



Effect of Fucoïdan Extracted From *Sargassum angustifolium* (Brown Seaweed) on Oxidative Stability of Butter Produced From Sour Cream



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Ahmad Alipour¹, Mohammad H. Marhamatizadeh^{1*} and Mahdi Mohammadi²

¹Department of Food Hygiene, Kazerun Branch, Islamic Azad University, Kazerun, Iran.

²Department of Biotechnology, Persian Gulf Studies and Research Center, Khalij Fars University, Bushehr, Iran.

FUCOIDAN extracted from seaweed, particularly *Sargassum angustifolium*, is considered as natural antioxidant agent. The present survey was aimed to assess the effect of fucoïdan extracted from *S. angustifolium* on oxidative stability of butter produced from sour cream. Fucoïdan was extracted from *S. angustifolium* samples. Prepared fucoïdan powdered were weighted in 0.05, 0.1, 0.3, 0.5 percent and added to butter samples. All samples were then kept in the refrigerator up to 60 days. The DPPH radical scavenging effects of extracted fucoïdan and also acidic and peroxide values of butter samples were evaluated. Fucoïdan (100 µg/mL) had the uppermost DPPH radical scavenging effect (56.46±5.27%), while that of 12.50 µg/mL had the lowermost (34.75±3.26%). Acidic value of butter samples had been increased during the storage period ($P<0.05$). Butter samples of the control group had the highest acidic value in the 60th day of the storage (0.40±0.033 mg KOH/g butter), while those treated with the 0.5% fucoïdan had the lowest (0.17±0.013 mg KOH/g butter). Peroxide value was increased from day 0 to day 40 of storage period and then decreased. Butter samples of the control group had the highest peroxide value in the 40th day of the storage (20.71±1.65 meq/kg butter), while those treated with the 0.5% fucoïdan had the lowest (0.17±0.013 meq/kg butter). Using 0.5% fucoïdan caused lower increase in the levels of peroxide and acidic values of butter samples during the 60 days storage period. The antioxidant effects fucoïdan extracted from *S. angustifolium* seaweed is dose-dependent.

Keywords: *Sargassum angustifolium*, Fucoïdan, Oxidative stability, Sour cream butter.

Introduction

Oxidation caused by natural factors such as air, light and temperature is one of the important factors caused decrease in the shelf-life of butter during storage and processing. Oxidative stress caused severe decrease in the quality and safety of butter and make it inappropriate for routine consumption [1]. Thus, it is imperative to inhibit from the occurrence of oxidation on butter and other related products. Butter produced from the sour cream is a extensively popular fermented acidified dairy product which contains all the necessary main nutrients such as fat, protein,

minerals and some kinds of vitamins in appropriate proportions.

Severe complications of using synthetic antioxidant such as their teratogenic, mutagenic, toxigenic, and even lethal effects caused considerable decrease in their uses. Thus, the tendency to application of natural antioxidant agents including extracts and essential oils has been increased [2].

Sargassum angustifolium (*S. angustifolium*) is a brown seaweed distributed in the Persian Gulf, Iran. It is significant source of carbohydrates,

Corresponding author, Mohammad H. Marhamatizadeh Alavi, Email, Drmarhamati@gmail.com, Tel., +989177210645.

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protein, vitamins, minerals, and antioxidants [3]. Sulfated polysaccharides is the major component of the *S. angustifolium* and responsible for diverse biological effects. Sulfated hemi-ester groups of polysaccharides are devoted to the sugar units called fucoidan [4]. Fucoidan produces fucose-based and also galactose-based polysaccharides with fucose or fuco-oligosaccharide branches, and/or xylose, glucuronic acid, or glucose and even protein and acetyl replacements [3, 4]. Antitumor, anti-inflammatory, antiviral, antimicrobial, wound healing, and antioxidant effects of fucoidan extracted from diverse kinds of algae have been confirmed previously [5].

According to the high antioxidant effects of fucoidan extracted from *S. angustifolium* and high distribution of this algae in the Persian Gulf, the present research was aimed to study the effect of fucoidan extracted from *S. angustifolium* on oxidative stability of butter produced from sour cream.

Materials and Methods

Study design

All solvents and chemicals used in the present research were manufactured by the Merck company (Merck, Germany) with a high degree of analytical purity. The butter used in this study was made from sour cream and packed in 100 g containers. From June to August 2019, *S. angustifolium* brown algae was collected from the coast of Bushehr port, Iran. Samples of algae were directly transferred to the Marine Biotechnology Laboratory, Persian Gulf University, Bushehr, Iran,

Fucoidan extraction

Fresh *S. angustifolium* was dried in shade and powdered. After that, 20 g of the powdered *S. angustifolium* samples were mixed with 1000 mL ethanol (85%) to remove pigments and proteins and shake for 12 h at room temperature. then, samples were washed with acetone and centrifuged at 1800 rpm for 10 min. Then, 5 g of the achieved sample was mixed with 100 mL of distilled water at 65 °C for 1 h and centrifuged at 18500 rpm for 10 min. After that, the supernatant was collected and concentrated using oven. It was then mixed with 1% calcium chloride at 4 °C and kept steady overnight for alginate deposition. After centrifuge, the supernatant was collected and mixed with 96% ethanol and stored overnight at 4 °C. An achieved polysaccharide materials were then dehydrated by acetone and ethanol

(30%) and dried in room temperature. Achieved fucoidan was then filtered (0.45 nm, ALBET-NY-045-47-BL, Spain) and washed with acetone and ethanol and dried in room temperature [6].

Butter preparation from sour cream

After preparing the yogurt from the fresh milk, the yogurt was kept in the container for a few days to make it sour. Then, the existing cream which was acidified with lactic acid, was transferred to churn machine and after adding water, it was stirred and the resulting butter was collected [7].

Sample preparation

Prepared fucoidan powdered were weighted in 0.05, 0.1, 0.3, 0.5 percent and added to butter samples. The control butter group didn't contain any fucoidan contents. Samples containing fucoidan and control samples were packed in 100 g containers with two coats of oily toilet paper and thin aluminum foil. All samples were then kept in the refrigerator. Sampling was done at days 1, 20, 40, and 60 after storage.

Antioxidant effects of fucoidan

Antioxidant effect of fucoidan was measured on the first day of production. Antioxidant effects of fucoidan in butter samples were assessed using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay. Totally, 25 mg of DPPH was dissolved in 100 ml of methanol. Then, this solution was diluted with methanol (1,10). Then, various dilutions of fucoidan (12.5-100 µg/mL) were prepared. Then, 0.1 mL of the fucoidan and 3.9 mL of DPPH solution was shaken and incubated at room temperature in the dark for 30 min. The absorbance of samples was measured at 517 nm. Methanol was used to adjust zero and DPPH-methanol solution used as a control sample. L-ascorbic acid and Gallic acid were used as two positive controls. DPPH radical scavenging effect of fucoidan was measured using the following formula [8],

$$\text{Scavenging effect (\%)} = [1 - (\text{A Sample } 517) / (\text{A Control } 517)]$$

Acidic value

To measure the acidity of butter samples, 20 g of butter was dissolved in 100 mL of equivalent volume of ethanol-chloroform. This solution was titrated in the presence of phenolphthalein until the stable purple color appeared with 0.1 N potassium hydroxide [9].

Peroxide value

To measure the peroxide value, 5 g of butter was dissolved in 30 mL of acetic acid-chloroform

solution (3,2). Then, 0.5 mL of saturated potassium iodide solution were added to previous solution and placed in a dark place for one minute. After this period, 30 mL of water and 0.5 mL of 1% starch solution were added to it. The released iodine was titrated to 0.01% normal sodium to be discolored with sodium thiosulfate [9].

Statistics

All experiments were carried out in triplicates. Statistical analysis was performed using the SPSS software (Ver 21, USA). Analysis of Variance (ANOVA) and Duncan test were performed to obtain any statistical differences.

Results

DPPH radical scavenging activity

In this study, the effect of different concentrations of fucoidan extracted from *S. angustifolium* was evaluated on the quality of butter samples prepared from sour cream. Table 1 reveals the DPPH radical scavenging effects of diverse concentrations of fucoidan extracted from *S. angustifolium*. Fucoidan with concentration

of 100 µg/mL had the uppermost DPPH radical scavenging effect (56.46±5.27%), while that with concentration of 12.50 µg/mL had the lowermost (34.75±3.26%). Statistical significant difference was obtained amid the fucoidan concentration and its DPPH radical scavenging effect ($P < 0.05$).

Acidic value

Table 2 reveals the acidic value of butter samples of control group and those treated with diverse concentrations of fucoidan during the storage period. Acidic value of butter samples of all studied groups has been increased during the storage period ($P < 0.05$). The highest acidic value in the 60th day of the storage period was related to butter samples of the control group (0.40±0.033 mg KOH/g butter), while the at lowest was related to those treated with 0.5% fucoidan (0.17±0.013 mg KOH/g butter) ($P < 0.05$).

Peroxide value

Table 3 reveals the peroxide value of butter samples of control group and those treated with diverse concentrations of fucoidan during the

TABLE 1. DPPH radical scavenging effects of diverse concentrations of fucoidan extracted from *S. angustifolium*.

Fucoidan concentration (µg/mL)	DPPH radical scavenging effects (mean ± SD*)
12.50	34.75±3.26 ^{a**}
25	45.29±4.18 ^b
50	52.30±4.99 ^c
100	56.46±5.27 ^c

*Mean of data ± Standard Deviation.

**Dissimilar small letters in the column disclose statistical difference about $P < 0.05$.

TABLE 2. Acidic value of butter samples treated with diverse concentrations of fucoidan during the storage period.

Butter treatments	Acidic value (mg KOH/g butter) during the storage period (day)			
	1	20	40	60
Control	0.12±0.011 ^{D*a**}	0.19±0.012 ^{Ca}	0.28±0.022 ^{Ba}	0.40±0.033 ^{Aa}
0.05% fucoidan	0.11±0.010 ^{Ca}	0.16±0.014 ^{Bb}	0.20±0.018 ^{Bb}	0.29±0.021 ^{Ab}
0.1% fucoidan	0.11±0.010 ^{Aa}	0.14±0.012 ^{Ab}	0.17±0.016 ^{Ac}	0.22±0.020 ^{Ac}
0.3% fucoidan	0.10±0.010 ^{Aa}	0.13±0.011 ^{Ab}	0.15±0.012 ^{Ac}	0.18±0.015 ^{Ac}
0.5% fucoidan	0.10±0.010 ^{Aa}	0.12±0.011 ^{Ab}	0.14±0.012 ^{Ac}	0.17±0.013 ^{Ac}

*Dissimilar capital letters in each row disclose statistical difference about $P < 0.05$.

**Dissimilar small letters in each column disclose statistical difference about $P < 0.05$.

storage period. Peroxide value of butter samples of all studied groups has been increased during the storage period up to the 40th day of the storage period ($P<0.05$). The highest peroxide value in the 40th day of the storage period was related to butter samples of the control group (20.71 ± 1.65 meq/kg butter), while the at lowest was related to those treated with 0.5% fucoidan (9.10 ± 0.69 meq/kg butter) ($P<0.05$). The peroxide value of all butter samples had been decreased from 40th day of the storage to 60th day. However, the peroxide value of butter samples on 60th day of the storage period was not lower than that of the 20th day of the storage period.

Discussion

Due to the destructive effects of the consumption of synthetic antioxidants such as butyl-hydroxy toluene (BHT), Butylated hydroxyanisole (BHA) and tert-Butylhydroquinone (TBHQ), the tendency of humans to consume new sources with antioxidants properties such has been increased. Fucoidan is a compound polysaccharide originate from diverse species of brown seaweed, particularly *S. angustifolium*. High antioxidant effects of fucoidan introduces it as a useful antioxidant agent. Nevertheless, it's antioxidant potential didn't use to protect fat-based foods such as butter from oxidation [10].

The present survey was aimed to apprise the antioxidant effect of fucoidan extracted from *S. angustifolium* on oxidative stability of butter produced from sour cream. Findings revealed that treatment of butter samples with 0.5% fucoidan caused significant reduction in the acidic and peroxide values. Additionally, fucoidan (100 µg/mL) harbored the highest DPPH radical

scavenging activities. Furthermore, antioxidant effect of the fucoidan was dose-dependent and increase with the upsurge of the fucoidan concentration. Thus, the fucoidan, particularly in higher concentration can use as antioxidant agent to protect food items, especially butter against oxidation.

Generally, at higher concentrations of natural extracts and essential oils, particularly fucoidan, due to the presence of more antioxidant compounds such as phenolic and flavonoid compounds and, consequently, an increase in the number of hydroxyl groups present in the reaction medium, the probability of donating hydrogen to free radicals and subsequently the oxidation inhibitory potency of the extract is increased [11]. Based on the DPPH assay, the hydrogen donation due to the presence of antioxidants reduces the radical adsorption. It appears as a significant color variable from purple to yellow. Findings revealed that the peroxide value decreased from day 40 to 60 after the storage period. The main reason for this finding is maybe the conversion of primary compounds produced from the fat oxidation to secondary components (such as Malondialdehyde (MDA)) results in a reduction of the peroxide levels in butter samples.

Some surveys have been conducted in this field. Kordjazi et al. [12] disclosed that the total phenolic content of the fucoidan extracted from *S. angustifolium* was 9.73 ± 0.00 mgGAE/g which revealed its boost antioxidant effects. Additionally, they showed that the DPPH radical scavenging effect of the fucoidan extracted from *S. angustifolium* was considerable compared with the gallic acid and ascorbic acid. Devi et al. [13]

TABLE 3. Peroxide value of butter samples treated with diverse concentrations of fucoidan during the storage period.

Butter treatments	Peroxide value (meq/kg butter) during the storage period (day)			
	1	20	40	60
Control	4.43±0.34 ^{D*a**}	11.73±1.01 ^{Ca}	20.71±1.65 ^{Aa}	15.25±1.41 ^{Ba}
0.05% fucoidan	4.10±0.29 ^{Da}	9.27±0.85 ^{Cb}	16.16±1.44 ^{Ab}	11.61±1.12 ^{Bb}
0.1% fucoidan	3.95±0.32 ^{Da}	7.61±0.63 ^{Cc}	13.96±1.06 ^{Ac}	9.93±0.92 ^{Bc}
0.3% fucoidan	3.12±0.27 ^{Db}	6.09±0.54 ^{Cd}	11.21±0.97 ^{Ad}	8.40±0.83 ^{Bc}
0.5% fucoidan	2.36±0.19 ^{Cb}	5.82±0.45 ^{Bd}	9.10±0.69 ^{Ac}	6.33±0.53 ^{Bd}

*Dissimilar capital letters in each row disclose statistical difference about $P<0.05$.

**Dissimilar small letters in each column disclose statistical difference about $P<0.05$.

reported diverse DPPH scavenging activities amongst different seaweed species. Mehdinezhad et al. [14] reported that the total phenolic and antioxidant contents of *S. angustifolium* seaweed were 0.061 ± 0.0001 mg/g and 0.231 ± 0.01 IC50 mg/mL, respectively. Similar findings have been reported by Sadati et al. [15], Besednova et al. [16], Costa et al. [17], Lee et al. [18], Campanella et al. [19] and Athukorala et al. [20]. High antioxidant effects of fucoidan extracted from seaweeds was also reported from Brazil [21], New Zealand [22], India [23], Malaysia [24], Nigeria [25] and Korea [26]. Rocha de Souza et al. [27] reported that fucoidan (homofucan) from the edible seaweed *Fucus vesiculosus* harbored the highest antioxidant effects and rendering its edible nature, it can be used as a preservative in food stuffs. Fucoidan extracted from the brown seaweeds is edible and doesn't have any bad effects on the flavour and taste of the produced products [28, 29].

Diverse researches have been focused on the presence of food-borne bacteria in butter and dairy samples produced from contaminated milk [30-36]. Some others reported the high antimicrobial effects of fucoidan extracted from *S. angustifolium* [37, 38]. Thus, using fucoidan extracted from *S. angustifolium* on butter samples can decrease the risk of food-borne pathogens because of high antibacterial effects of fucoidan.

Conclusion

To our knowledge, the present survey is the first using of fucoidan extracted from *S. angustifolium* seaweed to protect from the lipid oxidation and additionally as an antioxidant in the butter samples prepared from sour cream. Findings of the current research revealed that application of 0.5% fucoidan caused lower increase in the levels of peroxide and acidic values of butter samples during the 60 days storage period. Additionally, fucoidan in higher concentrations harbored higher DPPH radical scavenging effects. Thus, the antioxidant effects fucoidan extracted from *S. angustifolium* seaweed is dose-dependent. This is an preliminary survey about the application of fucoidan extracted from *S. angustifolium* seaweed as an edible food preservation to inhibit from the lipid oxidation and increase the shelf-life of butter samples.

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Conflict of interest

Authors declared that they have no conflict of interest.

Ethical consideration

As the work was not conducted on human and animal participants, there was no need to any kinds of ethical consideration.

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