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ORIGINAL ARTICLE

Functional and sensorial properties of cookies enriched with *SPIRULINA* and *DUNALIELLA* biomass

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Abstract The effects of *Spirulina platensis* and *Dunaliella salina* biomass (1% and 2%) on formulated cookies were studied. Colour, hardness, moisture content, ash content, protein content, lipid content, total phenolic content and total antioxidant activity by CUPRAC were assessed, and a sensory evaluation of the cookies was performed. The results show that the cookies baked with added *Spirulina* were significantly harder and darker than the controls, and the *Dunaliella* addition did not affect the protein content as much as the *Spirulina* addition, but the effect on moisture content was significantly positive. Additionally, higher total phenolic content and CUPRAC values were found for the *Dunaliella*-enriched cookies. After the sensory evaluation, the *Dunaliella*-enriched cookies were more acceptable to consumers.

Keywords *Spirulina platensis* · *Dunaliella salina* · Cookies · Chemical composition · Physical properties · Microalgae · Food

Introduction

Microalgae have been used for many years in human and animal nutrition and have received increasing attention due to their nutraceutical and therapeutic functions. They have attracted great attention due to their important therapeutic applications; offer a protective mechanism against diabetes and obesity, and are a promising source of protein and other biologically active ingredients (de Morais et al. 2015; Khan et al. 2018).

Spirulina biomass contains a highly digestible protein with a significant amount of gamma-linolenic acid and is a major source of each essential amino acids (Khan et al. 2018). It is also a source of structurally diverse exopolysaccharides, chlorophylls and phycobiliproteins that have multiple pharmaceutical functions, such as antioxidant, antitumor, antihyperlipidemic, neuroprotective, hepatoprotective, antibacterial and anticoagulant activities (Bishop and Zubeck 2012; García-Segovia et al. 2017).

The other most cultivated microalgae, *Dunaliella* sp., synthesises high amounts of carbohydrates, β-carotene, glycerol, Vitamin C and Vitamin A under stress conditions as salinity, irradiance and nutrient-limitations (Bishop and Zubeck 2012). Dunaliella biomass has great benefits in terms of the molecular improvement of recombinant proteins and decreasing the risk of cancer (Borowitzka and Siva 2007), making it useful for therapeutic, dietetic and industrial applications.

Due to these characteristics and its approval as 'generally recognised as safe' by the FDA, microalgae, especially *Arthrospira* (*Spirulina*) *platensis*, are widely used in the food industry as an ingredient in specialty food bars, powdered nutritional drinks, popcorn, beverages, fruit and vegetable juices, frozen desserts and condiments.

Increased consumer demand on healthy and functional foods has led to the development of low-fat, low-salt, gluten-free and sugar-free foods. In the presented work, *Spirulina platensis* and *Dunaliella salina* microalgae biomasses were used in homemade sugar-free cookie formulations in order to study the influence of the microalgae biomass on chemical characteristics, textural properties and sensorial properties.

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Materials and methods

Materials and sample preparation

Spirulina biomass (Fig. 1a) was obtained from the cultivation of *Arthrospira* (*Spirulina*) *platensis* (UTEX LB 2340) at 25 ± 2 °C for 25-day cultivation in Spirulina medium with a 12:12 lightening period at 3200 lux and *Dunaliella* biomass (Fig. 1b) was obtained from the cultivation of *Dunaliella salina* (UTEX LB 1644) at 20 ± 2 °C for 30-day cultivation in Erdschreiber's medium with a 12/12 lightening period at 3200 lux, and all biomasses were freeze dried after harvesting at Food Biochemistry and Biotechnology Laboratory, Chemical and Process Engineering Department, Yalova University.

The cookie formulas used in the study were not commercially produced and obtained from people who avoid added sugar in their diet. Many recipes were ultimately used after reviewing after homemade recipes suitable for gluten-free and no-added-sugar diets. After several sensory analyses, the cookies (Fig. 2) were prepared according to the formulations as stated below. The control group was produced with whole-wheat flour (coded as CB), and in the experimental groups, flour was replaced with a proportion of 1% and 2% Spirulina (coded as BS1, BS2) and Dunaliella (coded as BD1, BD2) powder.

Whole-wheat flour, rice flour and semolina (Makarnalutfen, Kırklareli, Turkey), 27.9%, 30.04% and 9.12% respectively, were mixed with milk and butter (Torku, Konya, Turkey), 6.12% and 26.82% respectively, to obtain a homogenous structured dough. After a period, giving various pulp sheet, rolling pins were used to form the dough into the desired shape, and the cookies were baked at 175 °C for 25 min in an oven (MS 9619 Arçelik, Turkey). After cooling, some cookies were taken for physicochemical and sensorial analysis. Other samples were stored at − 80 °C until further analysis. All results are given as averages (n = 3). Each replicate was obtained from separately prepared doughs.

Analysis

Colour measurements of samples were determined using a chromameter (Konica Minolta CR-400/CR41, Japan) on the triple scale consisting of CIE colour values (L*, a*, b*), L* = 100 white, L* = 0 black; high positive a* = red, high negative a* = green; and high positive b* = yellow, high negative b* = blue (Gouveia et al. 2007). Measurements were taken from five places, including the centre and each quarter.

Textural measurements were made by adjusting the parameters as follows: the pre-test speed to 1.00 mm s^{-1}, the test speed to 2.00 mm s^{-1}, the torque to 50 N and the distance to 5 mm, using a texture analyser (TA XT Plus Texture Analyser) that corresponds to the hardness value. Each cookie was cut into a 2.5-cm sided cube, with the upper and lower crusts eliminated. A 75-mm diameter aluminium plate (P/75) was used for compression. Measurements were replicated eight times, in duplicate, at room temperature (25 °C) (Çiftçi 2018).

The proximate composition was determined according to the AACCI (1990) and AOAC (1990): protein (AACCI: 46.12) with a conversion factor of 6.25, lipid (AOAC: 948.22), ash (AACCI: 08.01) and moisture (AACCI: 44.01). Carbohydrates were quantified by difference.

The phenolic content of the samples was determined using the Folin–Ciocalteu method (Marcinkowska-Lesiak et al. 2018). All measurements were performed in triplicate, and results were expressed as gallic acid equivalents per g (mg GAE g^{-1}) per sample.

Total antioxidant capacity was analysed via spectrophotometer (Shimadzu UV-1280, Japan) using the CUPRAC method (Apak et al. 2004). All measurements were performed in triplicate, and results were expressed as gallic acid equivalent per g (mmol GAE. g^{-1}) per sample.

The sensorial evaluation of samples was carried out by untrained panellists (n = 20, men and women aged 15–50 years) in a standard panel room. The proof sheet contained a list of sensory descriptors (colour, taste, appearance, mouthfeel, crispness, hardness and overall

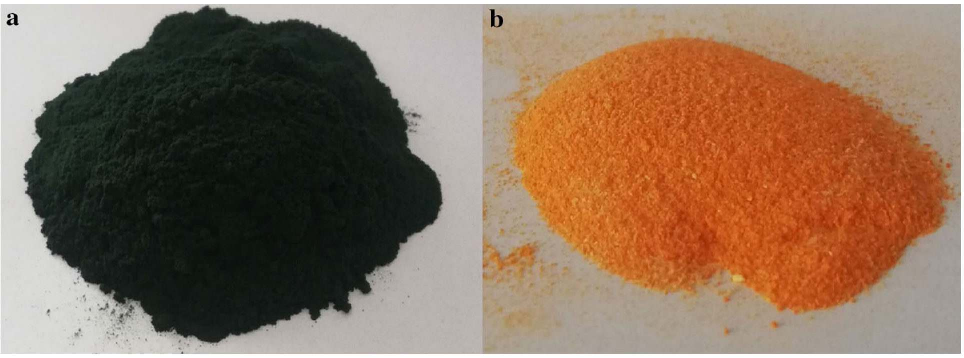

Fig. 1 Spirulina (Arthrospira platensis) (**a**) and Dunaliella salina (**b**) biomasses

Fig. 2 Cookies (**a**) control CB, **b** BS1; 1% Spirulina added, **c** BS2; 2% Spirulina added, **d** BD1; 1% Dunaliella added, **e** BD2; 2% Dunaliella added

acceptability). A 5-point tasting chart was used, with 1 representing highly unpleasant and 5 representing highly enjoyable.

Statistics

Data were submitted to variance analysis (ANOVA) and the difference among the means were determined using Tukey's test, with a confidence interval of 95% ($p < 0.05$).

Results and discussion

Physical properties

The colour parameters of the cookies were characterised as follows: green tonality for *Spirulina* and orange-yellow tonality for *Dunaliella*. The obtained parameters—lightness (L^*), greenness (a^*), yellowness (b^*) and chroma (C^*)—are presented in Table 1. The control samples presented positive and small a^* (1–2) values and high b^*

Table 1 Technological characteristics of cookies

	L^*	a^*	b^*	C^*	Hardness
CB	$49.24^{a} \pm 1.63$	$1.23^{c} \pm 0.33$	$28.46^{c} \pm 1.65$	$28.49^{c} \pm 1.13$	$12.30^{c} \pm 0.20$
BS1	$42.86^{c} \pm 1.23$	$-1.88^{d} \pm 0.38$	$16.24^{d} \pm 1.39$	$16.35^{d} \pm 0.88$	$13.20^{b} \pm 0.94$
BS2	$36.62^{d} \pm 1.39$	$-2.15^{e} \pm 0.43$	$9.39^{e} \pm 1.43$	$9.63^{e} \pm 0.89$	$15.38^{a} \pm 0.40$
BD1	$45.67^{b} \pm 1.43$	$4.37^{b} \pm 0.45$	$29.22^{b} \pm 0.93$	$29.54^{b} \pm 1.20$	$10.08^{d} \pm 0.48$
BD2	$48.84^{a} \pm 1.45$	$5.65^{a} \pm 0.33$	$31.54^{a} \pm 1.18$	$32.04^{a} \pm 1.26$	$9.23^{e} \pm 0.85$

Results are expressed as average ± standard deviation (n = 3). Different letters in the same column correspond to significant difference ($p < 0.05$)

(25–30) values, which indicated the main tonality. Despite the reduction in L^* for all samples, *Spirulina* and *Dunaliella* microalgae affected the colour parameters in the opposite way, as an increase in the *Spirulina* concentration led to lower chromatic parameters with negative a^* and hue values, while an increase in the *Dunaliella* concentration led to higher parameters for all samples (Table 1).

By increasing the *Spirulina* biomass concentration, lightness (L^*) significantly decreased ($p < 0.05$), with, as expected, significant green colour ($- a^*$) increases ($p < 0.05$) and the yellow colour (b^*) decreasing significantly ($p < 0.05$). Increase of $- a^*$ value is characteristic of the high chlorophyll content in blue–green algae, also expressed by Batista et al. (2017). These results are similar to research presented by Gouveia et al. (2007, 2008) in which *Chlorella vulgaris* and *Isochrysis galbana* biomass were used as colorants in cookies; as the microalgae concentration increased, L^*, a^* and b^* values decreased significantly. Achour (2014) and Figueira et al. (2011) also demonstrated that as more *Spirulina* is added, the green colour increases, but the luminosity also decreases.

As *Dunaliella salina* biomass is high in β-carotene and as the biomass is orange coloured, fortified cookies' a^* and b^* values increased significantly ($p < 0.05$) with the increasing algae concentration compared to the control samples. The addition of microalgae biomass resulted in much darker cookies in relation to the control where lightness (L^*) significantly decreased but yellowness (b^*) significantly increased as level of algae increased which is similar to the the findings of El-Baz et al. (2017).

The texture evaluation of the samples, including the control, relied on the hardness parameter; as can be observed in Table 1, the microalgae addition significantly ($p < 0.05$) affected the texture. The *Spirulina*-enriched cookies showed linearly increasing values with the biomass concentration. This positive effect of *Spirulina* biomass can be related to the high protein content (∼ 65%), which may have strengthened the cookie dough. Gouveia et al. (2007, 2008) stated that protein and carbohydrate molecules play a profound role in emulsion formation and water absorption processes and that firmness increases with biomass content, which may seem unexpected for *Dunaliella*-enriched cookies, as the hardness of the cookies decreased significantly ($p < 0.05$), unlike the outcome for the *Spirulina*-enriched ones.

Chemical composition

Table 2 presents the proximate chemical composition of the *Spirulina*- and *Dunaliella*-enriched cookies. While *Spirulina* has been used in many food products as an ingredient for improving the nutritional quality (Malik et al. 2013; Mazinani et al. 2016), there have been few studies of *Dunaliella*-enriched food products.

As the formulation of cookies is profoundly different from that for commercial cookies, the moisture content values in particular are different compared to other bakery foods. Moisture contents ranged from 10.37 to 13.08% and were significantly affected by the microalgae species and concentration ($p < 0.05$). There was no significant difference in ash content due to the microalgae species or concentration, even though the ash content of *Dunaliella* biomass is 42.81% (Table 2). This may be the reason for the low concentrations of microalgae biomass additions. Previous studies on cheese (Agustini et al. 2016), yogurt (Barkallah et al. 2017), ice cream (Malik et al. 2013) and croissants (Shalaby and Nabih 2013) have reported that the ash content has no significant relation to the microalgae concentration, in contrast to findings on bread (Menezes et al. 2015), cookies (Batista et al. 2017) and snacks (Lucas et al. 2017, 2018).

Owing to the fact that *Spirulina* biomass is richer in protein than *Dunaliella* biomass, the cookies with *Spirulina* showed a higher protein content (Table 2). There was a significant protein increase from 14.98 to 16.66% in all samples. The presented findings are similar to previous studies on noodles (Prabhasankar et al. 2009), bread (Menezes et al. 2015) and cookies (Batista et al. 2017). An 11.0% and 3.6% increase of protein content was found for the cookies with 2.0% added *Spirulina* and *Dunaliella*, respectively. These protein values are lower than those for enriched cookies reported by Batista et al. (2017), and differences in cookie formulations may be the reason.

Table 2 Proximate composition of biomasses and cookies

	Moisture	Ash	Protein	Lipid	Carbohydrate*
Spirulina	5.22 ± 0.00	10.71 ± 0.02	65.00 ± 0.09	2.52 ± 0.45	16.57
Dunaliella	6.16 ± 0.00	42.81 ± 0.06	22.18 ± 0.00	3.00 ± 0.37	25.85
CB	13.08^a ± 0.00	1.70^a ± 0.00	14.98^e ± 0.20	29.70^a ± 0.40	40.54
BS1	12.58^b ± 0.01	1.68^a ± 0.00	16.13^b ± 0.34	27.52^d ± 0.19	42.09
BS2	11.36^c ± 0.01	1.66^a ± 0.00	16.66^a ± 0.70	26.54^e ± 0.09	43.78
BD1	12.25^b ± 0.00	1.74^a ± 0.00	15.10^d ± 0.28	28.22^b ± 0.18	42.79
BD2	10.37^d ± 0.00	1.77^a ± 0.00	15.52^c ± 0.66	27.85^c ± 0.06	44.49

Results are expressed as average ± standard deviation (n = 3) and in percentage unit. Different letters in the same column correspond to significant difference (p <0.05)

*Carbohydrate content calculated by difference

While the protein content of the cookies increased, the lipid content decreased, as expected, from 29.70 to 26.54%. The *Spirulina*-enriched samples had the lowest lipid content, as they had a higher protein content. In contrast to the findings of Agustini et al. (2016), the change in lipid content was significant ($p < 0.05$). According to Figueira et al. (2011), enriching bread with *Spirulina* reduces the lipid content, as in Barkallah et al. (2017) and Şahin (2019).

Phenolic content and antioxidant capacity

Recent studies have reported high antioxidant activity of microalgae species (Barkia et al. 2019; Tibbetts et al. 2015). Phenols, flavonoids, tannins, lignins, phenolic acids and their derivatives are phenolic compounds and synthesised as secondary metabolites. They are considered as one of the most important compounds known as natural antioxidants and have attracted great interest for their health benefits (Machu et al. 2015).

The high bioactive compound contents of enriched cookies can be explained by the presence of large amounts of phycocyanin (173 µg g^{-1}) in *Spirulina* and of β-carotene (115 µg g^{-1}) in *Dunaliella* (Table 3). According to the results, microalgae-supplemented products are a good source of phenolic compounds. In addition, the concentration of microalgae in the cookies had a significant effect on the total phenolic content. Moreover, although the phycocyanin content of *Spirulina* is very high, the phenolic content was higher in the cookies fortified with *Dunaliella*. The reason is that despite the high levels of carotenoids, chlorophyll and phycocyanin in *Spirulina*, *Dunaliella* provides the highest concentration of natural carotenoids of all plants and algae (Tang and Suter 2011).

Similar trends have been observed regarding the total phenolic content of foods enriched with microalgae biomass (Rodríguez De Marco et al. 2014). Bolanho et al. (2014) reported an increase from 1.4 to 2.3 mg GAE g^{-1} in total phenolic content for 5.0% *Spirulina*-enriched (12 mg GAE g^{-1}) cookies; that value is low compared to the present study's findings on the *Spirulina*-enriched cookies (1.6, 2.1 and 2.5 mg GAE g^{-1} for the control, 1.0% *Spirulina* and 2.0% *Spirulina* cookies, respectively). Batista et al. (2017) evaluated *A. platensis*, *C. vulgaris*, *T. sueccia* and *P. tricornutum* biomass additions of 2.0% and 6.0% to cookies and found higher contents of phenolics for 6.0% *A. platensis*- and *P. tricornutum*-enriched cookies (0.90 and 0.62 mg GAE g^{-1}, respectively). Rózylo et al. (2017) studied in vitro antioxidant property changes of brown-algae-enriched gluten-free bread and reported that 6.0% and higher biomass additions significantly affected the total phenolics.

Table 3 Total phenolic content and antioxidant capacity of biomasses and cookies

	TPC (mg GAE g^{-1})	TAC (mmol GAE $g^{-1)}$ (CUPRAC)
Spirulina	34.22 ± 0.00	0.09 ± 0.02
Dunaliella	53.25 ± 0.00	0.12 ± 0.06
CB	1.60^e ± 0.00	0.50^e ± 0.00
BS1	2.10^d ± 0.01	0.85^d ± 0.00
BS2	2.50^b ± 0.01	1.20^b ± 0.00
BD1	2.30^c ± 0.00	0.92^c ± 0.00
BD2	3.00^a ± 0.00	1.76^a ± 0.00

Results are expressed as average ± standard deviation (n = 3) and in percentage unit. Different letters in the same column correspond to significant difference (p <0.05)

Compared to the control cookies, the incorporation of algae significantly ($p < 0.05$) affected the antioxidant capacity of all samples. In particular, the 2.0% addition of *Dunaliella* increased the antioxidant capacity about three-fold, from 0.50 to 1.76 mmol GAE g^{-1}. Phycocyanin is highly degradable during baking or cooking processes, which may explain the smaller effect of *Spirulina* incorporation compared to *Dunaliella* incorporation. Some researchers have also observed this small increase for *Spirulina*-enriched foods, which is due to phycobiliproteins (El-Baki et al. 2009; Rodríguez De Marco et al. 2014), and the present results are in agreement with those findings.

Sensorial evaluation

The sensory evaluation of the cookies is illustrated in Fig. 3. The panellists, in their comments, referred to the similar taste, flavour and texture of cookies that are commercially marketed, which is why the control samples were evaluated with small ranks. The *Spirulina*-enriched cookies, for all concentrations, scored 3 points, except on flavour. With higher *Dunaliella* content, the flavour attributes significantly decreased as compared to the *Spirulina*-enriched cookies. The overall acceptable results indicated that all samples had a good sensorial score, but the most preferable one was the 1.0% *Dunaliella*-enriched cookies.

In terms of flavour, the 1% *Dunaliella* samples were the top rated (4.70 ± 0.02) when compared to the *Spirulina*-enriched and control samples. As reported by Marcinkowska-Lesiak et al. (2018), higher concentration of *Spirulina* received lower scores than control samples for traditional Iranian cookies, a finding in accordance with the presented results.

For colour and appearance, again, all *Dunaliella* samples (1% and 2%) scored higher on colour (4.40 ± 0.11 and 4.40 ± 0.05) and appearance (4.10 ± 0.08 and 4.50 ± 0.06) due to their homogenous and attractive yellow-orange colour. The lower scores for the *Spirulina*-enriched samples may reflect that a green tonality is not an acceptable colour for bakery products.

Batista et al. (2017) have reported a preference for *A. platensis*-enriched cookies in relation to *C. vulgaris*. Spirulina addition to cookies were scored higher than Dunaliella addition in terms of crispness and hardness, and also found highly attributed for their flavour.

Fradique et al. (2013) have reported lower sensory scores, including “strange flavor” detection for pasta enriched with the marine microalgae *Isochrysis galbana* and *Diacronema vlkianum*. Although being a marine

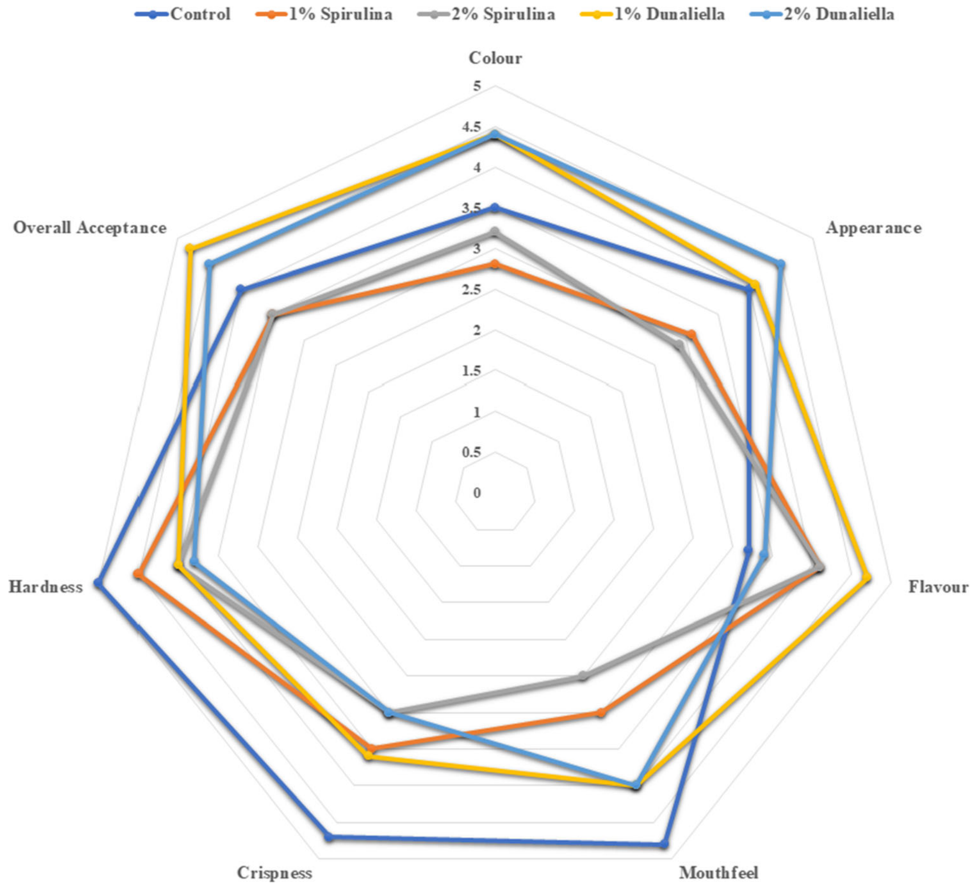

Fig. 3 Ranking of the cookies by panelists’ evaluations

microalga, *Dunaliella salina* enriched cookies were preferred in terms of mouthfeel, colour, appearance and general appreciation while Spirulina platensis addition scored lower.

Conclusion

In the context of healthy and nutritious functional food concerns, healthy new ingredients and improvements to new functional foods are receiving attention. These 'new' term can be fortified with microalgal biomass, which is high in protein, fatty acids, phenolics and/or antioxidants. In addition to the health and nutrition benefits, such foods should be delicious and aromatic. *Spirulina* (*A. platensis*) and *Dunaliella* (*D. salina*) are two of the most cultivated and consumed microalgae all over the world, as biomass itself or as an ingredient. In the presented study microalgae biomasses were used as an ingredient successfully. The results of this cookie enrichment study show that the higher protein content of microalgae species improves the nutrition value of enriched foods. The green *Spirulina* biomass was not preferred by the panellists for the natural grain-coloured cookies, in case of *Dunaliella* biomass. Therefore, *Dunaliella salina*, with its high phenolic content and antioxidant capacity, can be an option for improving functional cookies.

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