırılabilirlik (88.66 ila 165.18, N) özellikleri ile önemli bir renk değişimi (33.25, ΔE) gözlenmiştir.
Anahtar kelimeler: Az yağlı kurabiye, *Chlorella vulgaris*, mikroalg, reoloji, tekstürel özellikler

INTRODUCTION

"Functional foods" include foods and/or food ingredients that exert a positive effect on human health with reduction in the risk of chronic diseases, besides its nutritional functionality. Functional food development is progressing widely all around the world and, for the last two decades, microalgae have received an increasing interest and attention due to be a promising source for protein, fatty acids and other biologically active functional ingredients with significant therapeutic applications such as protective effect against diabetes and obesity. These advantages make microalgae a good raw material and ingredient for food production. *Chlorella* sp., with *Spirulina* sp., is the mostly cultivated microalgae for food applications all around the world. According to the WHO, using *Spirulina* and *Chlorella* biomasses together can provide high quality proteins for human consumption according to their basic amino acid profiles. *Chlorella* sp. provide nutrients including protein (11-58 %), carbohydrate (12-28 %) and lipid (2-46 %). They also contain important vitamins for the human body such as provitamin A, β-carotene, vitamin E, thiamine (B1), riboflavin (B2), niacin (B3), vitamin (B6), B12, biotin and folic acid. These nutritional benefits make the microalgae a valuable and innovative ingredient and can cause significant and interesting physicochemical changes and nutritional improving in food products (Uribe-Wandurraga, vd., 2020).

With the increase in the awareness of healthy foods, it has been understood that most of the main nutritional problems today are serious health problems associated with high consumption of fat and energy. The reduction of dietary fat has become a public health issue and a concern for most consumers. While fat consumption in the USA and Europe accounts for approximately 40% of the total daily calories, health experts recommend it not to exceed 30% (Zoulias, vd., 2002). This recommendation is made due to the association of high fat intake with obesity, cancer, high blood cholesterol, and coronary heart disease disorders. The WHO stated that the fat content of the processed food should be reduced by the food producers in order to reduce the rate of obesity in the world (Laguna, vd., 2012).

Cookies, containing high amount of sugar and fat and low water, are widely consumed among the baked food products all around the world. Cookie doughs mainly consist of high amount of shortening (20-30% on flour weight basis), which is not desirable for a healthier nutrition. Therefore, replacement and/or reduced fat cookies are more acceptable for consumers caring about their health. However, higher hardness and lower brittleness can be obtained from fat replaced or reduced cookies when compared to full fat counterparts (Laguna, vd., 2012). There are many studies focusing on the production of reduced-fat (low-fat) cookies by reducing the shortening amount. In the study of Zoulias, vd. (2000), the effect of sucrose replacement with polyols or fructose on dough rheology and properties of reduced-fat cookies were investigated. In another study, in order to investigate the effect of fruit powder addition (apple or apricot powder) on the quality of low-fat cookies, 10, 20, and 30% of fruit powders were used instead of wheat flour. The replacement of flour by apple and apricot powders in wire-cut cookie formulation resulted that the physical characteristics, total dietary fiber contents and textural properties of the cookies were significantly improved (Özboy-Özbaş, vd., 2010). Sokmen, vd. (2022) examined the bee pollen (BP) in the production of reduced-fat cookies (shortening amount was reduced by 30%), and wheat flour was replaced by 5, 10, 15% BP, and results revealed that the addition of BP increased the nutritional value, total phenol content and antioxidant capacity of reduced-fat cookies. As can be seen from the literature, the reducing of shortening amount of cookies is called "reduced or low fat" cookie production. In these studies, fat replacers were not used, and the additives such as bee pollen, fruit powders, polyols etc. were replaced with wheat flour, and the resulting cookies defined as "low-fat". It is well known that it is hard to produce low-fat cookies without affecting the structural, visual, color and sensorial properties of cookies. In addition, healthy ingredients such as proteins, fibers, antioxidants, vitamins, and minerals are included in the cookie production for healthier end-product (Fradinho, vd., 2015; Šaponjac, vd., 2016).

In previous studies, the microalgae biomass such as *Chlorella vulgaris*, *Isochrysis galbana*, *Dunaliella salina* and *Spirulina platensis* were used for coloring agent and functional food production purposes in cookies (Batista, vd., 2017; Gouveia, vd., 2007; Gouveia, vd., 2008; Sahni, vd., 2019; Şahin, 2020). To the best of our knowledge, rheological properties of reduced-fat cookie doughs with *Chlorella vulgaris* have not been studied so far, and in this study, the use of *Chlorella vulgaris* at various concentrations for the production of reduced-fat cookies was carried out. The detailed rheological characterization of cookie doughs, as well as color, physical, textural and sensory attributes of the cookies, were examined.

MATERIALS and METHODS

Materials

Chlorella vulgaris (UTEX 26) strains were purchased from the Culture Collection of Algae, Texas University (Austin, Texas, USA). Wheat flour consisting of 13.0% moisture, 9.80% protein, 0.65% ash and 24.0% wet gluten was kindly provided from Toru Un Inc. (Turkey). Other ingredients used in the formulation of cookies (powdered sugar, brown sugar, sodium bicarbonate, salt, skimmed milk powder, shortening) were purchased from the local market. All the chemicals used both for the analysis and microalgae medium were of analytical grade.

Production of *Chlorella vulgaris* biomass

Chlorella vulgaris (UTEX 26) in Proteose Medium was pre-cultured for 7 days and then cultivated in batch mode for 15 days in photobioreactor (PBR) at 25 °C with 12/12 lightening period at 3200 lux. The used medium was prepared with 2.94 mM of $NaNO_3$, 0.43 mM of K_2HPO_4, 1.29 mM K_2HPO_4, 0.3 mM of $MgSO_4.7H_2O$, 0.17 mM of $CaCl_2.2H_2O$, 0.43 mM of NaCl, and 1 g/L of proteose peptone. The biomass was harvested when the growth of microalgae achieved a stationary phase, centrifuged at 6000xg and then freeze dried (Teknosem, TRS2/2V, Turkey). The freeze-dried algal biomass was grounded with a coffee grounder and sieved from 212 μm. The freeze-dried biomass consisted of 5.83±0.08% moisture, 9.85±0.02% ash, 53.75±0.09% protein, 14.09±0.45% lipid and 16.48±0.07% carbohydrate (Aguirre ve Bassi, 2013).

Preparation of cookies

The cookies were prepared by AACC Method No: 10–54 (AACC, 1990). The baked cookies were left to cool before they were wrapped in aluminum foil and allowed to stand at room temperature until the analysis. For the production of low fat cookies, the amount of shortening in the cookie formulation was reduced by 30%. Cookies were prepared using the ingredients as indicated in Table 1. The dry ingredients except for flour were mixed thoroughly and the prepared dry mixture and shortening were transferred to the bowl of the mixer (Kitchen Aid, 5KSM150PSEAC model, USA) and mixed for a total of 3 minutes by stripping every 1 minute, and thus the cream was obtained. The cream was mixed for a total of 1 minute by stripping every 15 seconds. Flour or flour-biomass mixture was added to this mixture and stripped every 10 seconds, and the cookie dough was obtained by mixing for a total of 30 seconds. The dough was taken from the bowl of the mixer and rolled to 10 mm thickness by a roller. The rolled cookie dough was cut into 40 mm diameter and 10 mm height circle disks, and then cooked in the oven at 205 ± 2 ºC for 11 minutes. After cooling, cookies were stored in polyethylene bags in dark conditions at room temperature.

Table 1. The ingredients used for production of cookies (g/100 g of cookie dough).

Ingredients	Cnt	0.5%	1.5%	3.0%
Flour	50.42	49.91	48.90	47.39
Sucrose (fine granulated)	16.15	16.15	16.15	16.15
Brown sugar	5.79	5.79	5.79	5.79
Skimmed milk powder	0.50	0.50	0.50	0.50
Salt	0.63	0.63	0.63	0.63
Sodium bicarbonate	0.50	0.50	0.50	0.50
Shortening	13.41	13.41	13.41	13.41
Water	12.60	12.60	12.60	12.60
Chlorella spp.	0.00	0.50	1.51	3.02

Rheological characterization of cookie dough
The effect of *Chlorella vulgaris* incorporation on dynamic shear, creep-recovery and three intervals thixotropic tests (3ITT) of cookie dough were characterized at 25 °C, using a rheometer (Anton Paar, MCR302 model, Austria) equipped with parallel plate geometry (diameter 25 mm, gap 3 mm) and heating Peltier system. The dough was rolled with a pin until it reached a thickness of 3 mm and cut with a circular shape at 25 mm diameter. Dough samples were placed between plates and rested for 5 min to allow relaxation and stabilize temperature. Prior to dynamic shear analysis, stress sweep tests between 1 and 100 Pa were performed at a constant frequency (1 Hz) and temperature (25 °C) to determine the linear viscoelastic region, which was selected as 10 Pa (data not shown). Storage (G') and loss modulus (G'') were recorded between 1 and 100 rad/s angular frequency. During measurements, the moisture loss from the samples was prevented with a lid fitted on to measuring apparatus. The obtained results of G' and G'' values were subjected to non-linear regression analyses by power model, by which the viscoelasticity of food materials are widely described:

$$G' = K'(\omega)^{n'} \tag{1}$$

$$G'' = K''(\omega)^{n''} \tag{2}$$

where K' and K'' (Pa.s), n' and n'' and ω are constants, frequency exponents, angular frequency (rad s^{-1}), respectively (Rao ve Cooley, 1992).

The 3ITT of the cookie doughs was performed with the methodology of Saricaoglu, vd. (2019) for determination of deformation kinetics of cookie doughs. For this purpose, cookie doughs were tested at three intervals. The first interval was applied to cookie doughs as 10 Pa stress and 1 Hz frequency in which to LVR. High rotational shear deformation at 1000 s^{-1} was carried out for 30 sec in the second interval, and lastly, in the third interval, regeneration interval, the same parameters as to be in the first interval were applied. During the second interval, the sample did not slip away between the plate and probe. The following equations were used for the calculation of deformation ($\%D_r$) and recovery ($\%R$) degree at 30 sec after the last interval:

$$\%D_r = \frac{G'_i - G'_0}{G'_i} \times 100 \tag{3}$$

$$\%R = \frac{G'_{30}}{G'_i} \times 100 \tag{4}$$

where G'_i, G'_0 and G'_{30} are storage modulus values of initial, immediately after second interval and within the first 30 sec of third interval, respectively. Non-linear regression model (Eq. 5) was applied to data obtained from the third interval using Sigma Plot software (version 10, Systat Software Inc., CA, USA). An exponential function was well fitted to data and equilibrium storage modulus (G'_e) and maximum recovery time were estimated successfully.

$$G' = y_0 + \alpha \times \left(1 - e^{(-\beta \times X)}\right) \tag{5}$$

where y_0 and α are constants and β is recovery rate constant.

Creep-recovery test was performed at 25 °C and a constant shear stress of 100 Pa for 5 min, which was applied beyond the LVR (Pulatsu, vd., 2021). The applied stress was suddenly removed and the dough samples were monitored for 5 min for the recovery phase. All the rheological measurements were carried out in triplicate. The rheological data analysis was performed by the device software (Anton Paar, RheoCompass, v1.25, Austria) and Sigma Plot (Systat Software Inc., v10.0, CA, USA) was used for non-linear regression fitting of the data.

Characterization of cookies

Proximate Analysis

The protein, lipid, ash and moisture content of cookies were determined according to the AACCI (1990) and AOAC (1990) methods. Carbohydrate values were calculated using Atwater general factor system according to FAO (2003). All proximate analyses were repeated, at least in triplicate, and were performed after cookie preparation.

Spread ratio and color of cookies

Diameter and thickness of the cookies were determined immediately after baking the cookies using a digital caliper (Insize 102 model, China) and the spread ratio of the cookies was determined by calculating the ratio of diameter to thickness for each cookie according to AACC 10-50.5. (2000).

Color measurements based on triple scale of L^*, a^*, b^* values were determined with a colorimeter (Konica Minolta CR-400/CR41, Japan). L^* is the lightness, a^* and b^* range from green ($-a^*$) to red ($+a^*$) and blue ($-b^*$) to yellow ($+b^*$), respectively. Total color differences of baked cookies were evaluated by using control sample as reference with following equation:

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \qquad (6)$$

The triplicated measurements were taken from both inner and outer layer of cookies and the instrument was calibrated by using a standard white and black plate.

Texture profile analysis

Texture profile analysis of cookies were made by using a texture analyzer (Stable Micro Systems, TA HD Plus model, Godalming, UK) with a 100 kg load cell. All the tests were performed with a cylindrical probe at 36 mm diameter (P/36R). The pre-test and post-test speeds were 5 mm s^{-1} and the test speed was 1.0 mm s^{-1}. Compression distance was arranged as 7 mm and the maximum peak force determined from the first cycle was related to hardness (Hajas, vd., 2022; Soares, vd., 2023). Measurements were carried out at least with eight replicates from duplicated cookies at room temperature (25 °C).

Sensory evaluation

Baked cookie samples were evaluated in terms of sensorial properties by the panelists (n = 40, 15 men and 25 women aged 19–50 years) recruited among staff and students of Bursa Technical University. Cookies were placed in a plate and coded with three random digits, and offered to panelists in random order with the product evaluation sheet. The taste-free water was used for palate cleansing. Cookies were evaluated in terms of surface and cross-section appearance, taste, texture, overall acceptance and affordability using 9-point hedonic scale (1= dislike extremely; 2=dislike very much; 3=dislike moderately; 4=dislike slightly; 5=neither like nor dislike; 6=like slightly; 7=like moderately; 8=like very much; 9= like extremely) (Everitt, 2009).

Data analysis

The results of analyses were illustrated as mean ± standard deviation. All analyses were performed at least in triplicate per duplicated cookie samples. The statistical differences between cookie samples were evaluated by one-way Analysis of Variance (ANOVA) and mean differences were determined using Duncan's multiple comparison at a significance level of 5% with SPSS program (IBM Corp., version 21.0, USA). The non-linear regression analyses of rheological data were performed with Sigma Plot program (Systat Software Inc., v10.0, CA, USA).

RESULTS and DISCUSSIONS

Rheological properties of cookie dough

The rheological properties of cookie dough have high impact on the process ability and quality of cookies. The results of dynamic shear properties, elastic (G', Pa) and viscous (G'', Pa) modulus, give significant information about mechanical strength and extrusion capacity of cookie dough (Huang, vd., 2020). The dynamic shear spectra of *Chlorella vulgaris* incorporated cookie doughs is illustrated in Fig. 1. G' was greater than G'' in the entire measured frequency range indicating the prevalence of elastic properties over viscous properties. The dynamic spectra of cookie samples showed no cross-over point throughout the measured frequency range revealing the nature of solid-like behavior (Yang, vd., 2019). Control sample showed the lowest G' and G'' values for entire frequency range when compared with the values of microalgae enriched cookie doughs, which indicated that viscoelastic behavior was modified by additives, but elastic solid-like behavior was improved predominantly. This means that microalgae incorporated cookie doughs can more easily recover energy from deformation. The increase of microalgae concentration caused the increase of both G' and G'' values, considering that microalgae are a source of hydrocolloids, mainly proteins, which are generally recognized for their ability to enhance water absorption in doughs (Rosell, vd., 2001). It was also reported by Graça, vd. (2018) that the addition of 3.0 g *Chlorella vulgaris* based on wheat flour increased G' values, showing a possible strengthening of the dough structure due to viscoelastic protein matrix reinforcement depending on high protein content of microalgae.

G' and G'' values of cookie doughs showed an increasing tendency with rising oscillation frequency, and thus, it was possible to model these modulus as a power function of oscillatory frequency (Eq. (1) and (2)), and results are illustrated in Table 2. It is clear that the R^2 values of equations were higher than 0.97 which indicates that this power function can successfully describe the dynamic mechanical spectra of cookie doughs. The incorporation of microalgae at 0.5% did not significantly affect the K' values, whereas it was significantly increased when *Chlorella vulgaris* concentration increased to 1.5% and 3.0%. However, 0.5% microalgae addition increased the K'' values significantly, when compared to control. These results showed that elastic and viscous properties of cookie doughs were enhanced with the incorporation of *Chlorella vulgaris*. The slopes (n' and n'') of both moduli did not significantly affect from the addition of microalgae, and n'' values were higher than n' for all samples, which means loss modulus is more dependent on frequency change.

Table 2. Dynamic shear parameters of power-law functions describing the G' and G" values of microalgae incorporated cookies.

	$G' = K'(\omega)^{n'}$			$G'' = K''(\omega)^{n''}$		
Samples	K'	n'	R^2	K''	n''	R^2
Cnt	7.72±0.10^c	0.26±0.01	0.998	2.99±0.04^c	0.29±0.04	0.980
0.5%	7.94±0.12^c	0.27±0.01	0.997	3.21±0.05^b	0.28±0.04	0.984
1.5%	8.44±0.14^b	0.27±0.02	0.995	3.10±0.06^{bc}	0.30±0.08	0.979
3.0%	9.53±0.13^a	0.27±0.03	0.997	4.02±0.08^a	0.28±0.06	0.979

Values are means ± Standard Deviation. a-c Refers the significant differences between the values in the same column (p<0.05). *Cnt*: Control; K' and K'': constants; n' and n'': frequency exponents; ω: angular frequency (rad/s) R^2: determination coefficient of Eq. (1) and Eq. (2).

The complex viscosity (η^*, Pa.s), which can be obtained from dynamic shear measurements, is called as resistance against the flow of viscous liquid materials (Dimitreli ve Thomareis, 2008). The viscosity of cookie doughs should be low enough at high shear rates for processing and shaping, but the doughs should recover its high viscosity value after processing and shaping

(Uribe-Wandurraga, vd., 2020). It is clear in Fig. 2 that the complex viscosity of all cookie doughs decreased with increasing angular frequency, showing shear-thinning behavior, and microalgae incorporation increased the complex viscosity values of dough samples over the analyzed angular frequency ranges. The shear-thinning behavior of cookie doughs could be desired for better shaping and faster recovery after shaping (Huang, vd., 2019).

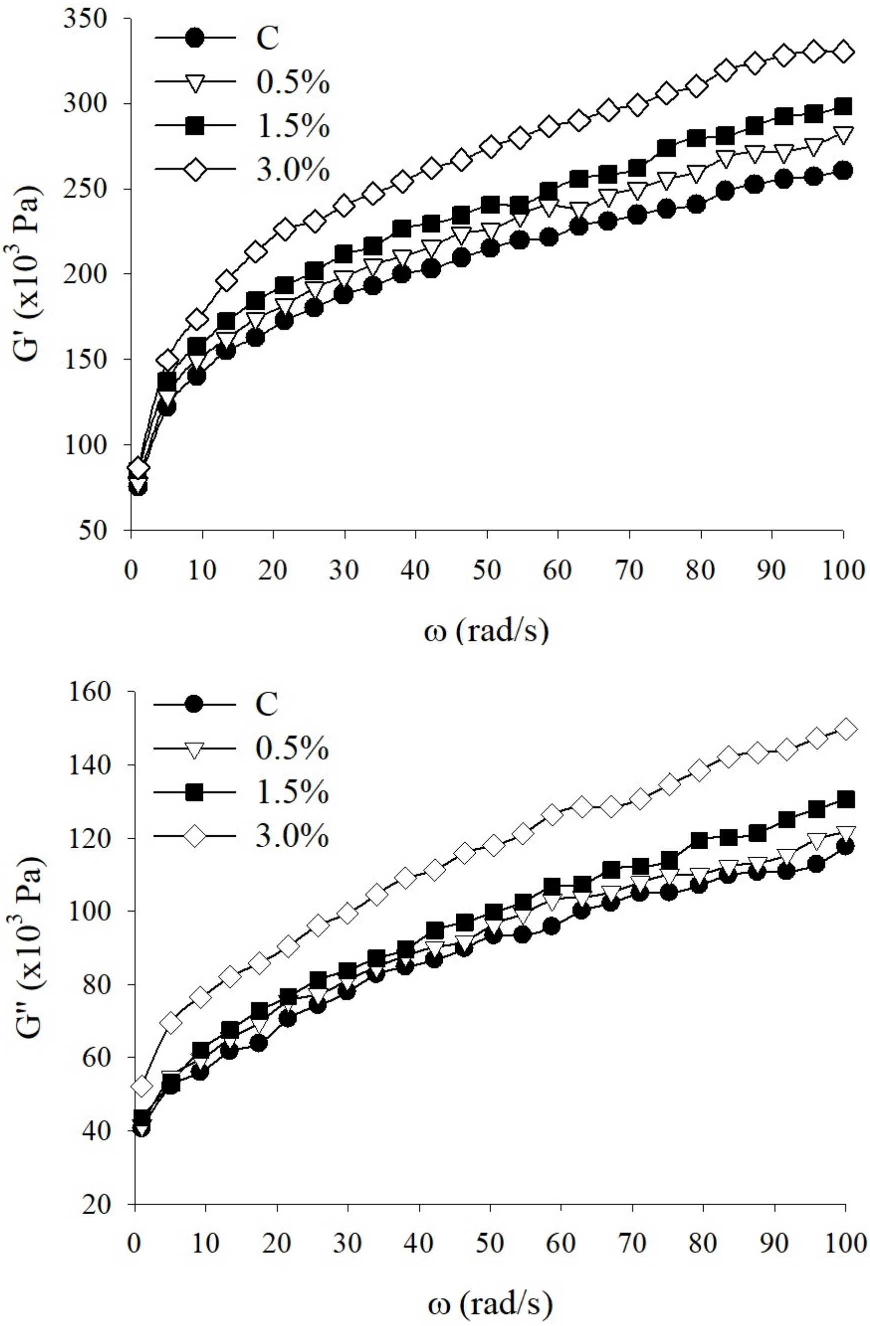

Fig. 1 Dynamic mechanical spectra of low-fat cookie doughs enriched with *Chlorella vulgaris*

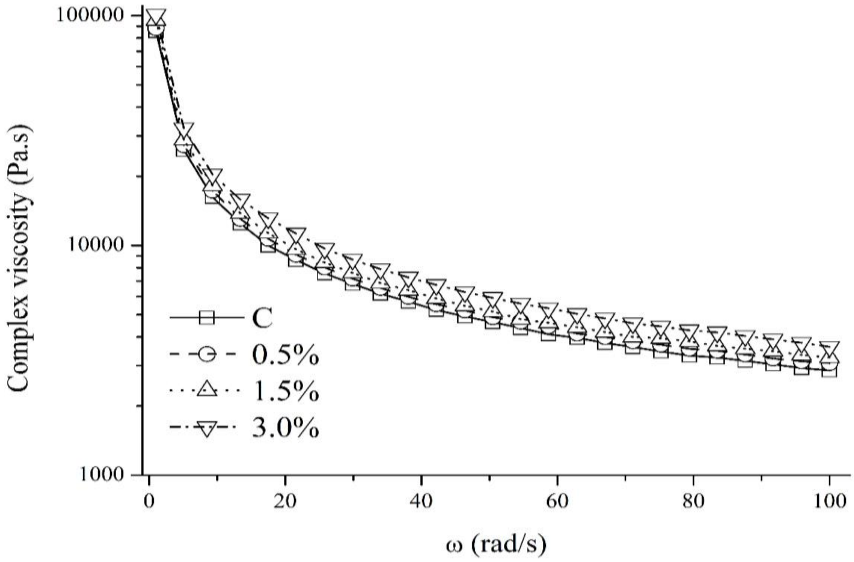

Fig. 2 The effect of microalgae concentration on complex viscosity of cookie dough

Three interval thixotropic test (3ITT) mainly consists of 3 different analyses stages containing reference, high shear deformation and regeneration. This test simulates the behavior of food materials during transporting, handling and processing (Saricaoglu, vd., 2019). As seen in Fig. 3, G' was higher than G'' indicating that cookie doughs, either control or microalgae enriched, had elastic character rather than viscous one. At the beginning of the third interval, 1.5% microalgae incorporated cookie doughs showed higher G'' than G', and then G' increased rapidly and showed higher values than G''. This result indicated that viscous character immediately after the second interval was dominant for cookie dough containing 1.5% microalgae, and this could be attributed to broken internal structure. Microalgae incorporated cookie dough at 0.5% showed the highest decrease both for G' and G'' probably due to the easily destroyed weak electrostatic and hydrophobic bonds with high shear deformations (Isanga ve Zhang, 2009).

The results related to 3ITT as well as non-linear regression analyses (Fig. 4) of 3rd interval are given in Table 3. Storage modulus of samples before deformation (G_i') significantly increased with the addition of *Chlorella vulgaris* up to 1.5%, and then increased insignificantly. The addition of protein rich component in cookie dough caused a significant increase due to binding water as observed before in dynamic shear properties. After high shear deformation, the lowest initial storage modulus (G_0') was observed from 0.5% *Chlorella* supplemented sample, and the increase of concentration from 1.5 to 3% significantly increased the G_0' values. This means that the addition of *Chlorella* to cookie doughs improves the recovery of samples after high shear deformations such as processing, shaping and handling. The lowest D_r value (58.69%) was observed from control sample, and the addition of *Chlorella vulgaris* at 0.5 and 1.5% significantly increased the D_r values, whereas control and 1.5% microalgae added samples displayed similar values with 3% addition. The cookie doughs are prepared with mixing in a bowl and then the doughs are shaped prior to cooking. Considering the findings of present study, the addition of microalgae caused higher relative deformation, probably due to binding of water by proteins of *Chlorella vulgaris*, and hence displayed higher deformation after mixing. However, after 30 s of high shear deformation, samples showed high D_r values had high recovery percentage. This means that microalgae incorporated cookie doughs can be mixed with higher speed and/or longer time for better homogenization, because they can show high recovery after 30 s of deformations such as mixing, shaping. The results of 3rd interval were subjected to non-linear regression with high R^2 values ranging from 0.946 to 0.961. Equilibrium storage modulus (G'_{eq}), maximum storage modulus can be reached at maximum time (t_{eq}), increased insignificantly with increasing concentration. Time for reaching G'_{eq} was firstly decreased when compared to control, and increased to 32.31 min at 3% microalgae concentration. The maximum recovery rates (Rec_{max}) of samples increased insignificantly, and the highest value was observed at 3% addition. These results show that 0.5 and 1.5% *Chlorella vulgaris* incorporated cookie doughs can recover higher and faster than control and 3% microalgae added samples after high shear deformation.

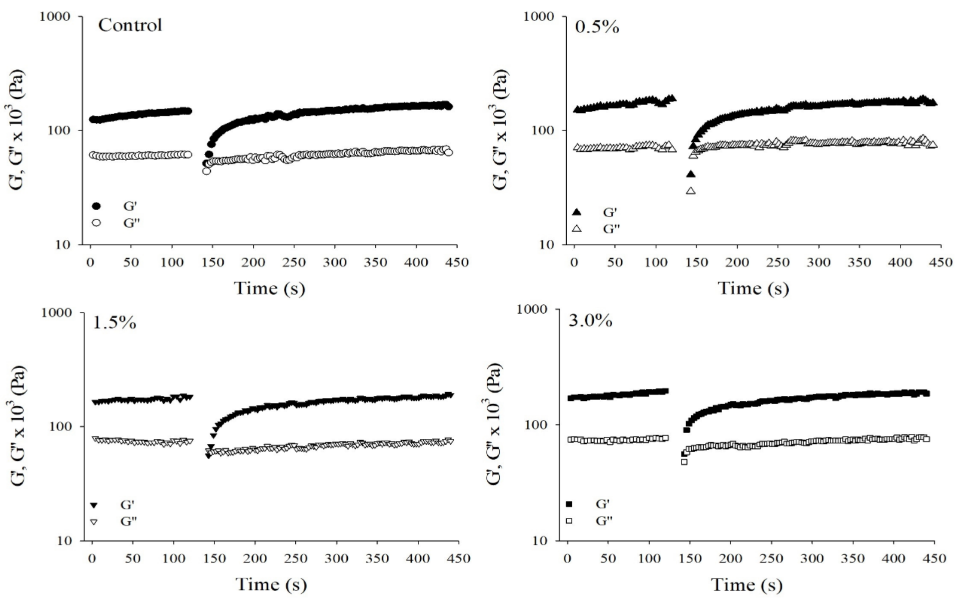

Fig. 3 Effect of *Chlorella vulgaris* addition on structural deformation and regeneration of low-fat cookie dough

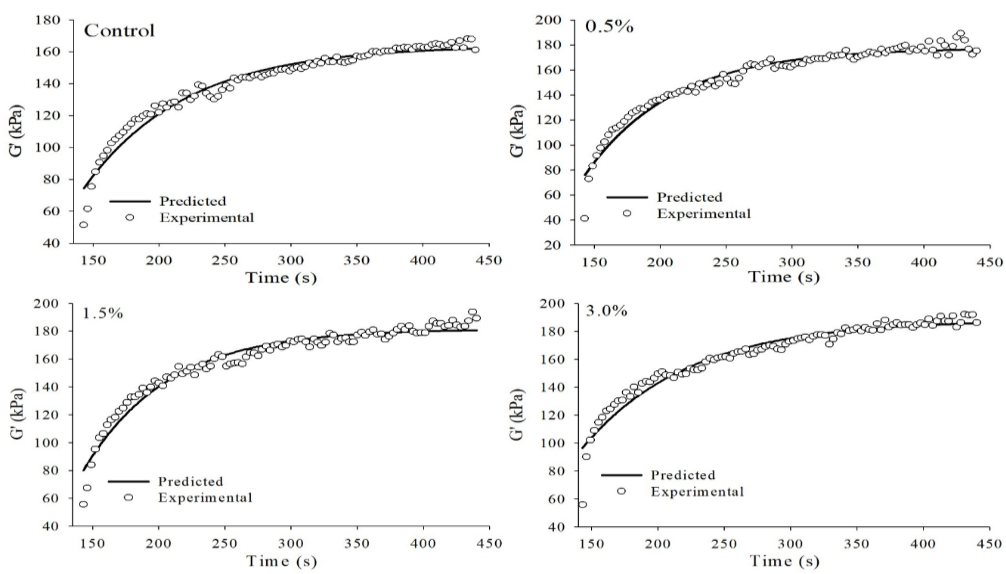

Fig. 4 Nonlinear regression modeling of recovery (third) interval of low-fat cookies with *Chlorella vulgaris*

Table 3. Effect of *Chlorella* concentration on 3ITT results and non-linear regression parameters of 3rd interval.

Parameters	Concentration (%)			
	Cnt	0.5	1.5	3.0
G_i' (kPa)	124.27±2.69[c]	151.66±3.27[b]	164.21±2.63[a]	169.61±3.82[a]
G_0' (kPa)	51.34±2.86[b]	41.14±2.45[c]	55.87±3.56[b]	65.87±3.39[a]
D_r (%)	58.69±1.41[c]	72.87±2.20[a]	65.98±1.63[b]	61.16±2.88[bc]
Rec (%)	66.95±2.91	72.88±1.39	68.12±3.01	66.12±3.18
$y_0 \times 10^5$	-4.05±0.09[b]	-6.74±0.18[c]	-7.86±0.14[d]	-3.54±0.17[a]
$a \times 10^5$	5.69±0.04[c]	8.52±0.13[b]	9.68±0.16[a]	5.42±0.07[c]
b	0.014±0.001	0.015±0.001	0.016±0.001	0.012±0.001
R^2	0.961	0.957	0.957	0.946
G_{eq}' (kPa)	163.99±14.14	177.99±5.66	181.99±1.41	187.99±9.90
t_{eq} (min)	28.63±1.51	26.30±2.50	24.77±2.21	32.31±3.83
Rec_{max} (%)	76.13±8.20	85.21±0.87	90.23±0.74	90.29±2.72

Values are means ± Standard Deviation. Values are means ± Standard Deviation. a-d Refers the significant differences between the values in the same row (p<0.05). *Cnt*: Control; G_i': Storage modulus of the sample before deformation; G_0': Initial storage modulus after high shear deformation; *Dr*: Relative deformation; *Rec*: Recovery percentage of sample after 30 s of 3rd interval; G_{eq}': Equilibrium storage modulus at *teq*; *teq*: Time required to reach equilibrium storage modulus; *Recmax*: Recovery percentage of sample at equilibrium storage modulus. a-d Means within the same row with different letters are significantly different at P< 0.05.

Creep and recovery tests can give significant information about internal structure of a system consisting of proteins and carbohydrates. Information about softness of a material can be estimated with creep compliance (*J[t]*) values, and the higher the *J(t)* value, the weaker the material structure, and vice versa (Sozer, 2009). Dynamic rheological measurements including creep-recovery tests at small-strains reveal the material characteristics of samples in the LVR where no structural deformation occurred. However, it is also necessary to determine the mechanical behaviour of the cookie dough outside the LVR since high shear forces act on the cookie dough during further processing. Hence, creep-recovery tests were performed outside the LVR. Creep-recovery results are illustrated in Fig. 5, and the increasing concentration of *Chlorella vulgaris* decreased the *J(t)* values which means stronger internal structure occurred. The addition of microalga biomass which is rich in proteins and polysaccharides caused the occurrence of a complex matrix. Proteins and carbohydrates are responsible from the water absorption in dough, and also dough firmness (Egea, vd., 2014; Gouveia, vd., 2007; Vieira, vd., 2020). Elastic and viscous parameters of cookie doughs were obtained by the application of four components Burgers model which is the most widely used model for foods such as ice cream mixes (Kurt, vd., 2016), emulsions (Dolz, vd., 2008), and cookie doughs (Uribe-Wandurraga, vd., 2020). The following equation called as Burgers model can be used for describing the relationship between creep compliance and time:

$$J(t) = \frac{1}{G_0} + \frac{1}{G_1}\left[1 - exp\left(\frac{-tG_1}{\eta_1}\right)\right] + \frac{t}{\eta_0} \qquad (7)$$

where *J(t),* G_0, η_0, G_1 and η_1 are overall compliance at any *t* time, instantaneous elastic modulus of Maxwell spring, Maxwell dashpot residual viscosity, elastic modulus of Kelvin-Voigt and internal viscosity of Kelvin-Voigt dashpot, respectively (Steffe, 1996). The creep parameters are summarized in Table 4, and as seen the Burgers model could be well used for describing the creep data due to high R^2 values. All the creep parameters were significantly increased with the increasing microalgae addition ($p<0.05$). The increasing G_0 and G_1 values, as well as η_0, showed that cookie doughs formed a highly elastic network upon increase of *Chlorella vulgaris* concentration. Internal viscosity (η_1) of samples displayed high values and it could be related with the interaction of ingredients. In addition, the increasing microalgae concentration resulted in the higher η_1 values probably due to high interaction between proteins of *Chlorella vulgaris* and wheat flour. Similar results were also determined by Peressini ve Sensidoni (2009) for inulin enriched cookie dough and by Pulatsu, vd. (2021) for pomace and insoluble dietary fiber added cookie doughs.

Table 4

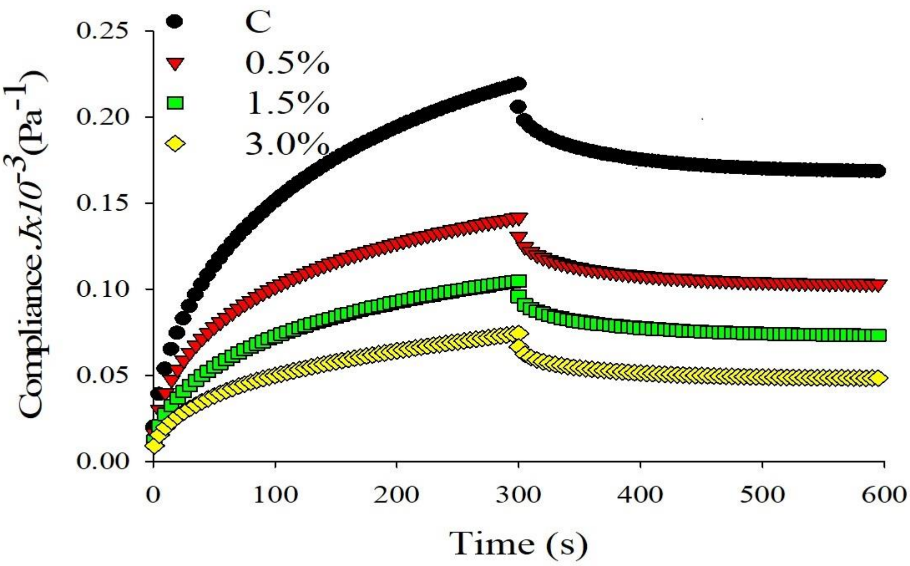

Fig. 5 Creep-recovery curves of low-fat cookie dough containing various amount of *Chlorella vulgaris*

Table 4. Burgers model parameters from creep data of cookie doughs

Samples	Creep parameters				
	G_0 *(kPa)*	η_0 *(Pa.s)*	G_1 *(kPa)*	η_1 *(Pa.s)*	R^2
Cnt	33.67±0.12[d]	3.68±0.30[d]	9.01±0.19[d]	179.61±5.65[d]	0.999
0.5%	42.19±0.26[c]	6.13±0.21[c]	14.14±0.05[c]	298.78±3.95[c]	0.999
1.5%	61.73±0.37[b]	7.71±0.62[b]	19.65±0.29[b]	391.36±9.89[b]	0.999
3.0%	83.13±0.17[a]	9.39±0.11[a]	32.37±0.28[a]	726.34±10.08[a]	0.999

Values are means ± Standard Deviation. a-d Means within the same column with different letters are significantly different at *p<0.05*. *Cnt*: Control; *G0*: instantaneous elastic modulus of Maxell spring; η_0: residual viscosity of Maxwell dashpot; *G1*: retarded elastic modulus of Kelvin-Voigt; η_1: internal viscosity of Kelvin-Voigt dashpot; *R2*: determination coefficient of Eq. (7).

The recovery behavior of cookie doughs was also illustrated in Fig. 5, and the increasing microalgae concentration caused the decrease of recovery compliance. The recovery phase of cookie doughs was well fitted to following model:

$$J(t) = J_\infty + J_{KV} exp(-\beta t^c) \quad (8)$$

where *t*, *β*, *C*, J_∞ and J_{KV} are time, parameters for determining the speed of recovery, the recovery compliance of Maxwell dashpot and Kelvin–Voigt element, respectively. When $t \to 0$, *J(t)* is equal to $J_\infty + J_{KV}$ which means the maximum deformation of the dashpots in the Burgers model. However, for $t \to \infty$, *J(t)* is equal to J_∞, which corresponds to the irreversible sliding of the Maxwell dashpot (Dolz, vd., 2008). Table 5 summarizes the results of recovery parameters, as well as the maximum compliance of creep phase and final recovery percentage. The maximum compliance J_{MAX} and J_∞ of samples were significantly decreased with increasing microalgae concentration. However, when compared with control J_{KV} and *β* were significantly decreased while C increased with the microalgae addition. The lowest final recovery percentage (23.30%) was observed from control while the highest value (33.79%) was 3% added cookie dough. These results clearly showed that the addition of *Chlorella vulgaris* to cookie dough caused more stiff dough structure, and take more time to recover. Similar results were also reported by Vieira, vd. (2020) for cookie doughs fortified with microalgae of *Arthrospira platensis*.

Table 5. Compliance of each element in the Burgers model, together with the final percentage recovery (R).

Samples	$J_{MAX} \times 10^{-4}(Pa^{-1})$	Recovery parameters					R (%)
		$J_{\infty} \times 10^{-4}(Pa^{-1})$	$J_{KV} \times 10^{-4}(Pa^{-1})$	$\beta\ (s^{-1})$	C	R^2	
Cnt	2.19±0.06^{a}	1.68±0.06^{a}	4.57±0.31^{a}	0.143±0.012^{a}	0.689±0.019^{b}	0.992	23.30±0.60^{b}
0.5%	1.42±0.07^{b}	1.03±0.08^{b}	0.53±0.06^{b}	0.026±0.003^{b}	0.937±0.011^{a}	0.990	27.52±2.37^{ab}
1.5%	1.05±0.09^{c}	0.73±0.03^{c}	0.23±0.03^{b}	0.017±0.004^{b}	0.989±0.009^{a}	0.989	30.29±3.88^{a}
3.0%	0.74±0.08^{d}	0.49±0.06^{d}	0.30±0.04^{b}	0.018±0.004^{b}	0.998±0.045^{a}	0.986	33.79±0.05^{a}

Values are means ± Standard Deviation. a-d Means within the same column with different letters are different ($p<0.05$). *Cnt*: Control; J_{MAX}: maximum deformation; J_{∞}: viscous part attributed to irreversible sliding of Maxwell dashpot; J_{KV}: elastic part related with Kelvin-Voigt unit; *B* and *C*: the parameters determining the recovery speed of system; R^2: determination coefficient of Eq. (8); *R*: final percentage recovery of system

Proximate composition, spread ratio and color of cookies

The proximate composition, diameter, spread ratio and color results of *Chlorella vulgaris* incorporated cookies are given in Table 6. As expected, dry matter, protein, ash and fat content of cookies were significantly increased with increasing microalgae concentration ($p<0.05$), whereas carbohydrate amount did not change significantly. The freeze dried biomass consisted of 5.83±0.08% moisture, 9.85±0.02% ash, 53.75±0.09% protein, 14.09±0.45% lipid and 16.48±0.08% carbohydrate. The moisture content of cookies which ranged from 5.11 to 6.80% was typical such a dried product. The main effects of microalgae biomass addition were observed from protein, ash and fat content, since freeze dried biomass was rich in protein, ash and lipid. The diameter and spread ratio of cookies significantly increased by the microalgae addition, but increasing microalgae concentration did not affect the diameter and spread ratio. Abboud, vd. (1985) reported that the spread ratio of cookies was not affected by fat type. However, in another study the replacement of shortening with flaxseed oil significantly increased the spread ratio of cookies (Rangrej, vd., 2015). In our study, 13.41% shortening was used for cookie production and this amount is very low when compared to other methods (Egea, vd., 2014; Gouveia, vd., 2007; Rangrej, vd., 2015; Uribe-Wandurraga, vd., 2020). Although we decreased the shortening amount during the production cookies, the increased spread ratio was probably due to the high fat content of the microalgae. Dinç, vd. (2014) stated that higher palmitic acid (C16:0) content made possible to interesterification and by this way palmitic acid had the tendency to crystallize in β' form which resulted in higher spread ratio. Large amounts of liquid oil in the crystal network can incorporate with β' crystals due to their relatively small size and therefore, smooth, continuous and homogenous products can be obtained (DeMan, 1994). It is earlier reported that *Chlorella sp.* consisted of 19.6% palmitic acid (Zhukova ve Aizdaicher, 1995), and therefore the addition of *Chlorella vulgaris* improved the spread ratio. The effect of defatted *Chlorella sp.* on spread factor of cookies was also reported by Sahni, vd. (2019), and they stated that the spreading of cookies can be attributed to the formation of syrup in the cookie dough during baking.

The surface and cross-sectional appearances of cookies were depicted in Fig. 6. It is clearly obvious that the addition of *Chlorella vulgaris* caused an apparent increase in green tonality. The color L^*, a^* and b^* values of *Chlorella vulgaris* were determined as 20.69±1.59, 0.47±0.08 and 3.57±0.25, respectively. The control cookies presented a dominant yellow chromaticity (positive b^*) for both inner and outer layer. The incorporation of *Chlorella* biomass resulted in a darker and green color parameter. By increasing the *Chlorella* biomass concentration, lightness (L^*) of inner and outer layer significantly decreased ($p < 0.05$), which means that darker cookies were occurred. The increased level of *Chlorella* biomass significantly decreased the a^* value at inner layer, and green color ($-a^*$ value) was dominant at 3%. However, at the inner layer of cookies, only control sample displayed positive a^* value, and the shift from positive to negative a^* value was observed depending on *Chlorella* biomass addition, and the greenness increased significantly ($p < 0.05$). The higher $-a^*$ value is typical due to high chlorophyll content of *Chlorella sp.*, as reported earlier by Batista, vd. (2017) and the addition of green and blue-green microalgae biomass decreases not only the red color (a^*) but also the luminosity (L^*) (Achour, vd., 2014; Figueira, vd., 2011). These results are similar to *Chlorella vulgaris* and *Isochrysis galbana* biomass added cookie samples in which microalgae biomasses were used as colorants in cookies (Gouveia, vd., 2007; Gouveia, vd., 2008). As expressed by Şahin (2020), high carotene and chlorophyll content increased the b^* values of cookies. The b^* values of inner layer increased significantly when compared to control samples, whereas *Chlorella* addition did not cause a significant change. The total color changes (ΔE) of *Chlorella* incorporated cookies are also given in Table 6. Control sample without microalgae addition was used as reference and as seen, the addition of *Chlorella* biomass significantly increased the color change. ΔE value can be classified as not noticeable (0-0.5), slightly noticeable (0.5-1.5), noticeable (1.5-3.0), well visible (3.0-6.0) and great (6.0-12.0) (Cserhalmi, vd., 2006). According to this classification, the inner and outer layers of cookie samples showed great color change ranging from 12.81 to 29.16 and 15.54 to 33.25, respectively.

Table 6. Proximate composition, spread ratio and color results of cookies

Parameters		Cookies			
		Cnt	0.5%	1.5%	3%
Proximate composition					
Moisture (%)		6.80±0.16[c]	5.86±0.28[b]	5.63±0.84[ab]	5.11±0.58[a]
Ash (%)		0.47±0.01[d]	0.49±0.01[c]	0.53±0.01[b]	0.55±0.01[a]
Protein (%)		5.55±0.05[d]	5.70±0.02[c]	6.18±0.02[b]	7.08±0.03[a]
Fat (%)		12.35±0.01[d]	12.51±0.01[c]	12.81±0.06[b]	13.37±0.11[a]
Carbohydrate (%)		74.83±0.23	75.45±0.30	74.85±0.72	73.89±0.77
Physical characteristics					
Diameter (cm)		6.18±0.13[b]	6.43±0.04[a]	6.53±0.03[a]	6.54±0.08[a]
Spread ratio		3.65±0.08[b]	4.14±0.20[a]	4.16±0.10[a]	4.19±0.14[a]
Color					
Outer layer	L^*	73.97±0.98[a]	64.00±0.45[b]	53.29±0.75[c]	47.52±0.20[d]
	a^*	10.92±0.65[a]	3.19±0.52[b]	2.59±0.39[b]	-0.51±0.05[c]
	b^*	28.76±0.71[a]	26.99±0.41[b]	25.90±0.73[bc]	24.59±0.66[c]
	ΔE	-	12.81±0.86[c]	22.51±0.75[b]	29.16±0.35[a]
Inner layer	L^*	80.91±2.02[a]	71.92±0.34[b]	56.61±1.13[c]	50.66±0.71[d]
	a^*	2.21±0.82[a]	-3.93±0.30[b]	-5.11±0.50[b]	-7.90±0.66[c]
	b^*	19.65±1.09[b]	30.51±0.94[a]	29.78±1.20[a]	28.88±1.08[a]
	ΔE	-	15.54±0.83[c]	27.39±0.58[b]	33.25±1.32[a]
Texture profile analysis					
Hardness (N)		198.69±10.81[c]	245.86±20.87[b]	273.84±6.62[b]	330.86±33.96[a]
Fracturability (N)		88.66±3.06[b]	102.28±9.96[b]	151.09±16.09[a]	165.18±16.54[a]
Springiness		0.372±0.025[b]	0.370±0.038[b]	0.386±0.016[b]	0.441±0.017[a]
Cohesiveness		0.030±0.006[c]	0.062±0.018[b]	0.079±0.007[b]	0.101±0.008[a]
Gumminess		5.86±0.82[c]	14.96±3.43[b]	21.61±1.30[b]	33.25±6.08[a]
Chewiness		2.19±0.38[c]	5.53±1.41[bc]	8.36±0.80[b]	14.73±3.29[a]
Resilience		0.011±0.003[b]	0.014±0.007[b]	0.020±0.003[ab]	0.024±0.004[a]

Values are means ± Standard Deviation. a-d Means within the same row with different letters are different ($p<0.05$).

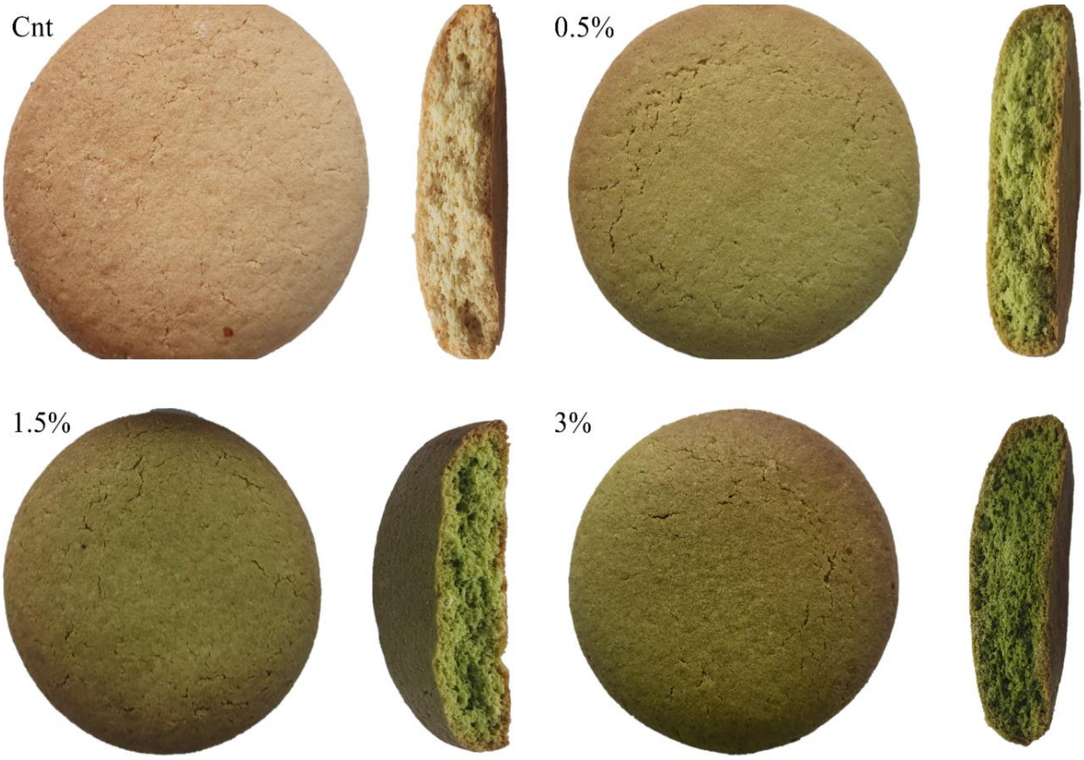

Fig. 6 Surface and cross-section visual appearances of baked low-fat cookies containing various amount of *Chlorella vulgaris*

Texture profile analysis

The effect of *Chlorella vulgaris* on the textural properties of cookies is also illustrated in Table 6. Hardness and fracturability are important parameters for consumer acceptability of baked cookies, and can be determined as the peak force that occurs during the first compression. Springiness denotes how well a product physically springs back after the first deformation, whereas cohesiveness is defined as how well the product withstands a second deformation relative to resistance under the first compression. Gumminess and chewiness are applicable for semi-solid and solid products, respectively and they are calculated using the hardness scores of samples. Resilience is defined as how well a product fights to regain its original height (Anonymous, 2021). The lowest hardness (198.69 N) was observed from the control, while the highest value (330.86 N) was determined from 3% microalgae added sample. The increasing *Chlorella* concentration caused higher hardness, but increasing concentration from 0.5 to 1.5% did not significantly increase the hardness. A linear and significant increase in hardness was also determined by Gouveia, vd. (2007) for cookies enriched with *Chlorella vulgaris* between 0.5 and 3% concentrations. Authors stated that protein and carbohydrate molecules of microalgae can play an important role on the water absorption, which promotes the firmness of cookies. In another study, the increasing concentration of defatted microalgae resulted in higher cookie hardness related with the ash content of cookie dough (Sahni, vd., 2019). Authors stated that gluten proteins in wheat constitute one third of glutamine and low content of acidic and basic amino acids which results in low charge density on protein surfaces. This low charge density surfaces of proteins makes them sensitive to mineral salts and mineral ions result in suppression of charge and allow molecular interactions via hydrogen bonding (Gujral, vd., 2008). Fracturability of cookies also increased with microalgae addition. However, fracturability values were statistically similar for control and 0.5%, and 1.5 and 3%. Similar fracturability results were also reported by Bashir, vd. (2020) for cookies produced with pearl millet and flaxseed flour. The highest springiness, cohesiveness, gumminess and chewiness values were observed from 3% *Chlorella* incorporated sample, whereas the lowest values were determined from control. Resilience results of cookies increased insignificantly up to 3% microalgae addition, and these results were also in accordance with the recovery percentage (Table 5) which 3% *Chlorella vulgaris* added cookie dough showed the highest %R value.

Sensory properties

The sensorial evaluations of cookies produced with *Chlorella vulgaris* at various concentrations are provided in Fig. 7. Cross-section appearance, taste, overall acceptability and affordability scores of cookies containing 0.5 and 1.5% microalgae did not significantly change when compared with control. However, the highest surface appearance was detected from the sample containing 0.5% *Chlorella*. The increasing concentration of microalgae at 3% significantly decreased all the sensorial properties, except for texture which decreased insignificantly. It is clear in the Fig. 7 that *Chlorella vulgaris* addition at 0.5 and 1.5% reached nearly 7 point for all sensorial attributes which shows consumers decided as "*like moderately*". Texture scores of samples were negatively in accordance with the hardness which increased with microalgae concentration while texture decreased. It can be concluded from the sensorial scores that 0.5 and 1.5% of *Chlorella* could be well used for the production of low-fat cookies, whereas 3% addition is less acceptable. The results of sensory analyses of microalgae based products such as cookies (Egea, vd., 2014; Sahni, vd., 2019), pasta (Fradique, vd., 2013; Zouari, vd., 2011) and yoghurt (Dubey ve Kumari, 2011) were in accordance with our findings.

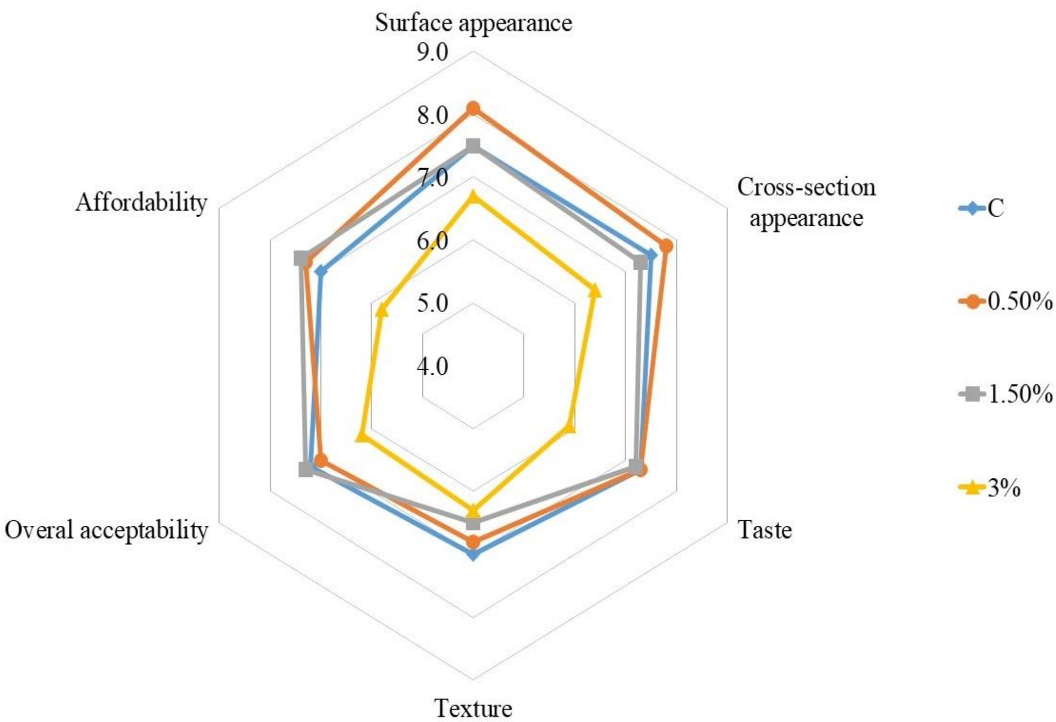

Fig. 7 The effect of *Chlorella vulgaris* addition on sensorial properties of low-fat cookies

CONCLUSIONS

Different concentrations of microalgae addition to the reduced-fat cookies significantly affected the properties of both cookie dough and cookies. Cookie doughs displayed viscoelastic characteristic due to higher G' than G'', and the increasing microalgae concentration significantly improved the viscoelasticity of doughs. Non-linear regression results and curves of 3ITT showed that the addition of *Chlorella* to cookie doughs improved the recovery of samples after high shear deformations such as processing, shaping and handling. Burgers and exponential decay models well explained the creep and recovery data, and via these tests the internal structure and elastic and viscous behavior of cookie doughs were better understood, and also the recovery results were in accordance with 3ITT results. Microalgae incorporation significantly increased the protein, ash and fat content of cookies, as well as spread ratio. *C. vulgaris* incorporated cookies presented mainly green tonalities that became significantly darker (L^*), greener (a^*) and less yellow (b^*) when increasing biomass concentration, and the inner and outer layers of cookies showed great total color differences (ΔE>12). *Chlorella* incorporation provided a significant structuring effect, in terms of cookies texture. Moreover, 0.5 and 1.5% microalgae added cookies sensorial pointed as nearly 7 for all sensorial attributes which means consumers "*liked moderately*" to cookies. This study suggests that low-fat cookies could be produced by using *Chlorella vulgaris* at 0.5 or 1.5%, and microalgae can also be considered as a suitable ingredient, enhancing textural, rheological and physical properties of low-fat cookies. In addition, with the improvement of sensory qualities with increasing microalgae concentration up to 1.5%, microalgae based low-fat cookies may become widely appreciated and consumed functional foods in the future.

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CONFLICT of INTEREST

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

AUTHORS' CONTRIBUTIONS

Ayse Neslihan Dundar: Conceptualization, Formal analysis, Investigation, Writing-Review & Editing. Oya Irmak Sahin: Conceptualization, Formal analysis, Investigation, Resources, Writing-Review & Editing. Furkan Turker Saricaoglu: Conceptualization, Formal analysis, Investigation, Writing-Original Draft, Visualization.

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