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Effect of Using Odourless Fish Oil on Some Physical, Chemical and Sensorial Properties of Blackberry Fruit Yoghurt*

Nazlı TÜRKMEN[1a]✉, Ebru ŞENEL[1b], Ceren AKAL[1c]

1. Ankara University, Faculty of Agriculture, Department of Dairy Technology, Ankara, TURKEY.
ORCID: 0000-0002-4219-8903[a], 0000-0003-0797-621X[b], 0000-0002-0441-541X[c]

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Abstract: Omega-3 fatty acids (α-linolenic acid-ALA, eicosapentaenoic acid-EPA and decosahexaenoic acid-DHA) are essential for humans. Especially EPA and DHA reduce the risk of death related with cardiovascular diseases, prevent many other diseases and fortify the immune system. Sufficent EPA and DHA can be intaken by consuming only seafood. According to the World Health Organization, the required EPA+DHA is 0.3-0.5 g/day for healthy people. But people having cardiovascular diseases should consume 1 g/day EPA+DHA according to the American Heart Association. The requirement of Omega-3 fatty acids is 650 mg per day in pregnancy and at least 300 mg should be DHA. In this study, it was aimed to enrich the product with Omega-3 by using odourless fish oil at different rates (0.3%, 0.5% and 1.0%) in the production of blackberry yoghurt. pH, titratable acidity values, fat, total dry matter and lactic acid contents, acid and peroxide values, viscosity and sensory properties of the samples were investigated. According to the results, there was no significant difference in physical and chemical properties of the fruit yoghurt except peroxide values (P>0.01). In sensory analysis, blackberry fruit yoghurt samples having 0.3% and 0.5% odourless fish oil were ranked as acceptable by the panelists. The daily requirement of EPA+DHA can be supplied by consuming approximately 250 g of the sample containing 0.3% of odourless fish oil and approximately 150 g of the sample containing 0.5%.

Keywords: DHA, EPA, Fish Oil, Fruit Yoghurt, Omega-3 fatty acids.

Kokusuz Balık Yağı Kullanımının Böğürtlenli Yoğurdun Bazı Fiziksel, Kimyasal ve Duyusal Özellikleri Üzerine Etkisi

Öz: Omega-3 yağ asitleri (α-linolenik asit-ALA, eikozapentaenoik asit-EPA ve dekosaheksaenoik asit-DHA) insanlar için esansiyeldir. Özellikle EPA ve DHA, kardiyovasküler hastalıklarla ilgili ölüm riskini azaltmakta, diğer birçok hastalığı önlemekte ve bağışıklık sistemini güçlendirmektedir. Yeterli EPA ve/veya DHA sadece deniz ürünleri tüketimi ile karşılanabilmektedir. Dünya Sağlık Örgütü'ne göre, sağlıklı insanlar için gerekli EPA+DHA 0.3-0.5 g/gün'dür. Ancak kardiyovasküler hastalıkları olan insanlar, Amerikan Kalp Derneği'ne göre 1 g/gün EPA+DHA tüketmelidir. Omega-3 yağ asitleri gereksinimi gebelikte ise günde 650 mg'dır ve bunun en az 300 mg'ının DHA olması gerektiği belirtilmektedir. Bu araştırmada, böğürtlenli yoğurt üretiminde farklı oranlarda (%0.3, %0.5 ve %1.0) kokusuz balık yağı kullanılarak, ürünün Omega-3 bakımından zenginleştirilmesi hedeflenmiştir. Örneklerin pH, titrasyon asitliği değerleri, yağ, kurumadde ve laktik asit içerikleri, asit ve peroksit değerleri, viskozitesi ve duyusal özellikleri araştırılmıştır. Elde edilen sonuçlara göre, örneklerin fiziksel ve kimyasal özelliklerinde peroksit değerleri dışında istatistiksel olarak anlamlı fark bulunamamıştır (P>0.01). Duyusal analiz sonuçlarına göre, %0.3 ve %0.5 kokusuz balık yağı bulunan böğürtlen meyveli yoğurt örnekleri panelistler tarafından kabul edilebilir olarak nitelendirilmiştir. Kokusuz balık yağını %0.3 oranında içeren örnekten yaklaşık 250 g, %0.5 oranında içeren örnekten ise yaklaşık 150 g tüketilmesi ile günlük EPA+DHA ihtiyacının karşılanması mümkündür.

Anahtar Kelimeler: Balık yağı, DHA, EPA, Meyveli yoğurt, Omega-3 yağ asitleri.

✉ Nazli Turkmen
Ankara University, Faculty of Agriculture, Department of Dairy Technology, Ankara, TURKEY.
e-mail: nazli.turkmen@ankara.edu.tr

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INTRODUCTION

In recent years, the production and consumption of functional foods have been increasing because of beneficial health effects. The Omega-3 fatty acids are some examples to essential fatty acids and they are ALA (α-linolenic acid), EPA (eicosapentaenoic acid) and DHA (decosahexaenoic acid) (1). ALA can be supplied from some gren plant tissues especially flaxseed, nuts and rapeseed (2) but EPA and/or DHA can be supplied only from sea foods (3). Generally, the Omega-3 fatty acids have beneficial properties, especially EPA and DHA reduce the risk of death connected to cardiovascular diseases (4-6). According to a research which is focused on the effects of Omega-3 fatty acids on cardiovascular diseases; the deaths caused by cardiovascular diseases are decreased by consuming fish, especially oily fish, twice a week (7). These two fatty acids prevent artherosclerosis, necrosis, stroke, migraine, allergic asthma, arthritis, diabetes, hypertension and strengthen the immune system. They also have beneficial effects against various intestinal and skin diseases, are protective against plenty of tumoral diseases, and they have therapeutic properties (8-10). The Omega-3 fatty acids are also closely related with Alzheimer's disease. The Alzheimer's disease is rarely seen in Japan and this is related to high Omega-3 fatty acids taken with fish based Japanese daily diet (11). Also, the depression cases were higher in New Zelland where average fish consuming is 20 g/day with 5.8% depression rate when compared to Korea where average fish consuming is 50 g/day with 2.8%. The fish consumption is 100 g/day per person in Japan therefore the depression cases are seen only at a rate of 0.12% (12).

According to the World Health Organization (WHO) and North Atlantic Treaty Organization (NATO) to take sufficient EPA+DHA, healthy people should consume fish twice a week and it is equal to 0.3-0.5 g/day EPA+DHA (7). According to the American Heart Association, the requirement of EPA+DHA is 1 g/day for people who have cardiovascular diseases and 2-4 g/day for people have high levels of tryglyceride (11).

Pregnants should consume 650 mg Omega-3 fatty acids per day and at least 300 mg of them should be DHA. Taking high levels of Omega-3 fatty acids during pregnancy provides the improvment in mathematical intelligence and the abilities of reading and writing in childhood (13). On the other hand, taking low levels of Omega-3 fatty acids during pregnancy causes behavioural disorder, sleep disturbance and hyperactivity (9). The sea foods taking part in daily diet of children is quite important as they contain high levels of essential fatty acids which are neccesary for the childs development (14). The retina, central nervous system, mathematical intelligence, reading, writing and pronunciation abilities improve with fish oil consumption in children (13,15,16).

Yoghurt rich in carbonhydrate, protein, fat, vitamin, calcium and phosphor has an important role in daily diet (17). There are many scientific data about regular consumption of yoghurt having a role in immune systems getting strong (18). To consume about two cups (approximately 400 gram) of yoghurt is enough to provide half of the daily calcium requirement for an adult (19). Yoghurt is an important milk product for the improvement of bones in children and it prevents osteoporosis in adults. Also, calcium intake in high levels has a role in protection against colon and rectum cancer (20). Digestion of yoghurt is easier than non-fermented dairy products. Especially, lactose intolerant people do not have the ability to digest lactose in non-fermented milks but they can consume yoghurt easily (17,21). Therefore, yoghurt has an important role in nutrition and it must have a place in daily diet in all ages.

Consumption of plain set type yoghurt is higher than fruit yoghurt in Turkey. However, fruit yoghurt consumption has been increasing lately especially by

children and young people. There are several research about yoghurt to be well-liked by the public with calcium, whey protein concentrate, inulin etc (22-24). There are also several other research about enriched yoghurt with Omega-3 fatty acids because of the beneficial effects of Omega-3 besides yoghurts natural beneficial effects (25-30).

The main purpose of this study is to provide the consumption of more Omega-3 fatty acids with fruit yoghurt by people needing more Omega-3 fatty acids; especially by children, pregnant women and people having cardiovascular diseases. For this purpose, odourless fish oil was used in yoghurt production. In the preliminary study, all of the panelists were able to sense the fishy taste and aroma of plain set type yoghurt even for the lowest concentration of odourless fish oil. Therefore the fruit extract was preferred in stirred type of yoghurt production. Different fruit extacts like blackberry, strawberry, apricot, raspberry and red berries extracts were used in the preliminary study and the yoghurt sample with blackberry fruit extract had the highest scores by the panelists. Therefore, blackberry fruit extract was used in fruit yoghurt production in the study. Different rates of odourless fish oil (0.3%, 0.5%, 1%) was added to the blackberry yoghurt samples and some chemical, physical and sensory attributes of the final product was investigated during 15 days of storage period.

MATERIALS and METHODS

Materials

Raw milk used for the production of yoghurt samples was obtained from Ankara University, Haymana Research and Application Farm, Turkey. Thermophilic yoghurt starter culture CH-1 (Chr. Hansen, Denmark), 99.9% pure odourless fish oil containing 160 mg/g of EPA and 100 mg/g of DHA (ASUKA Kim. San. Tic. A.Ş., Turkey), blackberry fruit extract (Yörsan A.Ş., Turkey) and milk powder with 95% dry matter (İzi Süt A.Ş., Turkey) were used in the production of blackberry yoghurt samples.

Production of Fruit Yoghurts

The raw milk was separeted into four equal parts and total fat content (milk fat+odourless fish oil) of milk was standardizated to 3%. The first part was the control sample (A) having ~3% only milk fat, the other three parts; B, C and D samples having 2%, 2.5% and 2.7% milk fat and 1%, 0.5% and 0.3% odourless fish oil respectively (Table 1).

Table 1. Sample codes and descriptions of the blackberry fruit yoghurt samples.
Tablo 1. Böğürtlenli yoğurt örneklerinin kodları ve tanımlamaları.

Sample code	Sample description
A	Control sample (blackberry yoghurt - 3% milk fat)
B	Blackberry yoghurt - 2% milk fat + 1% odourless fish oil
C	Blackberry yoghurt - 2.5% milk fat + 0.5% odourless fish oil
D	Blackberry yoghurt - 2.7% milk fat + 0.3% odourless fish oil

The cream was seperated from raw milk after milk was pre-heated to 40-50°C. The cream was added to the samples in appropriate amounts as explained above. After the total dry matter of the samples was standardized to 16% with milk powder, they were homogenized in 150-200 bar pressure. When addition of odourless fish oil was applied before homogenization, the fishy taste was more appreciable due to increase of the surface area of fat globules. Therefore, odourless fish oil was added to the samples after homogenization in suitable amounts as explained above and the samples were pasteurized in 85°C /15 min. The starter culture (3%) was added to the samples after they cooled to 43-45°C and then they were incubated in 43-45°C. The incubation was ended in ~4.6-4.7 pH and the samples were cooled to 20-30°C immediately. After addition of the blackberry fruit samples and blending, the samples were stored at +4°C. Steps of the fruit yoghurt samples production are given in Figure 1.

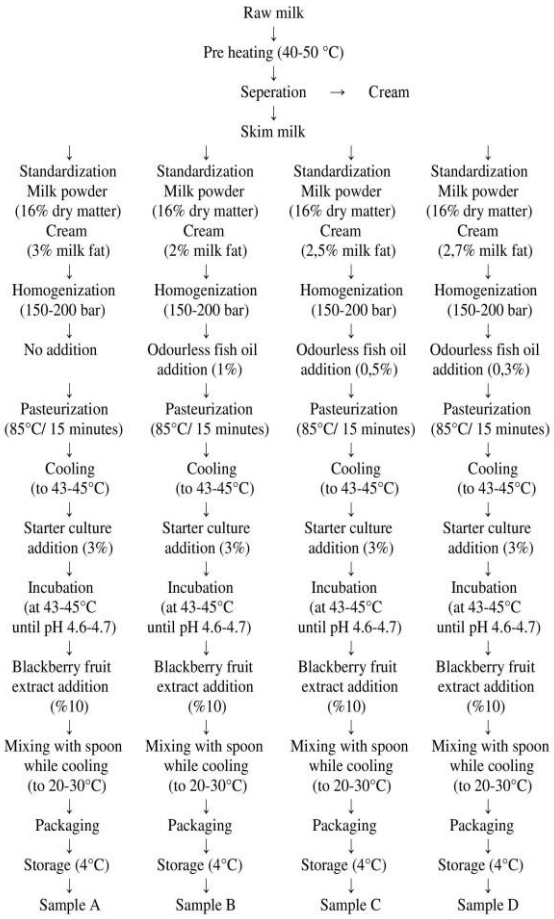

Figure 1. Steps of the blackberry fruit yoghurt samples production.
Şekil 1. Böğürtlenli yoğurt örneklerinin üretim basamakları.

The production of yoghurt samples were repeated three times. Physical, chemical and sensory analysis were performed on the 1st, 7th and 15th days of storage. Fat and dry matter contents of the samples were determined only on the 1st day and all the analysis were repeated three times in each production.

Chemical Analysis

Fat content of the samples were determined by Gerber method on the 1st day of storage (31). Total dry matter content of the samples were determined by ovendrying method on the 1st day of storage (31). pH values of the samples were measured by a pH meter-model Mettler Toledo (Analytical, Sonnerbergstasse 74, CH-8603, Switzerland) on the 1st, 7th and 15th days of storage. Titratable acidity of the samples was detected by titration method on the 1st, 7th and 15th days of storage. The results were calculated as Soxhelet-Henkel (°SH) (32). Lactic acid content of the samples was measured by spectrophotometric method (33). For this purpose, 25 g of yoghurt sample was mixed with 10 mL of barium chloride, 10 mL of 0.66 N sodium hydroxide and 5 mL zinc sulfate solution and the mixture was filtered through the Whatman 42 filter paper. 0.15 ml of the filtrate and 1 ml of the color solution were added to 10 ml of distilled water then the sample was read at 400 nm in spectrophotometer.

Determination of Acid and Peroxide Values

First, the milk fat extaction were performed from yoghurt samples (34). For this purpose, 250 g of yoghurt was filtered by crude filter during 2 hours in refrigerator, then the concentrated yoghurt sample was squashed with kieselguhr until it become a dry structure. The samples were washed 2-3 times with diethyl ether so the milk fat was dissolved in ether and then it was removed from the fat in rotary evaporator. Acid and peroxide value determinations were made in milk fat samples according to the method described by Downey (35). The acid value of the samples was determined by the titration method and peroxide value determination was made by spectrophotometric method.

Viscosity

Viscosity of the samples were measured using HAAKE brand VT 181/VTR 24 model viscosimeter at +4°C. Measurements were read in grade 1 and the results were calculated according to the formula:

Viscosity (cP) = Measurement x Grade x 100 (Probe constant)

Sensory Evaluation

Flavor, structure properties and acceptability of the samples were evaluated with multiple comparation analysis by 15 panelists (36).

Statistical Analysis

pH value, titratable acidity, lactic acid, acid and peroxide values and viscosity of the samples were evaluated by repeated measurement ANOVA. There were three levels as 1st, 7th and 15th days of storage factor and four levels as A,B,C and D of sample factorin research. Repeated measurements were analysed in levels of storage factor. The statistical differences in dry matter and fat content betweeen sample means were analysed by variance analysis. DUNCAN test was used to determine the difference among samples. The analysis were calculated by IBM SPSS Statistics 20 and multiple comparision were calculated by Mstat c package program.

RESULTS and DISCUSSION

Some properties of the raw milk used in the production of samples were detected. According to the results, fat content of the raw milk was determined to be 3.37 ± 0.07 g/100 g, non-fat dry matter content was 10.00 ± 0.00 g/100 g, pH value was 6.85 ± 0.06 and the titratable acidity was determined to be 7.37 ± 0.16 °SH.

Total dry matter and fat content of the control sample (A) and the other samples which have different levels of odourless fish oil (B, C, D) are given in Table 2.

Table 2. Total dry matter and fat content of the blackberry fruit yoghurt samples (n=3).
Tablo 2. Böğürtlenli yoğurt örneklerinin toplam kurumadde ve yağ içerikleri (n=3).

Sample	Total dry matter ($g.100g^{-1}$)	Fat ($g.100g^{-1}$)
A	15.71 ± 0.085	2.85 ± 0.050
B	15.57 ± 0.379	2.85 ± 0.050
C	15.22 ± 0.365	2.95 ± 0.050
D	15.45 ± 0.241	2.95 ± 0.050

The differences in total dry matter and fat content of yoghurt samples were not statistically significant (P>0.01) since the yoghurt milk was standardized.

pH and titratable acidity values, lactic acid contents, acid and peroxide values of the samples are given in Table 3.

Table 3. pH and titratable acidity values, lactic acid contents, acid and peroxide values of the blackberry yoghurt samples (n=3).
Tablo 3. Böğürtlenli yoğurt örneklerinin pH ve titrasyon asitliği değerleri, laktik asit içerikleri, asit ve peroksit değerleri (n = 3).

Sample	Storage (day)	pH value	Titratable acidity (ºSH)	Lactic acid content ($g.100g^{-1}$)	Acid value (mg KOH/g oil)	Peroxide value (mEq O_2/kg oil)
A	1	4.11 ± 0.065	48.20 ± 0.209	0.37 ± 0.016	9.10 ± 1.020	2.80 ± 0.788^{Ac}
A	7	4.09 ± 0.074	50.05 ± 0.834	0.42 ± 0.021	10.26 ± 0.679	8.14 ± 0.416^{Ac}
A	15	4.07 ± 0.060	54.27 ± 0.567	0.45 ± 0.029	9.78 ± 0.139	3.64 ± 2.150^{Ac}
B	1	4.18 ± 0.017	46.17 ± 2.320	0.34 ± 0.013	7.00 ± 0.546	72.80 ± 1.920^{Aa}
B	7	4.14 ± 0.046	49.25 ± 1.410	0.38 ± 0.037	6.88 ± 1.080	46.10 ± 3.13^{Ba}
B	15	4.11 ± 0.029	53.68 ± 1.710	0.42 ± 0.009	6.72 ± 0.430	44.97 ± 0.755^{Ba}
C	1	4.17 ± 0.018	45.31 ± 2.060	0.36 ± 0.018	8.25 ± 1.300	25.89 ± 0.556^{Ab}
C	7	4.12 ± 0.034	47.92 ± 0.906	0.37 ± 0.018	7.83 ± 0.253	24.67 ± 0.955^{Ab}
C	15	4.09 ± 0.021	52.31 ± 1.440	0.39 ± 0.016	11.18 ± 0.310	31.53 ± 4.630^{Ab}
D	1	4.18 ± 0.003	46.32 ± 1.490	0.37 ± 0.016	8.46 ± 1.320	7.84 ± 1.500^{Ac}
D	7	4.12 ± 0.040	48.67 ± 0.809	0.37 ± 0.027	9.16 ± 3.000	18.17 ± 2.410^{Abc}
D	15	4.10 ± 0.024	53.44 ± 0.934	0.39 ± 0.009	11.21 ± 0.582	10.76 ± 4.000^{Ac}

Within the storage days, values with different uppercase letters (A, B) are significantly different (P<0.05).
Within the samples, values with different lowercase letters (a, b, c) are significantly different (P<0.01).

pH value of the control sample was slightly lower than pH of the other samples, but the difference is not statistically important (P>0.05). However, the changes in pH value of the samples during the storage period was statistically important (P<0.01). The pH value of samples were decreased during storage period because of accumulation of lactic acid (37). These results were in accordance with the literature (38,39).

The differences among titratable acidity of samples was not statistically important (P>0.05), but during the storage period titratable acidity of samples increased. Also, many researches refered to the same results (24,38,40).

Lactic acid is one of essential compounds in yoghurt, responsible for the characteristic sharp and acidic flavour. The lactic acid levels of blackberry yoghurt samples were determined to be between 0.36-0.45 g.100g^{-1}. Addition of odourless fish oil did not effect the lactic acid content of yoghurt samples (P>0.05). However, the changes in the lactic acid levels of samples during storage period were found to be statistically important (P<0.01). Lactic acid content of all samples have increased during the storage. Similar results were obtained by some researchers (41,42).

No statistical difference was found in acid degree values neither among samples nor on different days of storage (P>0.01). Thus, addition of odourless fish oil did not effect acid degree values of the samples.

The differences between control sample and odourless fish oil added samples with respect to peroxide value was statistically important (P<0.01). The change in peroxide value of Sample B was statistically important (P<0.05) during the storage period but that of the other samples' were not. Also an interaction was found between the samples and storage period (P<0.01). Sample B had the highest peroxide value and the control sample had the lowest. This result arise from the differences of fatty acid composition of fish oil which has high amount of unsaturated fatty acids. Similar results were also obtained in a research on milk emulsions fortified with fish oil (8). Peroxide values of the odourless fish oil added samples were detected in high amounts although panelists did not state any oxidized taste during the sensory evaluation. Oxidized taste of the yoghurt samples may have been masked due to the blackberry fruit extract.

The use of odourless fish oil did not effect the viscosity of samples but control sample had higher viscosity than that of fish oil added samples. Viscosity values of all samples increased on the 7th day of storage but then decreased on the 15th day. Finally the viscosity value of samples were not importantly changed on the 15th day of the storage compared to the 1st day and this changes in viscosity of all samples during storage period was not found to be statistically important (P>0.05). The viscosity values of the yoghurt samples are given in Table 4.

Table 4. Viscosity values of the blackberry fruit yoghurt samples (n=3).
Tablo 4. Böğürtlenli yoğurt örneklerinin viskozite değerleri (n = 3).

Sample	Storage (day)	Viscosity (cP)
A	1	783 ± 109.0
A	7	900 ± 0.0
A	15	750 ± 28.9
B	1	767 ± 66.7
B	7	850 ± 76.4
B	15	750 ± 50.0
C	1	717 ± 60.1
C	7	800 ± 57.7
C	15	733 ± 44.1
D	1	767 ± 66.7
D	7	850 ± 76.4
D	15	750 ± 28.9

Panelists could not perceive any difference between the sample that had the lowest (0.3%) odourless fish oil (D) and control sample (A) on the 1st day of storage. Sample C, which had 0.5% odourless fish oil, was stated to be less tasty when compared to sample A by 12 of the panelists. Sample B having the maximum odourless fish oil was

evaluated less tasty than sample A by all of the panelists.

On the 7th day of storage, sample D was indicated almost the same as sample A by 10 of panelists while the other 5 pointed out sample D as more tasty than sample A. Sample C was perceived as less tasty than the control sample by 11 of panelists and 4 of the panelists could not find any differences between the samples. In this period, similar to the evaluation results on 1st day of storage, Sample B was also less tasty than sample A according to all of the panelists on the 7th day of storage.

Sample B, C and D were perceived quite similar to sample A with respect to the structural properties by all of the panelists on the 1st, 7th and the 15th days of storage. Sample B was generally unacceptable. Sample C was acceptable on the 1st and the 7th days of storage but unacceptable on the last day of storage. Sample D was the most liked sample during the storage. In general, samples' sensory quality increased until the 7th day of the storage, then decreased.

In conclusion, this study demonstrated that blackberry yoghurt enriched with odourless fish oil could be a good source of essential Omega-3 fatty acids consumption. Odourless fish oil addition did not significantly effect pH, titratable acidity, lactic acid, viscosity and acid degree value of yoghurt samples. However, its effect on peroxide value of samples was evident since unsaturated fatty acid amount was increased with addition of odourless fish oil. According to the sensory assesment, sample C and D were acceptable and the structural properties of none the samples were effected by the addition of odourless fish oil. The essential amount of the EPA+DHA is 0.3-0.5 g/day for healthy people and since the 250 g of sample D provides 0.25 g EPA+DHA and 250 g of sample C provides 0.4 g EPA+DHA it is easily possible that the requirement of EPA+DHA can be supplied by consuming approximately 250 g of the sample D and approximately 150 g of the sample C.

Conflict of interest

The authors declare that they have no conflict of interest.

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