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Fucoxanthin as a Biofunctional Compound in Goat Milk Yogurt: Stability and Physicochemical Effects

Maryuri T. Nuñez de González, Rahmat Attaie *, Selamawit Woldesenbet, Adela Mora-Gutierrez and Yoonsung Jung

Cooperative Agricultural Research Center, Prairie View A&M University, Prairie View, TX 77446, USA
* Correspondence: rattaie@pvamu.edu

Abstract: The food industry has increasingly added nutrients and other ingredients to products to enhance their health benefits. Fucoxanthin is recognized for its benefits in mitigating obesity, diabetes, hypertension, and inflammation. Therefore, addition of fucoxanthin into goat milk yogurt, its stability, and the physicochemical properties of yogurt during processing and storage was investigated. Yogurts with and without fucoxanthin were manufactured by mixing goat whole milk (82.85%, *w*/*w*), powdered goat milk (10.68%, *w*/*w*), and sugar (6.47%, *w*/*w*). Fucoxanthin (0.052 mg/g of yogurt mix) was added to the treatment. The mix was heated at 80 °C for 30 min, cooled, inoculated with a culture, and incubated at 43 °C for 5 h. Fucoxanthin in the yogurt mix and yogurt was quantified by an HPLC method. The recoveries of fucoxanthin from the mix before and after heating were 98.25% and 98.83%, respectively. However, less fucoxanthin (90.13%) was recovered from the freshly prepared yogurt than from the mix. Heating the yogurt mix did not affect the concentration of fucoxanthin but adding the inoculum to the mix reduced its concentration during fermentation. During the storage period, the concentration of fucoxanthin in yogurt remained the same. Fucoxanthin did not adversely affect the chemical composition and physicochemical properties of yogurt, but it influenced the color, decreasing lightness (81.47 ± 0.09), and increasing redness (7.67 ± 0.09) and yellowness (38.24 ± 0.09). Thus, goat milk yogurt can be an effective food matrix to deliver fucoxanthin to human diet.

Keywords: goat milk yogurt; fucoxanthin; stability; physicochemical properties

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1. Introduction

Yogurt is a popular fermented milk product worldwide, and its consumption is gaining popularity due to its nutritional and therapeutic functions [1]. Yogurt is produced through acid fermentation of milk, usually by a mixture of two specific bacterial strains, *Streptococcus salivarius* ssp. *thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*. Other yogurt starter cultures may include *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus lactis*, *Bifidobacterium longum*, *Bifidobacterium bifidus*, and *Bifidobacterium infantis*. During the fermentation process, these starter cultures metabolize the lactose in milk into lactic acid, causing a reduction in pH [2]. The growth of these starter cultures changes the milk components, affecting the physicochemical properties, sensory properties, and the shelf life of the yogurt. The important parameters of yogurt quality, including appearance, acidity, texture, and flavor, are affected by the type of culture and conditions of fermentation.

Milk and milk-based products provide a number of essential nutrients and can be effective matrixes for the release of bioactive compounds [3]. The fat in goat milk is more digestible, and the buffering capacity of goat's milk exceeds that of cow milk and human milk [4]. Moreover, goat milk is recommended for infants and adults who are sensitive or allergic to cow milk proteins [4]. Due to its naturally higher emulsifying capacity, goat milk can be an excellent carrier for delivering lipophilic carotenoids such as fucoxanthin to the human diet.

Probiotic containing yogurt has benefits due to its ability to improve gastrointestinal health and related disorders [5]. Interest exists in developing yogurts that have additional

health benefits (due to probiotic cultures, fortification, or supplementation with bioactive compounds) or have improved sensory characteristics [2]. Thus, we explored the possibility of supplementing goat milk yogurt with fucoxanthin and studied its effects on the physicochemical characteristics of yogurt.

Fucoxanthin, one of the most abundant carotenoids, is extracted from marine brown seaweeds, microalgae, and diatoms. Studies have shown that it has many health benefits, including activity against certain types of cancer, obesity, diabetes, and inflammatory conditions [6–14]. For example, in mice with type 2 diabetes, fucoxanthin at the dose of 400 mg/kg body weight exhibited an anti-diabetic effect and improved the lipid profile [15]. Furthermore, in obese premenopausal women who were non-diabetic and had non-alcoholic fatty liver disease, administration of 7.2 mg/day of fucoxanthin as a supplement resulted in increased energy expenditure and significant weight loss after 16 weeks [16]. In addition, in a 4-week clinical trial on Japanese adults, administration of fucoxanthin capsules (3 mg/day) reduced body weight, body mass index, and abdominal fats [17].

The biological activities of fucoxanthin that benefit human health are associated with its unique chemical structure, which includes an unusual allenic bond, epoxide group, and conjugated carbonyl chain, and its interaction with important biomolecules such as receptor proteins [6]. The potential bioactivities of fucoxanthin as a source of functional food, feed, and medicine have been investigated recently [18].

For fucoxanthin to be considered as an additive to yogurt, factors affecting its stability in such contexts should be considered. During the processing and storage of food, exposure to oxygen, heat, acidic conditions, and metals can potentially accelerate the degradation of fucoxanthin [19,20]. However, milk is reported as a suitable food matrix for the inclusion of fucoxanthin even after pasteurization and storage [21,22]. In terms of stability and bioavailability skimmed milk has been found to be a good food matrix for fucoxanthin [23].

To our knowledge, the stability of fucoxanthin during heat of processing to 80 °C for 30 min has not previously been studied in either cow or goat milks or during the fermentation process of dairy products. We expected that goat milk caseins will be more effective in preventing the chemical degradation of fucoxanthin in foods during the heat of processing and storage than cow milk caseins primarily due to the content of β-casein in goat milk. The β-casein component of goat milk protein is highly hydrophobic in nature and, thus, could form a thick protective layer preventing oxidation of fucoxanthin and enhance the chemical stability of this carotenoid in human foods. Hence, in the current study we produced goat milk yogurt, a novelty product, containing fucoxanthin and determined (1) the stability of the fucoxanthin during processing (heat treatment and fermentation) and storage of yogurt; and (2) the effects of supplemented fucoxanthin on the physicochemical characteristics of the yogurt.

2. Materials and Methods

2.1. Materials and Reagents

Food grade fucoxanthin (20% purity) was purchased from Shandong Jiejing Group Corporation (Rizhao, China). Commercial powdered goat milk and sugar were obtained from Hoosier Hill Farm LLC (Fort Wayne, IN, USA) and Sugar Imperial Co. (Sugar Land, TX, USA), respectively. Fucoxanthin standard, phenolphthalein, trichloroacetic acid (TCA), 2-thiobarbituric acid (TBA), sodium hydroxide (NaOH), tert-butyl methyl ether (TBME), and methanol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ethanol and petroleum ether were purchased from Fisher Scientific (Billerica, MA, USA). All chemicals and reagents used were of analytical-grade or HPLC grade. Deionized water, prepared by passing distilled water over a mixed bed of a cation-anion exchanger, was used throughout this study.

2.2. Manufacture of Yogurt

Goat milk samples were collected from the bulk tank of the milking parlor at the International Goat Research Center at Prairie View A&M University, Prairie View, TX, USA. Figure 1 shows the processing steps of goat milk yogurt production and the points where samples were taken for the physicochemical analyses. Yogurt with fucoxanthin (FXY) at a concentration of 0.052 mg/g of mix and yogurt without fucoxanthin (CY) were manufactured. This concentration of fucoxanthin was chosen to yield 7.9 mg/170 g serving, an amount purported to have health benefits for humans. Yogurt mixes were prepared with fresh goat whole milk (82.85%, *w*/*w*), powdered goat milk (10.68%, *w*/*w*), and sugar (6.47%, *w*/*w*) with or without fucoxanthin. The mix was homogenized (with a hand-held bio-homogenizer), heated at 80 °C for 30 min and then cooled and inoculated with 2% YF-L812 starter culture (Chr. Hansen Inc., Milwaukee, WI, USA). The mixes were incubated at 43 °C until the pH of yogurt reached 4.55; finally, after the end of fermentation the samples were stored at refrigeration temperature (4 °C) for stability studies.

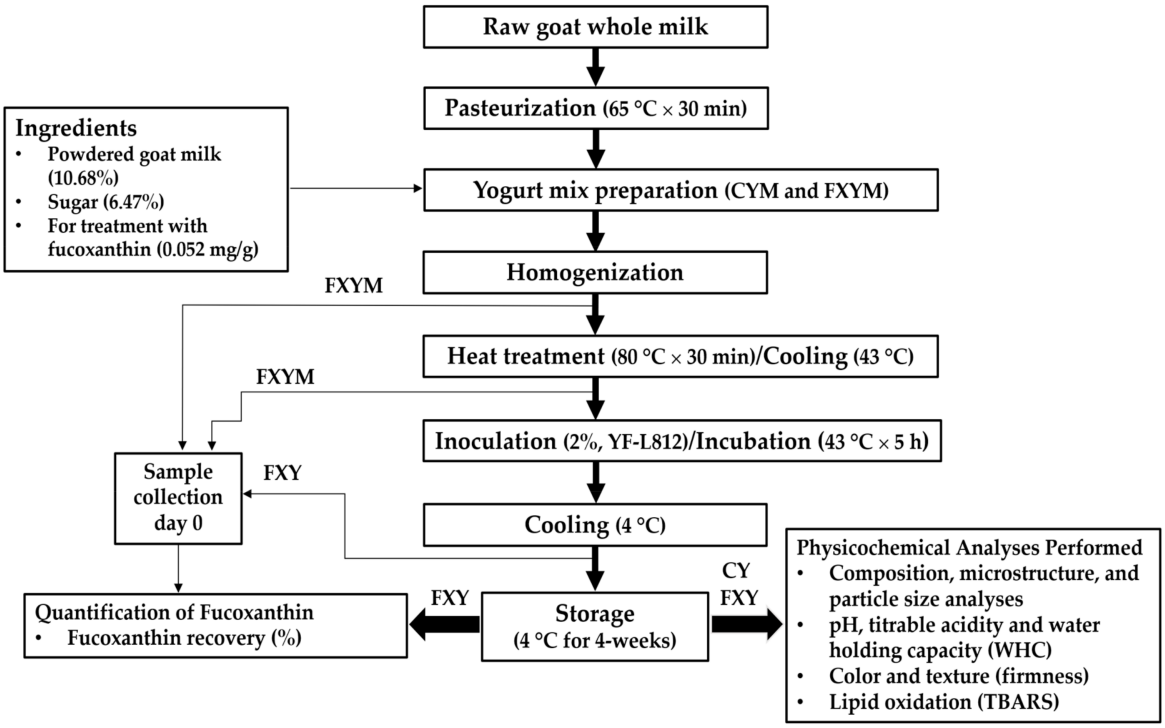

Figure 1. Processing and physicochemical analyses of goat milk yogurt. CYM = Control yogurt mix; FXYM = Fucoxanthin yogurt mix; CY = Control yogurt; FXY = Fucoxanthin yogurt.

2.3. Analysis of Composition of Yogurts

Samples of the control and treatment yogurts were used to determine the percentages of moisture, fat, protein, and ash according to AOAC procedures [24]. Chemical composition of the control and fucoxanthin supplemented yogurts were performed in duplicates on day 0 of manufactured products.

2.4. Quantification of Fucoxanthin

Extraction and quantification of fucoxanthin was carried out according to the method of Mok et al. [22] with some modifications. Briefly, 10-g samples of goat milk yogurt containing fucoxanthin were transferred into 100 mL (Pyrex) volumetric flasks and diluted to 100 g with distilled, deionized water. After thorough mixing of the diluted yogurt, approximately 2 g of this mixture was transferred into 10 mL test tubes, and 2 mL of ethanol (200 proof) was added for deproteination. One milliliter of petroleum ether and then 1 mL of tert-butyl methyl ether (TBME) were added to the extraction tube, and

the content was mixed using vortex for 30 s. The mixture was centrifuged (Avanti J-E centrifuge, Beckman Coulter Inc., Indianapolis, IN, USA) at 3500 rpm (1838 RCF× *g*) for 5 min to extract the fucoxanthin from the food matrix. The supernatant was collected into a 15 mL test tube. The addition of petroleum ether and TBME was repeated three times, and the supernatant was collected each time after the centrifugation step. All the collected supernatants from each sample were placed into a nitrogen evaporator (N-Evap Model 111, Organomation Associates, Inc., Berlin, MA, USA) for drying. The water bath temperature of the nitrogen evaporator was at 35 °C, and the flow rate of nitrogen gas was set at 2. The dried samples were dissolved into exactly 1 mL of 85% aqueous ethanol and then 1 mL of n-hexane was added to the extract to remove milk fat. Then 1 mL of n-hexane with milk fat was removed from the mixture and the samples were completely dried under nitrogen again. The dried samples were dissolved into exactly 1 mL of 90% ethanol and then transferred by 1 mL plastic syringe (Norm-Ject, Ace Glass Inc., Tuttlingen, Germany) using an 18-gauge needle (BD Precision Glide, Franklin Lakes, NJ, USA). The needle was removed and an Acrodisc 25 mm syringe filter with a 0.45 mm membrane (HT Tuffryn Membrane; Life Sciences, Lake Mary, FL, USA) was placed at the tip of the syringe. The extracts were filtered into HPLC vials before analysis.

Quantification of fucoxanthin was performed using an HPLC system (1260 Infinity, Agilent Technologies, MA, USA) and a YMC C-30 carotenoid column (250 × 4.6 mm i.d., 3 µm particle size, YMC America, Inc., Devens, MA, USA). The mobile phase consisted of a methanol and water solvent system with a flow rate of 0.7 mL/min and column temperature of 35 °C. The following solvent gradient program was used: The methanol/water ratio was increased from 90:10 to 100:0 over a 20 min period, and then 100% methanol was run for the last 5 min. The chromatogram obtained at 450 nm was used for quantitative analysis of fucoxanthin [22].

An analytical grade fucoxanthin stock solution (4 mg/1 mL with 99.5% purity, Sigma-Aldrich) was used to obtain concentrations of 4, 8, 12, and 16 ppm to construct a standard curve. The diluted concentrations of the stock solutions were injected into the HPLC system under the same running conditions as mentioned above. A standard curve (Y = ax + b) with the constant values of Y = 24.529X − 8.4328 and a correlation coefficient of R^2 = 0.9989 was used to calculate the quantities of fucoxanthin in each sample. The samples were analyzed in triplicate and the fucoxanthin recovery percent was reported.

2.5. Microstructure Analysis and Particle Size Determination

Confocal laser scanning microscopy (CLSM) was used to evaluate the microstructure of the control yogurt and fucoxanthin supplemented yogurt samples at 0 and 4 weeks of storage. A stock solution of Nile Red (Invitrogen, Carlsbad, CA, USA), 2 mg/mL (6 mM) in dimethylsulfoxide, was prepared and stored at 4 °C. Fast Green FCF (F-7252, Sigma-Aldrich, St. Louis, MO, USA) at a concentration of 1 mg/mL (1.2 mM) in water was prepared and stored at −20 °C. The staining solution was prepared by mixing 10 µL of Nile Red, 100 µL of Fast Green FCF, and 890 µL of water before use. Several drops of the staining solution were deposited in a coverglass-bottom (35 mm) Petri dish. Using a plastic transfer pipette with the tip cut off, yogurt was aspirated from the center of the sample that was contained in a 50 mL conical tube and a drop of the yogurt sample was applied onto the staining solution and allowed to settle and stain for 15 min at room temperature. Confocal imaging was performed on an inverted Leica (Leica Microsystems, Wetzlar, Germany) DMi8 microscope using Lecia SP8 laser scanning confocal system equipped with a white-light laser, hybrid detectors and HC PL APO CS2 20×/0.75 IMM water immersion objective. Pinhole size was set to 1 Airy unit. Imaging was performed in a line sequential mode, with Excitation/Emission set to 553/578–641 nm and 621/650–756 nm for Nile Red and Fast Green FCF staining, respectively. Micrographs are presented in this study as an overlay, in a 24-bit RGB TIFF format.

The particle sizes of the yogurts were determined using a Multisizer 4E Particle analyzer (Beckman Coulter, Brea, CA, USA). Yogurt samples (0.3 g) were diluted with

deionized water to 100 mL, and then one drop of tween 80 (Sigma-Aldrich, St. Louis, MO, USA) was added as a surfactant agent. The sample solution from the control yogurt (1150 µL) or the fucoxanthin supplemented yogurt (575 µL) was added into the graduated standard beaker of the analyzer containing 100 mL of electrolyte solution. A solution of 10 g/L of sodium chloride (33.3%, *v*/*v*) (Fisher Scientific, Fair Lawn, NJ, USA) was used as the electrolyte solution. The analysis was conducted at room temperature (~22 °C) using an aperture tube of 100 µm and with the stirrer of the analyzer running. The samples were analyzed in duplicates at weeks 0 and 4, and the particle size values were reported in µm.

2.6. Determination of pH, Titrable Acidity, and Water-Holding Capacity

The pHs of yogurt samples with and without fucoxanthin were determined throughout the 4-week storage period using an Accumet benchtop pH meter (Accumet AE150, Fisher Scientific, USA) at 25 °C. The pH meter was standardized with 4.00 and 7.00 Orion buffer solutions (Thermo Fisher Scientific, Chelmsford, MA, USA). The titrable acidity in the yogurt samples was determined according to the AOAC [24]. Briefly, a 20 mL sample of yogurt was mixed with 40 mL of boiled and cooled distilled water. Phenolphthalein (1% *w*/*v* in 95% ethanol) was used as an indicator. The mixture was titrated with standardized 0.1 N NaOH until the first color change persisted for 30 s, signaling the endpoint of titration (at pH 8.1–8.3). The results for titrable acidity were expressed in percentage of lactic acid.

The water-holding capacity (WHC) of the yogurt samples during the 4 weeks of storage at 4 °C were determined according to the procedure of Kim et al. [25]. Five grams of yogurt sample (Y) was centrifuged at 4000× *g* for 30 min at 4 °C (Avanti J-E centrifuge). The whey expelled (WE) was removed and weighted. The WHC was calculated using the following formula:

$$\text{WHC } (\%) = \left[\frac{\text{weight Y } - \text{ weight WE}}{\text{weight Y}}\right] \times 100 \quad (1)$$

2.7. Instrumental Color and Texture Analyses

The color components of yogurt samples (L* (lightness), a* (red-green), and b* (blue-yellow)) were determined by reflectance using a HunterLab colorimeter (ColorFlex Spectrophotometer, Hunter Associates Laboratory, Inc., Reston, VA, USA). The colorimeter was calibrated using a white tile (L* = 93.51, a* = −1.09, and b* = 0.90) with D65/10° Illuminant/observer. The color of each sample was determined in triplicate at room temperature (~22 °C) at the ends of weeks 1, 2, 3, and 4 of storage at 4 °C.

The firmness of yogurt samples was determined using a TA.XT.plus Texture Analyzer (Texture Technologies Corp., Hamilton, MA, USA) fitted with a 5 kg load cell and controlled by a computer. The samples were compressed using a TA-2A 90° cone probe. The test was conducted using the Return to Start test option with the following settings: 1 mm/s pre-test speed, 2 mm/s test speed, and 2 mm/s post-test speed. The trigger was set to auto at 5 g with a target distance of 15 mm according to the procedure of Texture Technologies Corp. [26]. The maximum force during the compression test was recorded as the firmness of the yogurt gel. The firmness of yogurt samples was determined weekly throughout the storage period, and the values were reported in Newtons.

2.8. Measurement of Lipid Oxidation

The 2-thiobarbituric acid-reactive substances (TBARS) content of the yogurt samples during the 4 weeks of storage was determined using the procedure of Semeniuc et al. [27]. Briefly, yogurt samples (2 g) were homogenized into 4 mL of sodium phosphate buffer (PB, pH 7) for 30 s. Then, 2 mL of trichloroacetic acid (30% *w*/*v*, TCA) was added and the resulting mixture was homogenized for 30 s. The mixture was adjusted to exactly 10 mL using a volumetric flask with PB and homogenized for additional 30 s. Samples were filtered using Whatman no. 1 filter paper (Sigma-Aldrich, St. Louis, MO, USA) to remove the precipitate. A volume of 5 mL of the clear filtrate was transferred into a glass screw-top

test tube, and 5 mL of 0.02 M aqueous solution of 2-thiobarbituric acid (TBA) was added. The samples were shaken for 30 s, heated in a water bath at 90 °C for 20 min, and then cooled for 30 min in a refrigerator (4 °C). A blank was prepared and treated like the yogurt samples with 4 mL PB, 1 mL TCA, and 5 mL TBA. The samples were evaluated weekly during the 4-weeks of storage at 4 °C. The absorbance of samples was measured at 530 nm using a Spectramax Max Plus spectrophotometer (Molecular Devices, Sunnyvale, CA, USA) and the results were expressed as TBARS. A fresh solution of TBA was prepared every week for the analysis of TBARS.

2.9. Statistical Analysis

Experimental data from the physicochemical analyses were analyzed by a two-factorial design within split plot design. The whole plot was repetition, and the split plot was a two-factorial design. The two factors were treatments (yogurt with and without fucoxanthin) and storage time (week 1, 2, 3, or 4), which were the main effects of the data. The data for the percent recovery of fucoxanthin at different stages of manufacturing of supplemented yogurt and during its storage were analyzed by a randomized complete block design. The PROC MIXED model procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC, USA) was used in this study. Analysis of variance was used to determine statistical differences ($p < 0.05$) among the main effects and their interactions. Least-squares means were used to identify significant differences between treatments. A total of three replicates were performed, and three samples from each replicate were analyzed.

3. Results and Discussion

3.1. Chemical Composition of Yogurts

The percentages of moisture, fat, protein, and ash from the yogurt samples are shown in Table 1. The fucoxanthin supplementation did not affect ($p > 0.05$) the proximate composition of treated yogurt compared to the control yogurt.

Table 1. Chemical composition of goat milk yogurts.

Composition (%)	CY	FXY
Moisture	73.86 ± 1.109	73.19 ± 1.109
fat	5.87 ± 0.501	6.53 ± 0.501
Protein	6.04 ± 0.289	5.39 ± 0.289
Ash	1.32 ± 0.073	1.25 ± 0.073

The values are least squares means with their standard errors (SE). CY: yogurt without fucoxanthin (control); FXY: yogurt with fucoxanthin at 0.052 mg/g of yogurt mix.

3.2. Stability of Fucoxanthin in Goat Milk Yogurt during Processing and Storage

The percent recovery of fucoxanthin from the yogurt mix before heating, after heating, and in the freshly prepared yogurt (on day 0) is presented in Table 2. The recoveries of fucoxanthin from the yogurt mixes before and after heating were 98.25 ± 1.53% and 98.83 ± 1.53%, respectively, which are not different ($p > 0.05$). However, statistically less fucoxanthin (90.13 ± 1.53%) was recovered from the freshly prepared yogurt than from the yogurt mixes. The degradation of fucoxanthin in the freshly prepared yogurt was less than 10% compared to the quantities recovered from the mixes. Heating the yogurt mix did not affect the concentration of added fucoxanthin ($p > 0.05$) but adding a starter culture to the mix reduced ($p < 0.05$) the concentration of fucoxanthin during fermentation. It is speculated that this reduction in percent recovery of fucoxanthin from yogurt could be due to the degradation of fucoxanthin by starter culture during growth and fermentation.

Table 2. Percent recovery of fucoxanthin in yogurt mix before heating, after heating, and in the freshly prepared yogurt.

Treatment	Recovery of Fucoxanthin (%)
Yogurt mix before heating	98.25 ± 1.53 [a]
Yogurt mix after heating	98.83 ± 1.53 [a]
Yogurt (freshly prepared)	90.13 ± 1.53 [b]

The values are least squares means with their standard errors (SE). The concentration of fucoxanthin was 0.052 mg/g of yogurt mix. Means with different letters are significantly different ($p < 0.05$).

In our study of goat milk yogurt, fucoxanthin was less stable than it was previously found in studies of pasteurized goat whole and skim milk. Nuñez de González et al. [21] found higher recovery yields of fucoxanthin in pasteurized goat whole milk (96.17 ± 1.5%) and skim milk (96.89 ± 1.5%) than yogurt. Similarly, Mok et al. [22] reported higher fucoxanthin recovery values in cow whole milk (95.37 ± 1.06%) and skim milk (93.25 ± 0.76%). Lactic acid fermentation, which decreases pH, could have reduced the stability of fucoxanthin in the yogurt. According to Hii et al. [28], fucoxanthin is susceptible to degradation by external agents such as light exposure and acidic pH and is least stable at pH 3. Zhao et al. [29] found that pH, followed by temperature and light are the most important factors affecting the stability of fucoxanthin in an oil-in-water emulsion.

During the storage period, the concentration of fucoxanthin (Table 3) in yogurt remained almost the same ($p > 0.05$), which could have been due to the fact that bacterial cultures are not very active at refrigeration temperatures. Overall, the concentration of fucoxanthin in yogurt remained stable during the 4-weeks of storage at 4 °C. Previously, we also found that fucoxanthin was stable in pasteurized goat whole milk and skim milk during 4-weeks of storage at 4 °C [21].

Table 3. Stability of fucoxanthin in yogurt during storage at refrigeration temperature based on (%) recovery.

Storage Time (Week)	Recovery of Fucoxanthin (%)
0	90.19 ± 1.30
1	90.73 ± 1.30
2	88.12 ± 1.30
3	87.64 ± 1.30
4	88.50 ± 1.30

The values are least squares means with their standard errors (SE). The concentration of fucoxanthin was 0.052 mg/g of yogurt mix.

3.3. Effects of Fucoxanthin on the Physicochemical Properties of Yogurt

The CLSM images of the control yogurt and the samples with fucoxanthin at 0 and 4 weeks of storage are presented in Figure 2. The microstructure of fucoxanthin supplemented yogurt gel exhibited a heterogenous protein network with smaller clumps and more frequent empty spaces at both 0 and 4 weeks of storage compared to the control. Thus, this type of microstructure produces a porous protein network that could favor syneresis or a decrease in the WHC and firmness of the treated yogurt. However, the control yogurt gels exhibited a more homogeneous and denser microstructure with well-distributed and more compact protein network that could reduce syneresis. It appears that the carotenoid molecules intervene with the formation of the protein network and gelation during fermentation. However, it elucidates the need to investigate in more detail the formation process of the protein network and gelation of fucoxanthin supplemented yogurt and its structure during the fermentation process.

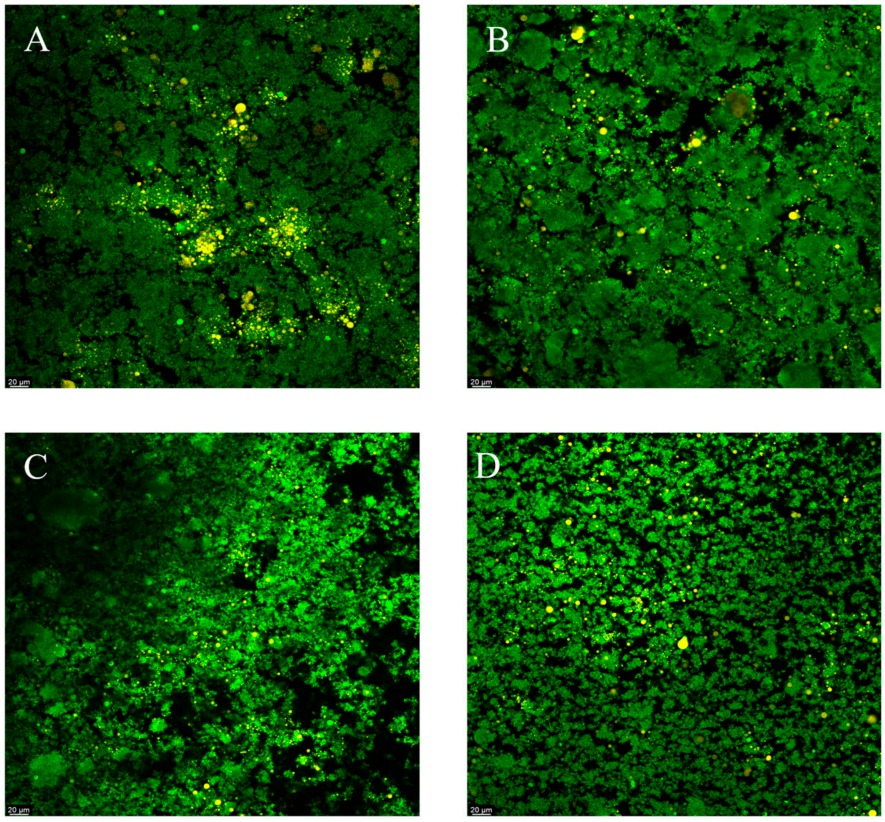

Figure 2. Confocal laser scanning microscopic images of yogurts. CY—Control yogurt without fucoxanthin; FXY—Yogurt with fucoxanthin at 0.052 mg/g of yogurt mix. (**A**) CY at week 0. (**B**) CY at week 4. (**C**) FXY at week 0. (**D**) FXY at week 4. Imaging was performed in a line sequential mode. Micrographs are presented as an overlay. The proteins networks are green, and the fat globules are yellow. Scale bar 20 µm.

The results of the mean particle size diameters of control yogurt and fucoxanthin supplemented yogurt at 0 and 4 weeks of storage are shown in Table 4. The diameters of particle sizes in yogurts ranged from 6.00 ± 0.162 µm to 6.65 ± 0.162 µm. We observed that the mean particle size diameter of yogurt supplemented with fucoxanthin was significantly larger ($p < 0.05$) than the control yogurt. This is probably due to the fact that the carotenoid molecules are lipophilic and mostly attach themselves to the fat globules and to a lesser extent to the protein matrix; hence, causing enlargement of diameters of dispersed fat particles in yogurt. The particle size diameters of yogurts were not affected ($p > 0.05$) by storage time at 4 °C.

Table 4. Effect of fucoxanthin supplementation on particle size of goat whole milk yogurt during storage.

Main Effect	Particle Size (µm)
Treatment	
CY	6.00 ± 0.162 [b]
FXY	6.65 ± 0.162 [a]
Storage time (week)	
0	6.15 ± 0.162
4	6.50 ± 0.162

The values are least squares means with their standard errors (SE). CY: yogurt without fucoxanthin (control); FXY: yogurt with fucoxanthin at 0.052 mg/g of yogurt mix. Means with different letters are significantly different ($p < 0.05$).

The effects of fucoxanthin supplementation on pH, titrable acidity, WHC, color, firmness, and lipid oxidation ($TBARS_{A530}$) of yogurt during storage at 4 °C were de-

termined. The yogurt supplemented with fucoxanthin had significantly ($p < 0.05$) higher pH (4.42 ± 0.06), lower acidity (0.73 ± 0.05%), and WHC (79.43 ± 2.76%) than the control sample (Table 5). The pH values of the yogurts with fucoxanthin and control were 4.29 and 4.42, respectively. In our study, the pH values of yogurt with and without fucoxanthin were in the same range as previously reported by others. For example, Domagała et al. [30] reported a pH of 4.55 ± 0.06 in fresh goat milk yogurt and 4.23 ± 0.06 in goat milk yogurt stored at 5 °C for 14 days.

Table 5. Effect of fucoxanthin supplementation on pH, titratable acidity, and water-holding capacity of goat whole milk yogurt during storage.

Parameters	Treatment (T)			Storage Time (ST, Weeks)					T × ST
	CY (n = 24)	FXY (n = 24)	SE	1 (n = 12)	2 (n = 12)	3 (n = 12)	4 (n = 12)	SE	(p-Value)
pH	4.29 [b]	4.42 [a]	0.06	4.44	4.35	4.30	4.31	0.12	S* (p = 0.006)
Acidity (% lactic acid)	0.82 [a]	0.73 [b]	0.05	0.73	0.70	0.92	0.74	0.11	NS (p = 0.950)
Water-holding capacity (%)	81.42 [a]	79.43 [b]	2.76	82.82	78.23	80.30	80.36	5.44	NS (p = 0.283)

The values are least squares means with their standard errors (SE). CY: yogurt without fucoxanthin (control); FXY: yogurt with fucoxanthin at 0.052 mg/g of yogurt mix; n: sample size. Means with different letters in the same row within each effect (treatment or storage time) are significantly different ($p < 0.05$). S*: significant; NS: not significant.

When pH values in the yogurt matrix increase, normally, the WHC also increases. In our study the supplementation of fucoxanthin into yogurt resulted in higher pH values ($p < 0.05$) than the control but produced significantly lower WHC. The reduction in WHC in fucoxanthin supplemented yogurt can be explained by the CLSM observation of yogurt gels. The micrographs show that fucoxanthin has affected the formation of the gel network inside the yogurt protein matrix, causing a more porous microstructure and producing a fluffier gel that when subjected to centrifugal force readily lost more water.

Regardless of whether fucoxanthin was present in the yogurts, no significant changes were observed in the pH, acidity, and WHC values during the 4-weeks of storage. However, for pH values only, there was an interaction between treatments (with or without fucoxanthin) and weeks of storage ($p = 0.006$).

Supplementation with fucoxanthin affected the color of yogurt (Table 6). It significantly decreased lightness (81.47 ± 0.09), and increased redness (7.67 ± 0.09) and yellowness (38.24 ± 0.09) (Figure 3). Significant treatment and storage time interactions were observed in redness ($p = 0.0003$) and yellowness ($p < 0.0001$) values. The color changes in treated yogurt, especially the yellowness, appear to have been due to the natural, orange-colored pigments of fucoxanthin. O'Sullivan et al. [31] indicated that the yellowness was significantly higher in yogurt by the addition of seaweed extract, due to presence of yellow pigments (rutin, fucoxanthin, or morin). The coloring properties of fucoxanthin may be useful in making peach, passion fruit, or orange yogurts, which are expected to have yellowish tones.

Texture (firmness) is one of the most important quality characteristics of a set yogurt. Consumers prefer a set yogurt that has a smooth texture and sufficient curd strength [1]. As shown in Table 6, yogurt supplemented with fucoxanthin was significantly less firm (0.61 ± 0.01 Newtons) than the control (0.71 ± 0.01 Newtons). These differences in values are expected from the observations of CLSM images of yogurt microstructures. The protein network in supplemented yogurt was less dense and compact than the control. The firmness of both yogurts, supplemented with fucoxanthin and without fucoxanthin (control), were higher than the values reported by Nguyen et al. [32] for goat skim milk yogurt (0.23 ± 0.02 N). Likewise, Domagała [33] reported that goat milk yogurt was softer (0.19 ± 0.01 N) than cow and sheep milk yogurts. As other researchers noted, the instrumental firmness of yogurt and its syneresis (whey separation) are influenced by the structure of protein matrix within the gel network of yogurt [33,34]. However, the differ-

ences in firmness between yogurts with and without fucoxanthin may be too small for consumers to perceive.

Table 6. Effect of fucoxanthin supplementation on HunterLab color, firmness, and lipid oxidation of goat whole milk yogurt during storage.

Parameters	Treatment (T)			Storage Time (Weeks)					T × ST
	CY (n = 24)	FXY (n = 24)	SE	1 (n = 12)	2 (n = 12)	3 (n = 12)	4 (n = 12)	SE	(p-Value)
HunterLab color									
L*	90.95 ^{a}	81.47 ^{b}	0.09	86.00	86.19	86.20	86.45	0.16	NS (p = 0.354)
a*	−2.05 ^{b}	7.67 ^{a}	0.14	2.76	2.89	2.83	2.75	0.29	S* (p = 0.0003)
b*	11.73 ^{b}	38.24 ^{a}	0.19	25.27	25.13	24.96	24.58	0.38	S* (p < 0.0001)
Firmness (Newtons)	0.71 ^{a}	0.61 ^{b}	0.01	0.62	0.73	0.70	0.61	0.01	NS (p = 0.724)
TBARS (at A_{530})	0.35	0.33	0.04	0.33	0.33	0.36	0.32	0.07	NS (p = 0.691)

L*= Lightness; a*= + red, − green; b* = + yellow, − blue. The values are least squares means with their standard errors (SE). CY: yogurt without fucoxanthin (control); FXY: yogurt with fucoxanthin at 0.052 mg/g of yogurt mix; n: sample size. Means with different letters in the same row within each effect (treatment or storage time) are significantly different (p < 0.05). S*: significant; NS: not significant.

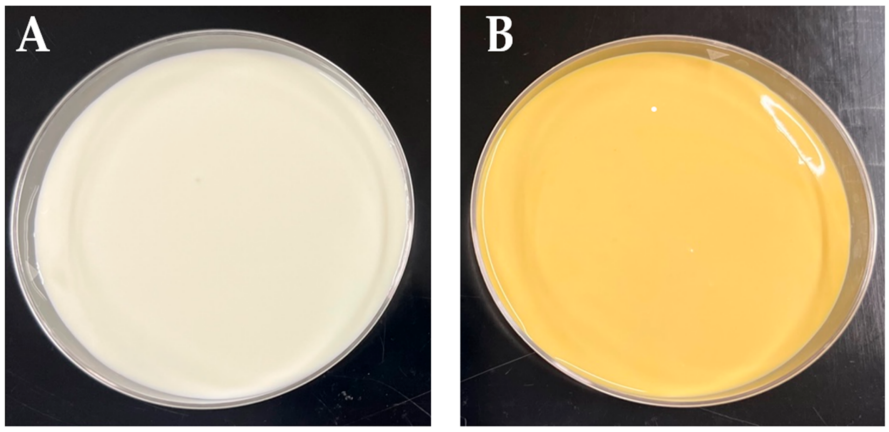

Figure 3. Yogurt products. (**A**) CY—Control yogurt without fucoxanthin at week 0; (**B**) FXY—Yogurt with fucoxanthin at 0.052 mg/g of yogurt mix at week 0.

Non-significant changes in the TBARS values were observed in yogurts with and without fucoxanthin and during their storage time (Table 6). Overall, fucoxanthin instrumentally influenced the firmness of yogurt, but it did not influence the lipid oxidation (as $TBARS_{A530}$) of the yogurt.

4. Conclusions

In this study, we have investigated the potential application of fucoxanthin as a biofunctional compound in goat milk yogurt. Heating the yogurt mix did not affect the concentration of added fucoxanthin (0.052 mg/g of yogurt mix) but adding cultures to the mix reduced its concentration (less than 10% compared to the control) during fermentation. Fucoxanthin did not adversely affect the physicochemical properties of the product during the 4-weeks of storage, but it influenced the color, decreasing lightness, and increasing redness and yellowness of yogurt. Fucoxanthin remained relatively stable in goat whole milk yogurt after fermentation and during its subsequent storage at 4 °C. Thus, goat whole milk yogurt can be used as a suitable matrix to deliver fucoxanthin to human diet. Further work seems necessary to optimize the amount of supplementation of fucoxanthin into goat milk yogurt and its effects on weight loss in human diet. Moreover, the sensory attributes and the consumer's acceptability of the goat milk yogurt supplemented with fucoxanthin need further evaluation.

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Abbreviations

The following abbreviations are used in this manuscript:

HPLC	High-Performance Liquid Chromatography
TCA	Trichloroacetic acid
TBA	2-Thiobarbituric Acid
NaOH	Sodium hydroxide
TBME	Tert-Butyl Methyl Ether
CYM	Control Yogurt Mix
FXYM	Fucoxanthin Yogurt Mix
CY	Control Yogurt (without fucoxanthin)
FXY	Fucoxanthin Yogurt (with fucoxanthin, 0.052 mg/g of yogurt mix)
TBARS	Thiobarbituric Acid Reactive Substances
WHC	Water Holding Capacity
AOAC	Association of Official Analytical Chemists
RCF	Relative Centrifugal Force (*x g*)
CLSM	Confocal Laser Scanning Microscopy
Y	Yogurt sample
WE	Whey Expelled
$TBARS_{A530}$	Thiobarbituric Acid Reactive Substances measured at 530 nm
N	Newtons

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