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CONTENTS

ORIGINAL PAPERS

FUMIGANT TOXICITY AND NYMPH PRODUCTION DETERRENCE EFFECT OF FIVE ESSENTIAL OILS ON ADULTS OF THE BLACK BEAN APHID, *APHIS FABAE* SCOP. (HEMIPTERA: APHIDIDAE)

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ABSTRACT

The black bean aphid, *Aphis fabae* is an oligophagous pest and due to direct damage in plant and virus transmission is important in sugar beet. This aphid is vector of 30 virus pathogens in different plants. The high toxicity of common pesticides for human and environmental contamination, along with the emergence of pest resistance, in recent year's efforts has been done for introducing low-risk compounds for the control factors of causing damage in plants. In this research, insecticidal activity and nymph production deterrent of five essential oils was studied on the black bean aphid in the laboratory conditions under 25 ± 2°C, 65 ± 5% RH and 16L:8D photoperiods. Mortality was evaluated at different concentrations that ranging from 1.18 to 108.82 µL/L air, and with 6 replications at the interim of 24 hours. The results showed that by increasing dose and time, mortality rate was also increased. The lowest LC_{50} value was related to *Mentha piperita.* These results showed that *M. piperita* oil was more toxic than other essential oils on the black bean aphid, *A. fabae*. The highest deterrence was related to *M. piperita* (and the lowest was belonged to *Satureja isophylla.* Obtaind results of the essential oils described here can be further studied and be used to control the black bean aphid.

KEYWORDS:
Fumigant toxicity, essential oil, *Aphis fabae,* nymph production deterrent

INTRODUCTION

The black bean aphid, *Aphis fabae* is one of the most important pests with broad host range. This aphid has caused damage to numerous host plants including broad bean, beet, beans, tomatoes, potatoes, buxus, etc. The black bean aphid is a vector of persistent viruses such as beet yellow virus, potato leafroll virus and nonpersistent viruses in beans, potatoes, beet, cucurbit and tomato [1]. This pest in plant is caused to reduce growth and dry matter [2]. Plant essential oils are aromatic connections obtained by steam distillation from medicinal and aromatic plants [3]. These oils are rich source of bioactive compounds and in terms of biological are considerable decomposition and low risk is for mammals. Important factor to limit their use is the high cost of oil production that it depends on the availability of plant and product quantity per hectare [4]. Plant essential oils contain compounds that showed ovicidial, repellency (anti-feeding), unproductive and toxic effects on insects. So far several plant species in the laboratory was tested to demonstrate its ability in control of stored products pests [5-9].

On the other hand, many studies showed that essential oils and plant extracts are effective in control of pests and these plants have not deleterious effects for mammals, therefore are consumed by human [10]. Toxicity of 20 essential oils against larvae of *Spodoptera littoralis* (Boisduval) have been studied by Pavela [11]. Essential oil *Cestrum parqui* L. is cause mortality on *Ceratitis capitata* [12]. Lee et al. [13] has examined toxicity of a number of essential oils on pests such as *Tetranychus urticae*, *Musca domestica*, *Ostrinia nubillalis.* Insecticidal effect of three plant essential oils of fennel, basil and thyme (*T. vulgaris*) were studied on two-spotted mite, *T. urticae* [14]. Comparison of insecticidal activity of essential oils of plant seed, *Khaya senegalensis* (Desr.) and insecticide of Primiphos methyl on *Callosobruchus maculates* F. showed that there is not significant difference between insecticidal activity and oviposition deterrence of essential oil of this plant with the insecticide.

Given that for humans and the environment is less dangerous and its preparation is cheaper, therefore to use it has been ordered for control pest [15]. Essential oils of plants, *Cymbopogon citrates*, *C. nardus*, *Alpinia calcarata*, *Cinnamomum zeylanicum* and *Murraya koinigii* is causing oviposition deterrence of *C. maculatus.* Those among men-

tioned compounds, essential oil of *C. citrans* and *C. nardus* are created highest oviposition deterrence in both condition contact and respiratory [16]. So use of the effective compounds and low risk is essential for pest control. Among the new alternative methods to reduce the use of classic pesticides, eco-chemicals control based on the relationship between plant- insect is one of the most promising methods [17]. Compounds with plant origin than conventional pesticides are very low risk for humans and the environment around him. This research was aims to study the deterrent effect and the fumigant toxicity of five essential oils on the black bean aphid, *A. fabae*.

MATERIALS AND METHODS

Rearing of *A. fabae*. For formation of colonies of the black bean aphid leaf disc method was used and on each leaf a number of viviparous female aphids were placed. For survival of leaves, end of leaves was placed in wet cotton. The leaves were replaced every two days once with fresh leaves. This method was continued until 6 to 7 generations of aphid. The black bean aphid were reared at 25 ± 2°C, 60% R.H. and a 16L:8D photoperiods.

Collecting Plants. Tested plants, including *Satureja isophylla*, *Mentha spicata*, *Mentha piperita*, *Salvia officinalis*, *Thymus carmanicus* (Lamiaceae) were prepared from of local medicinal stores of Tehran. Studied Plants before extraction were dried in laboratory conditions (temperature of 25 ± 3) for one week.

Extraction of the Essential Oil. For extraction of essential oil a hydro distillation apparatus Clevenger was used [4]. For extraction of essential oils, 100 g of grinded plant with one liter of water was poured in the Clevenger and extraction of essential oils was done at three hours. Extracted oils were dried over anhydrous sodium sulphate and in microtubes of 2 ml which were covered with aluminum coating were kept in a conventional refrigerator in 4°c and away from light until using in experiments.

Fumigant Toxicity Bioassay. Bioassay test was conducted using filter paper. The desired essential oil was released on the filter paper in the inner surface of containers. Mortality was evaluated at six different concentrations that ranging from 1.2 to 108.82 µL/L air, and with 6 replications at the interim of 24 hours. In each container 15 adult aphids were released on the acacia leaf with dimensions of 5×5 cm^2. Insects those were not able to move the legs and antenna were considered dead. Control container had no essential oils. To prevent release of volatiles, around the cap was covered with parafilm.

Nymph Production Deterrent. In this research, deterrent effect was tested at LC_{10} and LC_{25} concentrations for each essential oil. In each of tested containers, four adult aphids of 12 hours were placed on the acacia leaf. The desired essential oil was released on the filter paper in the inner surface of containers. Data was recorded daily until 72 hours. After counting, produced nymphs were removed from test containers. 10 replications were used in each experiment. Rate of nymph production deterrent was calculated from the following formula [18]:

Nymph production deterrent=

$$\left(1-\frac{NNt}{NNc}\right)\times 100$$

NN_t= Number of nymph on the treatment
NN_c= Number of nymph on the control

Statistical Analysis. The LC_{50} values with their fiducial limits were calculated by probit analysis using the SAS software [19]. The experiments were arranged in a completely randomize design and the data from nymph production deterrent were subjected to (PROC GLM) ANOVA ($p < 0.05$) after checking for normality. The means were separated using the Duncan's test at the 5% level.

RESULTS

Fumigant Toxicity Assay. By increasing dose, mortality rate and insecticidal activity was increased. For example *S. officinalis* oil at 8.82, 20.59, 38.24, 73.53 and 108.82 µL/L air produced 0,10, 30, 61.67 and 75% mortality, respectively. Those same trends were observed for other tested essential oils. The highest mortality of adult were recorded at the highest concentration the as 75% for all essential oils. At the lowest concentration, the mortality was not observed for all essential oils. However, none of these compounds were not caused the 100% mortality of the adult insects. Data regarding LC_{50} and LC_{90} of all tested plants against *A. fabae* is presented in Table 2. The most potent of the five tested plants against the black bean aphid was *M. piperita* with an LC_{50} of 9.29 µL/L air, whilst the least potent of the three tested plants was *S. officinalis* oil with an LC_{50} of 41.18 µL/L air. LC_{50} and LC_{90} parameters values showed that *M. piperita* oil compared to others have higher fumigant toxicity against adults of the aphid. Results of probit analysis showed that in all cases except in the essential oil of *S. isophylla* g- factor was less than 0.5%. Heterogeneity factor in *M. piperita* and *T. carmanicus* essential oil was less than one. This factor was greater than one for *S. isophylla* (4.03),

TABLE 1
Fumigant toxicity of five different essential oils against the black bean aphid, *A. fabae*.

Plant essential oils	LC_{50} (µl/l) Confidence interval	LC_{90} (µl/l) Confidence interval	g-factor (95%)	Heterogeneity	Slope ± SE	X^2 (df)	p-value
S. isophylla	14.29 (8.53 – 33.06)	38.12 (22.18 – 127.06)	0.589	4.03	3.018±0.362	12.08 (3)	0.002
M. spicata	11.27 (9.88 – 12.82)	21.08 (17.76 – 26.88)	0.055	0.80	4.714±0.563	2.41 (3)	0.002
M. piperita	9.29 (6.47 – 13.06)	19.29 (13.59 – 43.35)	0.245	1.87	4.043±0.460	5.60 (3)	0.000
S. officinalis	41.18 (32.47- 51.35)	81.59 (63.18- 130.06)	0.142	1.10	4.315±0.486	3.30 (3)	0.001
T. carmanicus	10.66 (8.76 – 12.76)	33.00 (26.00 – 45.71)	0.043	0.77	2.616±0.278	3.08 (4)	0.000

TABLE 2
LC_{10} and LC_{25} values of five different essential oils against the black bean aphid, *A. fabae*

	Essential oils				
	S. isophylla	*M. spicata*	*M. piperita*	*S. officinalis*	*T. carmanicus*
LC_{10} (µl/l air)	5.35	6.02	4.49	20.79	3.41
LC_{25} (µl/l air)	8.53	8.11	6.29	28.71	5.88

TABLE 3
Analysis of variance of the effect of five different essential oils on nymph production deterrent of the adults of the black bean aphid, *A. fabae*.

Resources changes	df	Mean Square	F
Essential oils	4	0.48	16.46**
Concentrations	1	0.56	19.31**
Essential oils × Concentrations	4	0.01	0.65ns
Error	90	0.02	
Total	100		

** Significantly different at 1%
ns not significantly different

TABLE 4
Mean (±SE) of nymph production deterrent of five different essential oils on the adults of the black bean aphid, *A. fabae*.

	Concentration (µL/L air)	
Essential oils	LC_{10}	LC_{25}
S. isophylla	6.18±10.60 Ba	11.45±13.83 Da
M. spicata	26.12±15.48 Ab	45.21±18.88 Aba
M. piperita	35.75±18.25 Aa	52.00±17.42 Aa
S. officinalis	5.87±8.33 Ba	23.45±15.28 CDa
T. carmanicus	23.15±18.00 Aa	34.90±16.54 BCa

Capital letters indicate the mean comparison in each column.
Small letters indicate the mean comparison at different concentrations

M. piperita (1.87) and *S. officinalis* essential oil (1.10) on the results of probit analysis represents g-factor action (95%) in correction of LC50 values were.

Effect of Essential Oils on Nymph Production Deterrent. The effect of different essential oils on nymph production deterrent of the adults of *A. fabae* is shown in Table 3. The results of the analysis of variance showed that there is significant difference between deterrent effect of different essential oils (F=16.46, df=1). Also there is significant difference between deterrent effect in different concentrations (F=19.31, df=1). Based on the results of Table 3, interaction effect of different essential oils in different concentrations showed no significant difference (F=0.56, df=4). The maximum deterrence percentage is related to *M. piperita*, so that in 6.29 µL/L air and 4.49µL/L air 52% and 35.75% detergency of the black bean aphid was recorded, respectively. The lowest deterrence percentage (11.45%) was calculated in 8.53 µL/L air for *S. isophylla*, and in 20.79 µL/L air for *S. officinalis*, (5.87%) (Table 4).

DISCUSSION

Among insects, aphids as important pests of agricultural crops are raised in the world that due to resistance, since many control methods have been used against them [20]. Use of pesticides is one of the primary methods in aphids control and have an important role in pest control, but improper use of them are causing adverse effects on non-target organisms and the environment [21]. Also, high speed of development, high rate of population in-

crease, parthenogenesis, viviparity and polymorphism in aphids are causing rapid resistance to different pesticides, so that use of these chemicals is limited [22].

Essential oils due to volatility and very short-term persistence in the environment, as biocompatible pesticides can be considered as one of the best alternatives to chemical pesticides in aphid's control. In present study, the highest LC_{50} value was calculated for essential oil of *S. officinalis* and the lowest LC_{50} value was for *M. piperita.* The results showed that between studied essential oils, *S. officinalis* oil had the highest toxicity on *A. fabae*.

Salehitabar and Hasanshahi [23] was calculated LC_{50} values of essential oil of *Cinnamomum zeylanicum* Blume (7.48 µL/L air) on the black bean aphid. In other study, insecticidal effect of essential oil and leaves powder of *Chenopodium ambrosioides* L. on *Callosobruchus maculates* F. showed that in both cases, oviposition deterrence and reduction in emergence of adult in first generation was observed [24]. Klingauf et al. [25] showed that anise and eucalyptus essential oils are caused 100% mortality in rose-grain aphid, *Metopolophium dirhodum* (Walker). They reported that mortality rate was depended on essential oil concentrations and time. Also in this research it was observed that by increasing dose and time, mortality rate and insecticidal activity of oil was increased. Işık and Görür [26] were evaluated effect of seven plant essential oils (*Juglans regia*, *Juniperus excels*, *Juniperus oxycedrus*, *Foeniculum vulgare, Rosmarinus officinalis, Laurus nobilis* and *Pimpinella anisum*) on the cabbage aphid, *Brevicoryne brassicae*. These essential oils reduce reproductive potential and led to increased mortality in aphid population.

Effect of essential oils of *Thymus vulgaris* L., *Veronica officinalis* L. and *Agrimonia eupatoria* L. on the cabbage aphid, *B. brassicae* showed that essential oil of *T. vulgaris* caused about 85 % mortality in aphid population [27]. Tomova et al. [28] studied effect of essential oil of *Tagetes minuta* L. against three aphid species; So that their results confirmed that this essential oil significantly reduced fertility rate of the aphids. Motazedian et al. [29] were studied toxicity and repellency effects of three essential oils of *Mentha longifolia*, *Salvia officialis* and *Myrtus communis* (Myrtaceae) against *Tetranychus urticae* Koch and were concluded that *M. longifolia* (LC_{50}=20.08 µL/L air) possesses the highest lethal activity whereas *S. officialis* the lowest (LC_{50}=60.93 µL/L air).

Miresmailli et al. [30] were studied toxicity of essential oils of *Rosmarinus officinalis* against *Tetranychus urticae* Koch. LC_{50} value of *R. officinalis* against *T. urticae* grown on barley was 10 µL/L air and against *T. urticae* grown on tomato was 13 µL/L air. Also effect of composition of oil was examined on mortality of *T. urticae,* separately. The results showed that mortality effect of all compounds in *R. officinalis* essential oil was more than the effect of each compound separately.

Essential oil of *Rosmarinus officinalis* plant at concentration of 10 percent was caused repellency on adult of the onion thrips [31]. Essential oils of *Eucalyptus globules* against *Aphis gossypii* had insecticidal property and its LC_{50} value was obtained equal to 2000 PPM [32]. According to report of Hori (33-34) essential oils of peppermint, thyme, garlic and onion had repellency effect on *Myzus persicae*. Insecticidal activities of 53 plant essential oils were tested against *Trialeurodes vaporariorum* by Choi et al. (35) and it was found that the highest mortality in adults, nymphs and eggs were recorded for Bay, caraway seed, clover leaf, lemon eucalyptus, lime dis 5 F, pennyroyal, peppermint, rosewood, spearmint, and tea tree. Also there is significant difference between mortality rates of essential oils with other oils. In our study, the highest deterrence percentage of *A. fabae* is related to LC_{10} of *M. piperita* (35.75%) and the lowest was belonged to *S. officinalis* (6.18%) and *S. isophylla* (5.87%).

Based on studies by Liu et al. (36), *Artemisia princeps* Pamp and *Cinnamomum comphora* (L.) essential oils have lethal and repellency effect on *Sitophilus oryzae* and *Bruchus rugimanus* (Bohem). These two essential oils have synergistic effect on each other and when the oils are incorporate (1:1), their lethal and repellency effect is much higher. Onion essential oil has insecticidal property on the black bean aphid (37). Hasanshahi et al. (38) was studied fumigant toxicity of five essential oils on *B. brassicae* and found that the highest toxicity was related to *Artemisia dracunculus* L. essential oil. LC_{50} value for *A. dracunculus* oil was calculated equal to 6.25 µL/L air in the laboratory conditions. Nemati et al. (39) stated that, LC_{50} value for essential oil of *Foeniculum vulgare* Mill. on *A. fabae* in the laboratory conditions was 3.56 µL/L air.

In overall, results of this study showed that studied essential oils especially *M. piperita* had good effect on the black bean aphid, *A. fabae* and due to low risk of being this compounds for humans and other mammals, also their low viable in nature can be proper substitute for chemical pesticides. Also appropriate composition of the oil can be used for control of aphid in integrated pest management methods.

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DETERMINATION OF THE PRESENCE OF *CRONOBACTER SAKAZAKII*, *LISTERIA MONOCYTOGENES*, *SALMONELLA* SPP. AND *ENTEROBACTERICEAE* COUNT IN ICE CREAMS AND INFANT FORMULAS

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ABSTRACT

The purpose of this study is the determination of the presence of *Cronobacter sakazakii*, *Listeria monocytogenes*, *Salmonella* spp. and *Enterobactericeae* count in ice creams and infant formulas, and present its importance with regard to hygienic quality in dairy products. For this purpose, 25 unpackaged and 65 packaged ice cream samples, and 90 packaged infant formulas have been analysed. Among the unpackaged ice cream samples 9 was determined to have *C. sakazakii* and four was determined to have *L. monocytogenes*, and one packaged ice cream was determined to have *C. sakazakii*. *Enterobacter cloacae* was determined in 13 infant formulas. None of the ice cream and infant formulas samples were determined to have *Salmonella* spp. It was concluded that the detection of *C. sakazakii* and *E. cloacae* in ice creams bears importance for public health.

KEYWORDS:
Ice cream, infant formula, *Cronobacter sakazakii*, Enterobacteriaceae, *Enterobacter clocae*

INTRODUCTION

Cronobacter sakazakii is an opportunistic pathogen that has been associated with neonatal infections such as meningitis, brain abscess, bacteremia [1, 2]. Nevertheless, it has also been reported in adult infections [1, 3, 4, 5, 6]. Their presence in foodstuff poses serious health risks for consumers and a safety concern for the food industry. *C. sakazakii* causes serious neonatal infections such as meningitis, septicemia and enteritis [7, 8] and several contagious diseases. Although there are numerous bacteria species that contaminate powdered infant formula (e.g., *C. sakazakii*, *Enterobacter* spp., Klebsiella, Yersinia, Citrobacter, Staphylococcus, *Streptococccus* spp.), *C. sakazakii* is the most common type [9, 10]. Contamination of powdered infant formula and milk has been associated with *C. sakazakii* [11] and it has been reported as the principal reason of neonatal meningitis [12]. The rate of death due to *C. sakazakii* infection ranges between 40%-80% in newborns, and death is experienced after a short while in these cases [13, 14]. It can also result in serious sequelae such as hydrocephalus and quadriplegia. Moreover, it manifests neurological problems among survivors [15, 16].

On 2002, FDA has warned health providers in USA against the possibility of infection due to *C. sakazakii* in newborn infants fed with powdered infant formula. On 2004, *Mycobacterium paratuberculosis* and *C. sakazakii* have been taken into the program of the study group as substantially dangerous pathogens on the 23rd ISO/TC 34 SC 9 and 11th CEN/TC 275/WG 6 meeting performed in Parma, Italy [12].

Several measures were taken in European Community International risk management such as the revision of Codex Alimentarius for the implementation of guidelines and certain food safety criteria [17]. In a study reviewing the risk assessments performed by FAO/WHO about several powdered infant formulas, it was determined that more studies are required despite previous scientific studies [18]. Since there is still insufficient information on Cronobacter ecology in our day, these microorganisms should also be addressed in foodstuffs other than infant formula [19]. In this context, convenience foods on the market have been scanned for the presence of Cronobacter and other Enterobacteriaceae, and it was observed that Cronobacter could survive in food manufacturing [20, 21, 6]. There is a small number of studies performed for investigating the presence of *C. sakazakii* in various foods.

The hypothesis of this study is to address the necessity of evaluating ice cream and similar foods, that are consumed and enjoyed by all segments of society, against the presence of *C. sakazakii* similar to the way it is performed for infant formulas.

MATERIALS AND METHODS

In this study, 90 ice cream samples (n = 23 unpackaged vanilla, n =26 unpackaged fruit, n =16 unpackaged chocolate flavored ice cream, n=8

packaged vanilla, n=9 packaged fruit, n=8 packaged chocolate flavored ice cream) and 90 infant formulas, which were brought to the laboratory through cold chain, have been analysed for the presence of *C. sakazakii, Salmonella* spp., *L. monocytogenes*, and the number of *Enterobactericeae*.

Performance of *Salmonella* spp. Analysis as per ISO 6579 Method. Under aseptic conditions, 25 g were weighed from samples into TEMPO® stomacher bag (BioMe ´rieux), 225 ml Buffered Peptone Water (BPW-LAB M- LAB204, UK) was added, and the samples were incubated at 37°C for 24 hours after being homogenized for 3 seconds in the homogeniser. 0.1 ml was inoculated from incubated samples into Rappaport Vassiliadis (R.V.S-LAB M- LAB086) broth media, and incubated at 41.5°C for 24 hours. Afterwards, petri plates were incubated at 37°C for 24 hours after drawing lines on Xylose Lysine Deoxycholate Agar (X.L.D- LAB M- LAB032) with a sterile disposable loop (ISO 6579-1:2017). After the incubation, API® 20E (BioMe´rieux-REF 20100) Biochemical Confirmation and Oxidase tests (Liofilchem-REF 88029N) were applied on black colonies suspected for *Salmonella* spp. presence.

Performance of *Listeria monocytogenes* Analyses as per ISO 11290-1 Method. Under aseptic conditions, 25 g were weighed from samples into TEMPO® stomacher bag (BioMe ´rieux), 225 ml Listeria Demi Fraser Broth (Liofilchem s.r.i,- REF 499020) was added, and the samples were incubated at 30°C for 24 hours after being homogenized for 3 seconds in the homogeniser. 0.1 ml was taken from incubated samples and inoculated into Listeria Fraser Broth (LAB M- LAB212, UK) media. Following 24 hours of incubation at 37°C, petri plates were incubated at 37°C for 24 hours after drawing lines on Ottaviani Agosti Agar (ALOA-LAB M-HAL010) with a sterile disposable loop (ISO 11290-1:2017). Microgen Listeria (LAB M-MID 67, UK) Biochemical Confirmation and Hemolysis (LAB M-MID 67, UK) tests were performed on blue colored colonies surrounded with a hemolysis zone in ALOA agar, which were suspected for the presence of *L. monocytogenes*.

Performance of *Cronobacter sakazakii* Analyses as per ISO 22964 Method. Under aseptic conditions, 25 g were weighed from samples into TEMPO® stomacher bag (BioMe ´rieux), 225 ml Buffered Peptone Water (BPW-LAB M-LAB204) was added, and the samples were incubated at 37°C for 18 hours after being homogenized for 3 seconds in the homogeniser. 0.1 ml from incubated samples and 0.1 ml Vancomycin (BioMe´rieux-REF 1551150) supplement were inoculated in Cronobacter selective broth (CSB-Liofilchem-REF 610389), and incubated at 41.5°C for 24 hours. From the incubated broth, drawing was performed on Chromogenic™ Cronobacter Identification Agar (CCIA- Liofilchem-REF 610390, ITALY) with a sterile disposable loop, and they were incubated at 41.5°C for 24 hours (ISO 222964:2017). For biochemical confirmation, API® 20E (BioMe´rieux-REF 20100) and Oxidase tests (Liofilchem-REF88029N) were performed on turquoise colored colonies suspected for the presence of *C. sakazakii*.

Determination of Enterobactericeae Count as per ISO 21528-2 Method. Under aseptic conditions, 10 g was weighed from the samples into TEMPO® stomacher bag (BioMe ´rieux), 90 ml Buffered Peptone Water (BPW-LAB M- LAB204, UK) was added, then 1 ml (10^{-1}) was inoculated from the samples into sterile disposable petri plates under aseptic conditions after being homogenized for 3 seconds in the homogeniser, and 12-15 ml of Violet Red Bile Glucose Agar (V.R.B.G.A. LABM-LAB088) media was poured on top as a double layer. Petri plates were incubated at 37°C for 24 hours. After the incubation, confirmation procedure was performed with Glucose OF Medium (Liofilchem-REF 610388) media on violet colored colonies suspected for *Enterobactericeae* . In the result of confirmation, the tubes that were containing media was evaluated as glucose-positive upon a change in their color into yellow (ISO 21528-2:2017). Colonies were counted in cfu/g in the petri plates with positive confirmation results, and the results were evaluated.

Statistical Analyses. Microbiological counts in cfu/g were converted to log 10 base. Statistical significance of the mean values among the groups was determined with «One Way ANOVA» analysis. After checking their homogeneity with Levene's Test, ANOVA Method was used to determine whether there was a significant difference between groups. Tamhane's T2 test was used as a Post- Hoc test algorithm, which is a multiple comparison test.

RESULTS

In this study, microbiological analysis results of ice creams have been evaluated according to ice cream type and according to the presence of packaging. While *Enterobactericeae* count of vanilla, chocolate and fruit ice creams did not have any statistically significant difference ($p>0.05$) (Table 3), a significant difference was observed between ice creams presented for consumption with or without packaging ($p<0.05$) (Table 4, Figure 3). In a total of 90 ice cream samples, 10 (11%) samples were detected to have *C. sakazakii;* 4 (4%) samples were detected to have *L. monocytogenes*, and none of the ice cream samples were determined to have

Salmonella spp. Determination of *C. sakazakii* in one sample (3%) among the industrially-manufactured 26 ice creams was deemed as noteworthy. The distribution of pathogenic microorganisms in ice creams is presented in Figure 1 and 2, and statistically significant percentages are presented in Table 1 and 2.

According to the microbiological analysis results of 90 infant formulas in this study, no statistical difference was determined with regard to *Enterobactericeae* count (cfu/g), and the presence of *C. sakazakii, L. monocytogenes*, *Salmonella* spp. ($p>0.05$) (Table 5); however, the presence of *Enterobacter cloceae* in 13 infant formulas (14%) has suggested the presence of risk factors. The level of significance was determined as $p<0.05$ in infant formulas with regard to *E. cloacae*. The distribution of pathogenic microorganisms in infant formulas is presented in Figure 4.

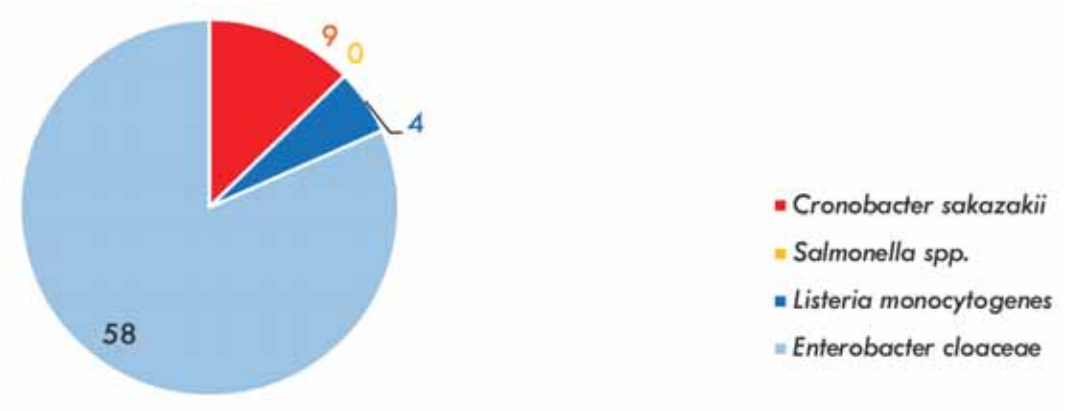

FIGURE 1
Percentage Distribution of Pathogenic Microorganisms in Unpackaged Ice Cream (64 unpackaged ice creams)

FIGURE 2
Percentage Distribution of Pathogenic Microorganisms in Packaged Ice Creams (26 packaged ice creams)

TABLE 1
Pathogenic Microorganism Percent in Vanilla, Chocolate and Fruit Ice Creams

				POSITIVITY PERCENT
	Vanilla	Chocolate	Fruit	MEAN
Listeria monocytogenes	9.7%	0%	2.9%	$X^2_1=3.23$, $p<0.05$
Cronobacter sakazakii	9.7%	12.5%	11.4%	$X^2_2=1.95$, $p<0.05$
Enterobacter cloacae	87.1%	91.7%	91.4%	$X^2_3=0.44$, $p<0.05$

TABLE 2
Pathogenic Microorganism Percent in Unpackaged and Packaged Ice Creams

	POSITIVITY PERCENT		
	PACKAGED	UNPACKAGED	MEAN
Listeria monocytogenes	0%	6.3%	$X^2_1=1.70$, $p<0.05$
Cronobacter sakazakii	3.8%	14.1%	$X^2_2=1.95$, $p<0.05$
Enterobacter cloacae	88.5%	90.6%	$X^2_3=0.96$, $p<0.05$

TABLE 3
Statistical Significance with regard to *Enterobactericeae* Count in Vanilla, Chocolate and Fruit Ice Creams

TYPE	n	Mean	Standard Error	P value
Vanilla	31	1.97	0.30	
Chocolate	24	1.86	0.39	0.44
Fruit	35	1.46	0.26	

TABLE 4
Statistical Significance with regard to *Enterobactericeae* Count in Unpackaged and 239 Packaged Ice Creams

TYPE	n	Mean	Mean Standard Error	P value
Unpackaged	64	2.43	0.19	0.0001
Packaged	26	0.05	0.05	

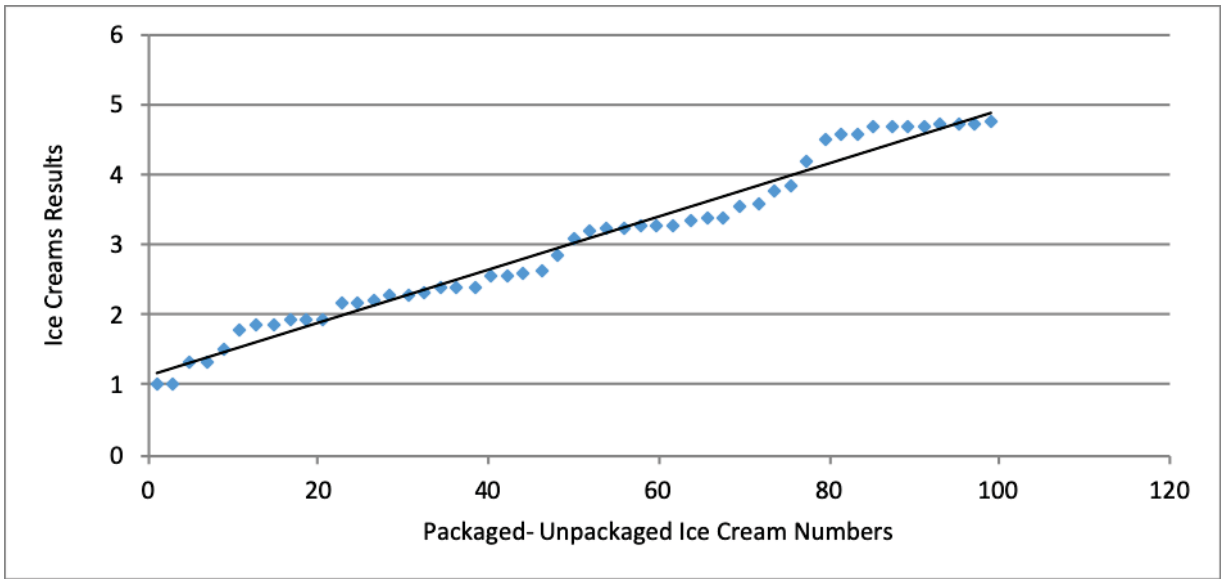

FIGURE 3
Regression Analysis between Unpackaged and Packaged Ice Creams with regard to *Enterobactericeae* Count

FIGURE 4
Percentage Distribution of Pathogenic Microorganisms in Packaged Infant Formulas (90 infant formulas)

TABLE 5
Pathogenic Microorganism Percent in Infant Formulas

	POSITIVITY PERCENT	
	PACKAGED	MEAN
Enterobacter cloacae	14 %	X^2_2=1.90, p>0.05

DISCUSSION

In this study, 90 ice cream samples and 90 infant formulas were taken into analysis for the detection of *C. sakazakii, Salmonella* spp., *L. monocytogenes*, and the number of *Enterobactericeae* in order to assess whether ice creams samples were safe for public health, in addition to infant formulas.

According to the results of the study; no statistical difference was observed with regard to *Enterobacteriaceae* count when ice creams were classified as vanilla, chocolate and fruit ($p>0.05$). Statistical differences were determined between ice creams which were presented with or without packaging ($p<0.05$). While *C. sakazakii* was determined in 10 samples (11%) and *L. monocytogenes* was determined in 4 samples (4%) in a total of 90 samples, no samples were determined to have *Salmonella* spp. No statistical significance was determined in infant formulas with regard to *Enterobacteriaceae* count ($p>0.05$). *E. cloacae* was determined in 13 infant formulas (14%), and it was deemed to be statistically significant in the comparison with other pathogens ($p<0.05$). *C. sakazakii, L. monocytogenes* and *Salmonella* spp. was not determined in any of the formulas.

Similar to our study, El-Gamal et al. [22] have reported in their study performed in Egypt that they have determined the presence of *C. sakazakii* in 30% of 20 Domiati cheese samples, 4% of 25 ice cream samples,4% of 25 yoghurt samples, and 24% of 50 powdered infant formula samples. The investigators determined *L. monocytogenes* in three of 6 Domiati cheese samples (50%) in the same study, and they did not determine this bacteria in ice cream, yoghurt and infant formula samples.

Heparkan et al. [23] have detected *C. sakazakii* contamination in 3 of 25 milk powders (12%) and 1 of 15 whey powders (6.6%) in their study performed on milk-containing infant formulas and infant formula additives.

Baumgartner et al. [6] have determined *Cronobacter* spp. in three of eight food categories. They have indicated that the highest contamination frequency was in sprouts, and fresh herbs / salads. It has been reported in studies performed in recent years that *Cronobacter* spp. contamination level reported in infant formula and powdered milk is approximately 2%-2.5% [21, 24].

El Khair et al. [25] have reported that 16% of fruit ice creams, 4% of chocolate ice creams, and 2% of hazelnut ice creams were contaminated with *E. sakazakii* among 32 types of ice cream obtained from 20 different ice cream makers in Palestine.

Mathews et al. [26] have determined *C. sakazakii* contamination in 4 of 150 unpackaged ice creams (2%) and 2 of 150 packaged ice creams (1%) they have collected in Gabarone, South Africa.

Leuschner et al. [27] have isolated *E. sakazakii* in 8 of 58 infant formula samples (14%) collected from 11 countries.

In their study performed in Netherlands between years 2001-2005, Kandhai et al. [28] have determined *Cronobacter* spp. in 7 of 175 milk powders (4%), 8 of 395 ready-to-use powder formulations (2%) for infants younger than 1 year of age, 1 of 5 ready-to-use powder formulations (20%) for infants older than 1 year of age, and one of other 182 ready-to-use powder formulations (0.5%).

El-Sharoud et al. [29] have reported the presence of *Cronobacter* spp. in 5 of 37 skimmed milk powders (13.5%), 4 of 10 sahlep drinks (40%), 2 of 35 infant formula milks (5.7%), and 4 of 10 Domiati cheeses (40%) in Egypt and Ireland.

It is recognized that all of the study results stated above complies with the results of this study. It is important to consider that studies to be performed on *Cronobacter* spp. (*C. sakazakii*) should not be limited with infant formulas, these microorganisms should be addressed in other foods apart from infant formulas since the information on *Cronobacter* ecology is still insufficient in our day [19], and that it should be addressed based on various food products consumed commonly by public in each country.

It is crucial to add that our study supports the comment of FAO/WHO [30] on which states “All *Cronobacter* species have been retrospectively linked with clinical infection cases in infants or adults, and therefore all species should be considered to be pathogenic”.

There is a small number of studies on the presence of *C. sakazakii*, *Salmonella spp*., *L. monocytogenes* and *Enterobacteriaceae* count in ice creams, and unfortunately, it was observed that the studies related to *C. sakazakii* are mostly limited to infant formulas.

The results reveal that *C. sakazakii* is at least as risky as *Salmonella* spp. and *L. monocytogenes* in ice creams, which are enjoyed by all age groups especially in summer. Around the world, pathogenic microorganisms that pose any risk for ice creams are stated as *Salmonella* spp., *L. monocytogenes and Enterobacteriaceae* according to microbiological criteria. However, it was intended to state in this study that *C. sakazakii and E. cloacae* risks should be eliminated in ice creams on the market for the protection of public health.

CONCLUSION

Our results show that *C. sakazakii* can be present in various food products, and therefore foodstuff can be the source of all potential contaminations. The fact that the concentration and prevalence values of *Cronobacter* spp. in foodstuff may show differences according to manufacturing type

manifests as another possibility. The highest risk arises during the manufacturing and preparation of food products for consumers [28]. *C. sakazakii* contamination is caused by the operations before, during and after the process (e.g., use of contaminated additives after pasteurization, manufacturing environment before packaging), and external contamination sources during the reconstitution of the formula and other steps of the preparation (e.g., use of non-hygienic equipment) [31]. Possible sources of these bacteria in ice creams may generally be nose, hands, skin, and clothes worn during processing. Coughing, speaking and sneezing during ice cream preparation causes the proliferation of microorganisms through droplets, and results in the disruption of the quality of the product during transport, storage and sales operations.

The fact that unpackaged ice creams have high microbiological bacteria count compared to industrially-packaged ice creams and the presence of pathogenic microorganisms suggests that businesses should control all factors during raw material procurement, processing, packaging and storage, and secure the manufacturing with regard to food safety and hygiene.

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UTILIZATION OF SOME DAIRY BY-PRODUCTS AND BIO-YOGHURT IN MAKING PROBIOTIC YOGHURT DRINK

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ABSTRACT

Probiotic yoghurt drink (PYD) was prepared from bio-yoghurt supplemented with 2% skim milk powder (SMP) or whey protein concentrate powder (WPC) and diluted with water or sweet whey (1:1). Analysis of the product revealed that its total solids was not affected by type of supplement, but increased when whey was used as a diluent. Fat and protein contents were significantly increased when WPC and whey were used. Ash content was slightly higher and carbohydrate was slightly lower in case of using SMP, while whey significantly increased both of them. Acidity and pH were not affected (P>0.05) by supplements, diluents and storage, while the caloric values were significantly higher in the PYD made using WPC than that made with SMP. Whey caused an increased impact on energy while storage caused the opposite. Counts of Bifidobacteria and *L.acidophilus* were not affected by type of supplement, but were always higher when water was used. Organoleptically, the properties were not affected by types of supplement, but significant higher scores were given when whey was used. Slightly less scores were given for the stored product when compared with the fresh one.

KEYWORDS:
Dairy by-products, probiotics, yogurt drink

INTRODUCTION

Utilization and/or disposal of some dairy by-products are major concerns to dairy specialist all over the world. Skim milk, whey and buttermilk are known as the industry's principal byproducts-residues from the manufacture of cream, cheese and butter respectively [1]. Especially, whey represents an important environmental pollutant since the worldwide whey production in 2016 was 200 million ton [2, 3].

The use of such by-products or their different preparations as an ingredient in formulated foods and dairy products is quite important. Composition, properties and classes of SMP [4], whereas nutritive value, functional properties and application of whey protein preparations [2, 5, 6]. Therefore, the present study dealt with the use of skim milk powder (SMP), whey powder (WP) and whey protein concentrate (WPC) in a powder form in making probiotic yoghurt drink (PYD) since popularity and importance of yoghurt and its related products are well-known. Yoghurt drink (YD) or drinking yoghurt or drinkable yoghurt is considered as stirred yoghurt with a low viscosity [7]. This product especially after using probiotic bacteria in its making has a growing area of interest based on its convenience, portability and ability to deliver all of the health benefits as well as nutritional ones [8,9,10]. The use of some dairy by-products and their effects on viability of the probiotic bacteria used in making YD were the objectives of the present study.

MATERIALS AND METHODS

Materials. fresh cow's milk was obtained from Sakha Animal Prod. Res. Stat., Animal Prod. Res. Inst. Low-heat SMP (Valio, LTD, Finland), WPC powder (Lactalis Ingredients, USA) and whey powder (Portiant Dairy, Inc. USA) were purchased from the local market. Yoghurt starter (YC-XII-DVC) consisting of *S.thermophilus* and *L. delbruekii ssp. bulgaricus* as well as probiotic culture (ABT-2, Probio Tec., DVC) consisting of *Bifidobacterium* BB_{12}, *L. acidophilus* - LAS and *S. thermophilus* were purchased from Chr. Hansen Lab., Copenhagen, Denmark. CMC (Central Drug House, Ltd. India) used as a stabilizer and food-grade salt were obtained from the local market.

Manufacture of yoghurt drink. It was done from bio-yoghurt supplemented with 2% SMP or WPC and fermented using both of the pre-mentioned starters (1:1) [7]. Yoghurt samples were diluted (1:1) with pre-boiled water or reconstituted sweet whey (7% w/w) before adding stabilizer (0.5%) and salt (0.5%) and filling into glass containers.

Analysis methods. The fresh and cold stored (10 days) YD samples were analysed for total solids (TS), fat (Gerber method), protein (Kjheldal method), ash, acidity (as lactic acid) and pH as described

[11], whereas carbohydrate was calculated by difference (Carbohydrate = TS – [Fat + Protein + Ash]). Caloric value was calculated from the following equation [12]:

E = 370 F + 170 P + 168 L + 18.

Where E = Energy (kJ/kg), F = Fat content (%), P = Protein content (%), L = Lactose or carbohydrate content (%).

Counts of Bifidobacteria and *L. acidophilus* were carried out on Deman, Rogosa-Shape Agar (MRS) and MRS based agar media respectively [13]. Evaluation of the organoleptic properties was mainly done [14].

Statistical analysis. it was performed using the SPSS version 10 program [15]. Results were subjected to ANOVA and Duncan's test to determine the significant differences among the means at 0.05 level.

RESULTS AND DISCUSSION

Table (1) shows chemical composition of the prepared yoghurt drink (YD) as affected by type of supplement and type of diluent. Total solids (TS) of fresh or stored YD was not affected by the first factor (P>0.05). This was expected since SMP and WPC were in a powder form and were added with the same ratio (2%) to the used yoghurt milk. The use of whey as a diluent significantly increased TS that could be attributed to the used liquid whey contained some solids rather than water. Presence of more fat (6%) and protein (80%) in WPC when compared with those of SMP (1.2% and 11%, respectively) increased fat (P≤0.05) and protein (P>0.05) of the fresh or stored YD. The values of fat and protein contents were always higher in the product prepared using whey than that made using water.

Ash content was slightly higher (P>0.05) in the product made using SMP than WPC. This could be explained on the basis of containing SMP more ash (8%) than WPC (4%). This was true in case of using water or whey as a diluent. The opposite was noticed with respect to carbohydrate content since slightly higher values ($P > 0.05$) were mostly recorded in YD made using WPC, while the values were significantly higher when whey was used as a diluent in making YD comparing to water. It may be of interest to note that the changes in all the prementioned constituents during the cold storage period (10 days) were not significant (Table 1). The present findings are in agreement with those given by [16] who found that the chemical composition of zabady drink was affected by type of diluent (water or whey) and with those given by [17]who reported the same impact of SMP and WPC on composition of YD.

The acidity and pH shown in Table (2) revealed an increase in acidity of the product due to supplementation with WPC or due to dilution with whey. Such increase as well as the changes in pH were insignificant, while advancing storage had also insignificant impact in this respect. Impact of diluents agrees with [16], while the changes in acidity and pH are in agreement with[18,19] who attributed such changes in YD to post acidification and accumulation of lactic acid by lactic acid bacteria during storage.

Owing to the difference in the chemical composition of YD due to the different supplements or diluents used, the caloric value (Table 2) of the prepared product differed significantly. WPC significantly increased the caloric value. The values were much higher ($P \leq 0.05$) in case of using whey comparing with water. This was true in the fresh and stored product. However, the changes due to storage were insignificant. Impact of diluents agrees with the results given by [16] in this respect.

TABLE 1
Chemical composition (%) of fresh (A) and stored (B) probiotic yoghurt drink (PYD) as affected by supplementation with skim milk powder (SMP) or whey protein concentrate (WPC) and by dilution with water or whey (Average ±SE of 3 replicates)*.

Property		Water		Whey	
		SMP	WPC	SMP	WPC
Total solids	(A)	8.01 ± 0.09^{bA}	8.08 ± 0.06^{bA}	10.36 ± 0.05^{aA}	10.36 ± 0.05^{aA}
	(B)	8.20 ± 0.07^{bA}	8.30 ± 0.07^{bA}	10.50 ± 0.12^{aA}	10.51 ± 0.08^{aA}
Fat	(A)	1.52 ± 0.01^{cA}	1.62 ± 0.01^{bA}	1.63 ± 0.05^{bA}	1.72 ± 0.01^{aA}
	(B)	1.52 ± 0.01^{cA}	1.62 ± 0.01^{bA}	1.64 ± 0.02^{bA}	1.72 ± 0.02^{aA}
Protein	(A)	2.22 ± 0.00^{bA}	2.24 ± 0.01^{bA}	2.51 ± 0.01^{aA}	2.53 ± 0.01^{aA}
	(B)	2.24 ± 0.00^{bA}	2.25 ± 0.00^{bA}	2.53 ± 0.00^{aA}	2.55 ± 0.00^{aA}
Ash	(A)	0.58 ± 0.03^{aA}	0.48 ± 0.01^{aA}	0.64 ± 0.04^{aA}	0.54 ± 0.01^{aA}
	(B)	0.59 ± 0.02^{aA}	0.49 ± 0.01^{aA}	0.65 ± 0.03^{aA}	0.55 ± 0.00^{aA}
Carbohydrate	(A)	3.71 ± 0.06^{bA}	3.75 ± 0.04^{bA}	5.61 ± 0.07^{aA}	5.61 ± 0.05^{aA}
	(B)	3.87 ± 0.04^{bA}	3.93 ± 0.08^{bA}	5.72 ± 0.07^{aA}	5.73 ± 0.06^{aA}

* Averages with unlike small superscripts (due to treatments) and averages with capital superscripts (due to storage period) differed significantly (P≤0.05).

TABLE 2

Acidity (%), pH and caloric value (kJ/100g) of fresh (A) and stored (B) probiotic yoghurt drink (PYD) as affected by supplementation with skim milk powder (SMP) or whey protein concentrate (WPC) and by dilution with water or whey (Average ±SE of 3 replicates)*.

Property		Water		Whey	
		SMP	WPC	SMP	WPC
Acidity	(A)	0.47±0.04^{aA}	0.51±0.03^{aA}	0.64±0.04^{aA}	0.66±0.02^{aA}
	(B)	0.51±0.02^{aA}	0.57±0.04^{aA}	0.69±0.03^{aA}	0.72±0.3^{aA}
pH	(A)	5.08±0.04^{aA}	5.09±0.02^{aA}	4.93±0.04^{aA}	4.95±0.02^{aA}
	(B)	4.97±0.01^{aA}	4.95±0.03^{aA}	4.83±0.05^{aA}	4.90±0.4^{aA}
caloric value	(A)	157.4±0.08^{dA}	161.6±0.09^{cA}	197.9±1.01^{bA}	202.0±1.01^{aA}
	(B)	160.4±0.08^{dA}	165.2±1.01^{cA}	200.1±1.05^{bA}	204.2±0.09^{aA}

* Averages with unlike small superscripts (due to treatments) and averages with capital superscripts (due to storage period) differed significantly (P≤0.05).

TABLE 3

Counts of Bifidobacteria and *L. acidophilus* (log CFU/ml) of fresh (A) and stored (B) probiotic yoghurt drink (PYD) as affected by supplementation with skim milk powder (SMP) or whey protein concentrate (WPC) and by dilution with water or whey (Average ±SE of 3 replicates)*.

Property		Water		Whey	
		SMP	WPC	SMP	WPC
Bifidobacteria	(A)	7.66±0.18^{aA}	7.64±0.12^{aA}	7.29±0.20^{bA}	7.28±0.12^{bA}
	(B)	7.17±0.20^{aB}	7.16±0.16^{aB}	6.82±0.14^{bB}	6.80±0.13^{bB}
L. acidophilus	(A)	7.51±0.12^{aA}	7.47±0.15^{aA}	7.21±0.16^{bA}	7.18±0.11^{bA}
	(B)	7.12±0.13^{aB}	7.07±0.15^{aB}	6.76±0.17^{bB}	6.71±0.11^{bB}

* Averages with unlike small superscripts (due to treatments) and averages with capital superscripts (due to storage period) differed significantly (P≤0.05).

Counts of Bifidobacteria and *L. acidophilus* (Table 3) seem to be not affected by the use of SMP or WPC but significantly increased in the product made using whey comparing with water. This was true in case of fresh and stored YD. However, less significant ($P \leq 0.05$) counts were recorded in the stored product than the fresh one. It may be of interest to note that the counts of the pre-mentioned bacteria were always higher than the minimum recommended count to transfer the probiotic effect to consumer. Such probiotic bacteria as mentioned [20] are live micro-organisms that when administered in adequate amounts (10^6 - 10^7 CFU/ml) confer health benefits. Suitability of using yoghurt starter with probiotic bacteria agrees with the studies given in the literature for making bio- yoghurt and its related products[21, 22, 23]. The decreasing impact of whey and advancing storage on counts of the probiotic bacteria could be attributed to development of more acidity in the YD prepared using whey, while activity of the starter used increased acidity and decreased pH during storage.

This trend agrees with the results given by [22-25], reported the same for concentrated yoghurt. In general, [26] reviewed the factors affecting viability of probiotics in dairy bio-products which included the strains used, culture conditions, chemical composition of the product, final acidity, availability of nutrients, growth promoters and inhibitors, dissolved oxygen and fermentation time and storage temperature. Recently [27] demonstrated the factors responsible for survival of probiotics in foods in general and the technologies used to stabilize their viability during processing and storage.

The organoleptic properties of YD (Tables 4 and 5) included flavour, body and texture, acidity and general appearance were not affected by using SMP or WPC ($P > 0.05$) but the scores given for the pre-mentioned properties were always significantly higher in case of using whey as a diluent comparing with water. This may be due to the pleasant acidic taste of the product that caused by developing more acidity in the presence of whey as previously shown in Table (2). Such scores were always slightly less in the stored product (Table 5) when compared with those given for the fresh one (Table 4). This agrees with the trend found by [17]. However, the accepted YD with presence of probiotic bacteria is of great importance for health. This confirmed the finding of [10] who mentioned that YD was an acceptable vehicle to deliver the probiotic health effects even at the end of 30 days storage period.

CONCLUSION

The present study concluded that YD was prepared from bio-yoghurt supplemented with 2% SMP or WPC had effects on the viability of the probiotic bacteria beside that the organoleptically properties were not affected by the types of supplement.

TABLE 4

Scoring of the organoleptic properties and container of fresh probiotic yoghurt drink (PYD) as affected by supplementation with skim milk powder (SMP) or whey protein concentrate (WPC) and by dilution with water or whey (Average ±SE of 10 panelists)*.

Property**		Water		Whey	
		SMP	WPC	SMP	WPC
Flavour	(45)	37±0.92^{b}	36±1.02^{b}	41±1.00^{a}	41±0.82^{a}
Body and texture	(30)	25±0.79^{b}	26±0.84^{b}	27±0.43^{a}	27±0.22^{a}
Acidity	(10)	7±0.29^{b}	7±0.35^{b}	8 ±0.24^{a}	8±0.23^{a}
Appearance	(10)	7±0.23^{b}	7±0.19^{b}	8±0.05^{a}	8±0.07^{a}
Container and Closure	(5)	5±0.00^{a}	5±0.00^{a}	5±0.00^{a}	5±0.00^{a}

*Averages with unlike superscripts differed significantly (P≤0.05).
** The values in parenthesis represent the maximum attainable score.

TABLE 5

Scoring of the organoleptic properties and container of stored probiotic yoghurt drink (PYD) as affected by supplementation with skim milk powder (SMP) or whey protein concentrate (WPC) and by dilution with water or whey (Average ±SE of 10 panelists)*.

Property**		Water		Whey	
		SMP	WPC	SMP	WPC
Flavour	(45)	37±1.00^{b}	36±0.93^{b}	39±0.20^{a}	38±0.28^{ab}
Body and texture	(30)	26±0.31^{a}	26±0.27^{a}	26±0.19^{a}	26±0.08^{a}
Acidity	(10)	5±0.08^{b}	5±0.26^{b}	7±0.20^{a}	6±0.61^{ab}
Appearance	(10)	6±0.08^{a}	6±0.08^{a}	6±0.08^{a}	6±0.08^{a}
Container and Closure	(5)	5±0.00^{a}	5±0.00^{a}	5±0.00^{a}	5±0.00^{a}

*Averages with unlike superscripts differed significantly (P≤0.05).
** The values in parenthesis represent the maximum attainable score.

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YOGURT ICE CREAMS PRODUCED BY ADDING DIFFERENT FRUITS: PRODUCTION AND CHARACTERISTICS

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ABSTRACT

Fruit yoghurt ice cream samples were produced with four different fruits as blackberry; raspberry, black mulberry and strawberry in which 15% and 30% concentrations of fruits were used. Fruit adding to yoghurt ice cream was decreased dry matter and fat ratios of samples but increased ash ratios. Acidity of samples produced with 30% black mulberry addition was higher at significant level ($p < 0.01$) than that of other samples. Similarly, pH of black mulberry samples was lower as statistically ($p < 0.01$) compared to the other samples. Overrun ratio of samples varied from 21.28% to 36.2%. Viscosity of samples produced with 15 % fruit addition was higher ($p < 0.01$) than that of 30% fruit added samples. Fat destabilization ratio varied between 8.50% and 14.10%. The longest first dripping times were found in the samples added black mulberry and strawberry, and as fruit amounts increased, first dripping times of samples increased. Panelists preferred the samples of 30% fruit addition to 15% fruit addition.

KEYWORDS:
Yoghurt Ice Cream, Fruits, Viscosity, Fat Destabilization, Overrun

INTRODUCTION

Yogurt is a fermented dairy product obtained with the activation of two lactic acid bacteria named as *Streptococcus thermophilus* and *Lactobacillus delbruecki* sp., *bulgaricus*. Yoghurt is a food of high biological value. Frozen yoghurt is a frozen dessert made with yoghurt and other ingredients. In 2012, the consumption of available frozen yoghurt reached 23.9 lb. (10.83 kg) for per person in the USA. Different ingredients and freezing methods have resulted in different types of ice creams. Anthocyanins are them pigments responsible for the red and blue colors of plant organs such as fruits, flowers, and leaves. The main dietary sources of anthocyanins are red fruits, certain vegetables, and red wine [1]. Black mulberry contains the anthocyanins as 54.11 mg L^{-1} [2]. Mulberry fruit contains 28.42 mg kg^{-1} ascorbic acid, 193.85mg kg^{-1} anthocyanins and 1308.07 mg kg^{-1} total phenolic matter [3]. The bioavailability of anthocyanins from raspberry extract was high in vitro digestion and all of the anthocyanins in raspberry survived gastric system [1]. Frozen yoghurt or yoghurt ice cream is an alternative of ice cream. Yoghurt ice cream contains both the nutritional value of yoghurt and the delicious taste of ice cream. Fat, sugar, acid and dry matter contents are the four variables of frozen yoghurt [4]. Consumers prefer a frozen yoghurt with low acidity and high sugar concentration [5]. The samples of yoghurt ice cream with vanilla and strawberry jam (15%, 20% and 25%) had the 28.60-45.60 SH acidity, 21.77 %-31.63% overrun, 1670-1970 s first dripping time and 5744-6266 s complete melting time [6]. The ice cream mix with black mulberry juice had the 74.50 % average overrun and 1.52 g min^{-1} melting ratio [2].

It has been reported that the viscosity, overrun and the total melting time of the yoghurt ice cream samples were between 9.766 Cp and 11.600 Cp, 25.39 % and 34.28 % and 82.33 min. and 87.66 min., respectively [7]. To add in this , researchers [7] found that the TAMB, psychrophilic and lactic acid bacteria counts of samples were between 4.64 log CFU g^{-1} and 4.80 log CFU g^{-1},3.49 log CFU g^{-1} and 4.01 log CFU g^{-1} and 2.61 log CFU g^{-1} and 3.89 log CFU g^{1}, respectively. The vegetable marrow adding to ice cream was increased the viscosity, first dripping time and complete melting time of ice cream samples, but decreased overrun value [8]. In this study, the samples of fruit (blackberry, raspberry, black mulberry and strawberry) yoghurt ice cream were made and the physical, chemical and organoleptic properties of the samples were determined. The objective of the study was to investigate the effects on the quality of yoghurt ice cream of the fruits rich in term of phenolic matter.

MATERIALS AND METHODS

Ingredients used at study. Cow's milk (11.93 % Total Solids (TS), 3.3% butterfat and 8.30 SH acidity were obtained from the Ataturk Univer-

sity Pilot Factory. Fruits (blackberry, raspberry, black mulberry and strawberry), stabilizer (salep), emulsifier (lecithin), nonfat dry milk (96.17% dry matter, 1.00 % fat and 6.83 SH acidity), cream (68.3% fat and 9.75 SH acidity) were purchased from a local market.

Production of yoghurt ice cream. First, the yoghurt ice cream mixes were prepared [9]. For this, the yoghurt samples were produced using S. thermophilus and L. delbruecki ssp bulgaricus. Then, other ingredients (1% stabilizer (salep), 0.5% emulsifier (lecithin), 2.5% cream, 2.5 % non-fat dry milk and 18% sucrose) were added to yoghurt and yoghurt ice cream mixes were prepared. Fruits at pulp form were added to mixes brought to 40°C at two different ratios as 15% and 30%. The samples of fruit yoghurt ice cream were produced in freezing machine (-5°C−-7°C). Then, the plain and fruit yoghurt ice cream samples were packaged in plastic cups. The samples of fruit yoghurt ice cream were then hardened at -18°C in a deep freezer. Yoghurt ice cream samples were produced in duplicate.

Analysis of samples of fruit yoghurt ice cream. Microbiological analysis. Ten- gram ice cream mix samples were homogenized with 90 kg sterile solution of 0.85 % sodium chloride. Then, decimal dilutions were prepared in 9 ml sterile NaCl (0.85%). The Total Aerobic Mesophilic Bacteria (TAMB) was enumerated on Plate Count Agar (PCA) after incubation at 32°C for 72 hours. The number of lactic acid bacteria of samples was enumerated on MRS (Lactobacillus) Agar. The numbers of the psychrophilic bacteria was enumerated on PCA incubated (72 hours) in the refrigerator (5 ±1°C) [10].

Physical and chemical measurements. The dry matter ratio, fat ratio and acidity (SH) of milk, non-fat dry milk, cream, yoghurt and yoghurt ice cream samples were determined. The pH of mix samples was measured with pH meter [11]. The viscosity of samples was measured at 4±1°C with spindle No: 4 at 20 rpm [12].The overrun ratio of mix samples are measured as a comparison of the weight of the mix and fruit yoghurt ice cream. Then, results were calculated using formula [13].

Overrun (%) =

-weight of yoghurt ice cream – the weight of mixes /the weight of yoghurt ice creams

The fat destabilization index in the melted ice cream samples was determined by a method based on the procedure [14]. For first melting time analysis, the 10 g sample of ice cream was put to a filter and waited at 18±2 °C for first dripping. The time of first dripping was determined as the second [6].

Sensory assessment. The sensory analysis of ice-cream samples were determined based on Hedonic type scale before Sensory analysis was carried out to determine the best sample using six panelists [15]. Sensory analyses of the ice-cream samples were carried out by ten selected food technology staff and high School food technology department students at university, comprised of females and males. Each panelist was trained before evaluation in order to familiarize with the sensory analysis, samples and methodology. Sensory evaluation was performed in a room with appropriate temperature (25 °C) in opensitting. The samples of ice cream were given to the panelist in frozen condition. Hedonic type scale was used for evaluating the samples. The highest score was accepted by nine.

Statistical Analysis. One-way analysis of variance (One-way Anova) was used for the determination of the differences between the ice cream samples and the effects of storage. Accordingly, statistical analysis software SPSS version 15.0 (SPSS Inc. Chicago, Illinois) was used. The significant data as a result of the analysis of variance (ANOVA) were tested according to the Duncan multiple comparison tests at $p < 0.01$ level.

RESULTS AND DISCUSSION

Results of the microbiological analysis. The results of microbiological analysis of yoghurt ice cream were given in Table 1.

TABLE 1
Microbiological analysis results of yoghurt ice cream added different fruits

Yoghurt ice cream samples	Amounts of fruit (%)	TAMB (log CFU g^{-1})	Lactic acid bacteria (log CFU g^{-1})	Psychrophilic bacteria (log CFU g^{-1})
Plain (control)	-	6.50^{a}	3.51^{a}	2.35^{ab}
Blackberry	15	6.43^{a}	2.80^{a}	2.42^{b}
	30	5.85^{a}	3.20^{a}	2.45^{b}
Raspberry	15	6.33^{a}	3.60^{a}	2.50^{b}
	30	6.14^{a}	3.27^{a}	2.45^{b}
Black mulberry	15	6.21^{a}	3.42^{a}	2.45^{b}
	30	5.62^{a}	3.27^{a}	2.50^{b}
Strawberry	15	6.30^{a}	3.38^{a}	2.26^{a}
	30	6.26^{a}	3.32^{a}	2.24^{a}

a,b means that the differences between the samples of yoghurt ice cream were significant ($p<0.01$).

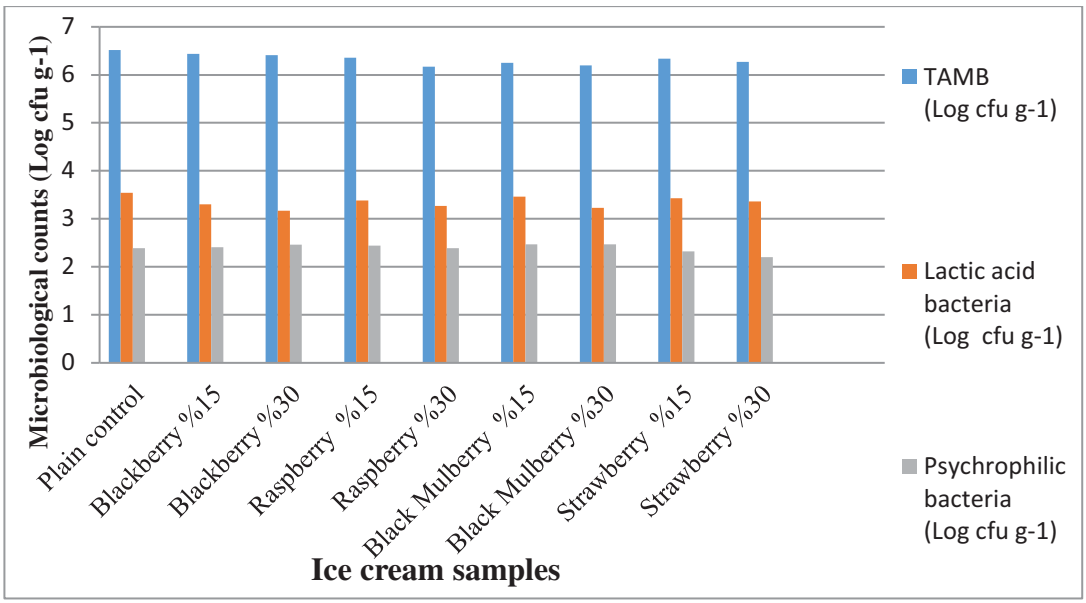

FIGURE 1
Microbiological analysis results of samples of yoghurt ice cream added different fruits

TABLE 2
The results of physical and chemical analysis of samples of yoghurt ice cream

Yoghurt Ice Cream Sample	Amount of fruit (%)	Dry Matter (%)	Fat (%)	Ash (%)	Acidity (SH)	pH	Overrun (%)	Viscosity (Pa.s)	Fat Destab. (%)	First Dripping Times(s)
Plain (control)	-	37.25^{b}	8.10^{b}	0.47^{a}	47.81^{a}	4.55^{b}	27.01^{b}	7.01^{a}	13.80^{d}	603^{a}
Blackberry	15	33.71^{aB}	6.15^{aA}	0.55^{bB}	48.82^{bA}	4.35^{bB}	21.95^{aA}	15.00^{bB}	8.35^{aA}	903^{bA}
	30	33.07^{aA}	5.85^{aA}	0.50^{bA}	50.47^{bB}	4.34^{bA}	27.10^{aB}	10.85^{bA}	10.00^{aA}	1144^{bB}
Rasp berry	15	33.59^{aB}	6.30^{aA}	0.54^{bB}	48.50^{bA}	4.35^{bB}	23.87^{abA}	12.75^{bB}	11.05^{bcA}	933^{bA}
	30	32.83^{aA}	6.05^{aA}	0.51^{bA}	50.13^{bB}	3.93^{bA}	26.87^{abB}	9.95^{bA}	10.55^{bcA}	1097^{bB}
Black Mulberry	15	34.11^{aB}	6.15^{aA}	0.54^{bB}	52.44^{cA}	3.75^{aB}	32.32^{cA}	13.05^{bB}	9.00^{abA}	1035^{cA}
	30	31.97^{aA}	5.95^{aA}	0.50^{bA}	55.62^{cB}	3.40^{aA}	36.01^{cB}	9.78^{bA}	10.55^{abA}	1285^{cB}
Strawberry	15	33.99^{aB}	6.10^{aA}	0.52^{bB}	48.19^{aA}	4.33^{bB}	26.61^{bA}	15.70^{bB}	11.25^{cA}	957^{bcA}
	30	32.98^{aA}	5.75^{aA}	0.50^{bA}	48.24^{aB}	4.23^{bA}	28.20^{bB}	9.25^{bA}	12.30^{cA}	1267^{bcB}

a,b, c means that the differences between the samples of yoghurt ice cream were significant ($p<0.01$).
A, B means that the differences between the fruits amounts were significant ($p < 0.01$).

The Total Aerobic Mesophilic Bacteria (TAMB) counts of samples changed between 5.62 log CFU g-1 and 6.43 log CFU g^{-1}(Table1). The TAMB counts of plain samples were higher than that sample of fruit yoghurt ice cream. But, the differences between TAMB counts of the samples of yoghurt ice cream and fruits amounts were no significant ($p > 0.05$). The TAMB counts determined by [7] were lower than that of our findings. The lactic acid bacteria count of samples was between 2.80 log CFU g^{-1} and 3.60 log CFU g^{-1}(Table1). The lactic acid bacteria counts of plain (control) samples were generally found as higher than that of fruit yoghurt ice creams (Fig.1). But, the differences between lactic acid bacteria counts the samples of yoghurt ice cream and fruits adding amounts were no significant as statistical ($p > 0.05$). The lactic acid bacteria counts found by [7] were higher than our findings. The psychrophilic bacteria counts of samples were ranged between 2.24 log CFU g^{-1} and 2.50 log CFU g^{-1} (Table1). The psychrophilic bacteria numbers of plain yoghurt ice cream samples and fortified with strawberry were lower statistically ($p < 0.01$) than that of other yoghurt ice cream samples.

Results of Physical and Chemical. The results of physical and chemical analysis of samples of yoghurt ice cream were given Table 2. The dry matter ratio of control yoghurt ice cream samples was higher as statistical ($p < 0.01$) than that of samples of fruit yoghurt ice cream. The dry matter ratio of samples added 15% fruit were higher significant level ($p < 0.01$) than that of 30% fruit adding (Table2). The dry matter ratio of control yoghurt ice cream samples was found of higher than that of fruit yoghurt ice cream samples. This can be originated from the fact that the dry matter ratios of fruits added to ice cream samples were low. The fat ratio of samples varied between 5.75% and 8.10 %. As fruit ratio added to the ice cream increased, the fat ratio of ice cream samples decreased. The ash ratio of samples was ranged from 0.47% to 0.55%. But, as fruit ratio added to ice cream increased, the ash ratio of samples increased too. The lowest acidity (47.81 SH) of ice cream samples was found at

samples of control ice cream. The acidity of samples added black mulberry were higher at a significant level ($p < 0.01$) than that of others [16]. The acidity of yoghurt ice cream produced with different stabilizers was between 47.50 SH and 52.83 SH [16]. The acidity values found by [16] were parallel with our results. The acidity of strawberry yoghurt ice-cream [6] was lower compared to our findings. The pH of yoghurt ice-cream samples varied from 3.40 to 4.55. The pH of black mulberry yoghurt ice cream samples was lower at a significant level ($p < 0.01$) than that of other samples. Control samples had the highest pH level (Table 2).

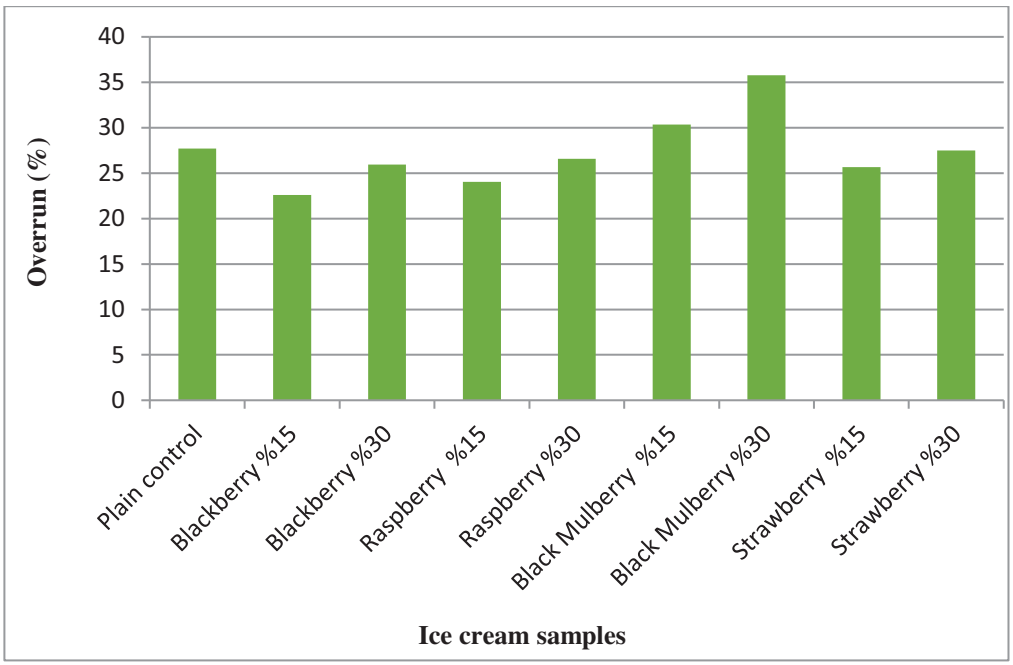

FIGURE 2
Overrun (%) values of ice cream samples added different fruits

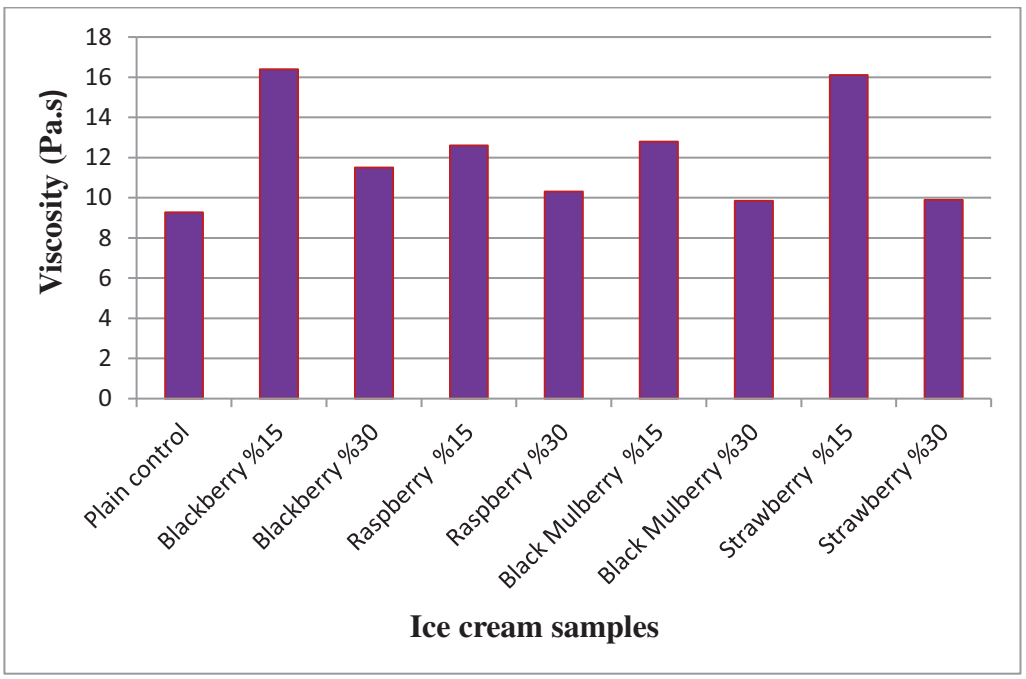

FIGURE 3
Viscosity values of ice cream samples added different fruits

Overrun ratio shows the amount of air incorporated to ice cream during the manufacturing process. The overrun ratio of the samples varied from 21.95% to 36.01% and a positive correlation was observed between the fruit and overrun ratios which were in accordance with the findings of [6]. As fruit amount of ice cream samples increased, the overrun increased significantly ($p < 0.01$) too (Fig.2). The viscosity of samples of yoghurt ice cream was altered between 7.01 Pa.s and 15.70 Pa.s and the lowest viscosity was observed in the control yoghurt ice cream sample (Fig.3). Interestingly, 15 % fruit addition resulted in higher viscosity at a significant level ($p < 0.01$) compared to the 30 % fruit added samples. The vegetable marrow adding was increased the viscosity [8]. Grape wine addition [17] was increased the viscosity of ice-cream mix as parallel to our founding. The state has sourced the interaction between components like pectin in fruits and sugars [18]. The viscosity of yoghurt ice cream was between 9.76 Pa.s and 11.60 Pa.s which were parallel to our observation [7]. The lower fat destabilization shows very good mix emulsification. We want lower fat destabilization in ice cream. The fat destabilization ratio varied from 8.35% to 13.80% and decreased at a significant level ($p < 0.01$) by the fruit addition (Fig.4). The fruits added to ice cream mix have decreased the emulsification of ice cream. The first dripping time of samples was between 603 s and 1285 s. The longest first dripping time was found in samples added black mulberry (Fig. 5). The vegetable marrow adding to ice cream mix was increased first dripping time [8]. The first dripping time found by [6] was longer than our findings.

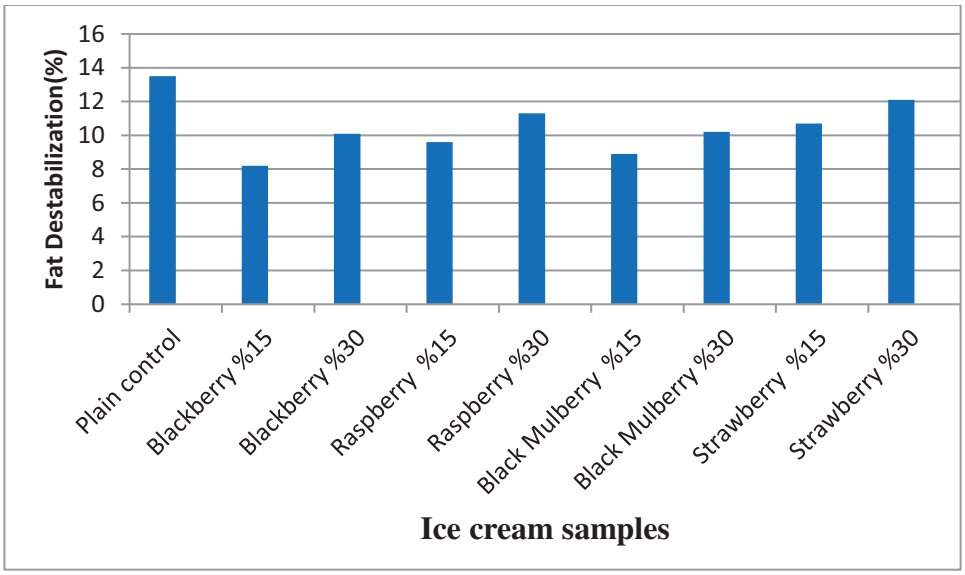

FIGURE 4
Fat destabilizations values of ice cream samples added different Fruits

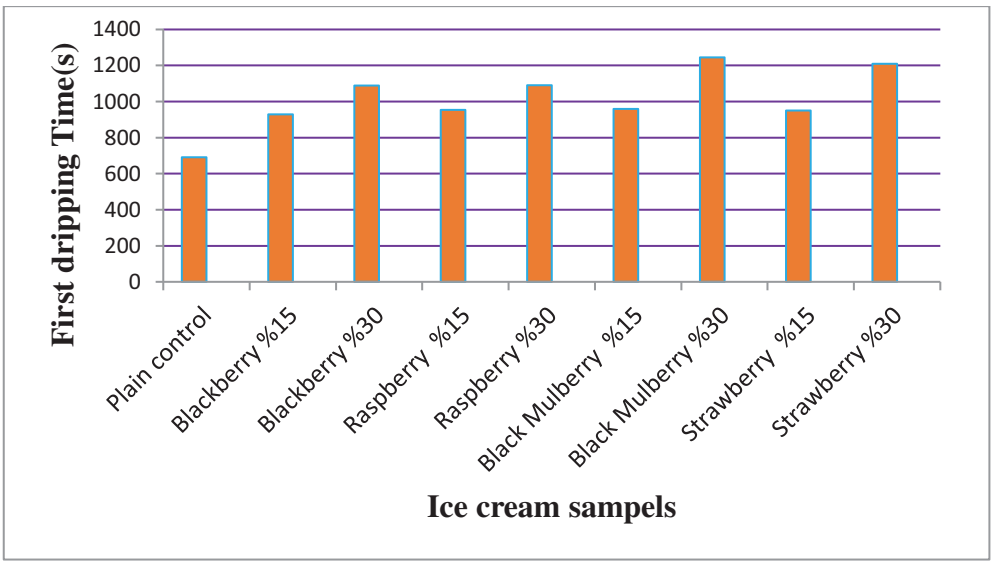

FIGURE 5
First dripping times of yoghurt ice cream samples added different fruits

TABLE 3
Organoleptic analysis results of yoghurt ice creams made with different types of fruits (Maximum score was 9)

Yoghurt ice cream samples	The amount of fruit (%)	Color	Structure and consistency	Taste and flavor	Feeling in the mouth	General Acceptability
Plain (control)	-	6.7^{a}	7.1^{c}	7.7^{b}	5.8^{a}	6.8^{a}
Blackberry	15	5.8^{aA}	5.6^{aA}	6.0^{aA}	6.5^{bA}	6.0^{aA}
	30	7.7^{aB}	6.8^{aB}	7.6^{aB}	7.6^{bB}	7.1^{aB}
Raspberry	15	6.4^{aA}	6.4^{abA}	7.5^{bA}	6.9^{bA}	6.8^{aA}
	30	7.1^{aB}	7.1^{abB}	8.3^{bB}	7.3^{bB}	7.4^{aB}
Black mulberry	15	8.5^{bA}	6.3^{abA}	6.6^{aA}	5.3^{aA}	6.7^{aA}
	30	8.8^{bB}	7.0^{abB}	6.4^{aB}	5.6^{aB}	7.0^{aB}
Strawberry	15	5.7^{aA}	7.0^{cA}	6.4^{aA}	6.4^{bA}	6.2^{aA}
	30	6.8^{aB}	7.3^{cB}	7.4^{aB}	7.1^{bB}	7.0^{aB}

a, b, c means that the differences between the samples of yoghurt ice cream were significant (p 0.01)
A, B means that the differences between the fruits amounts were significant ($p < 0.01$)

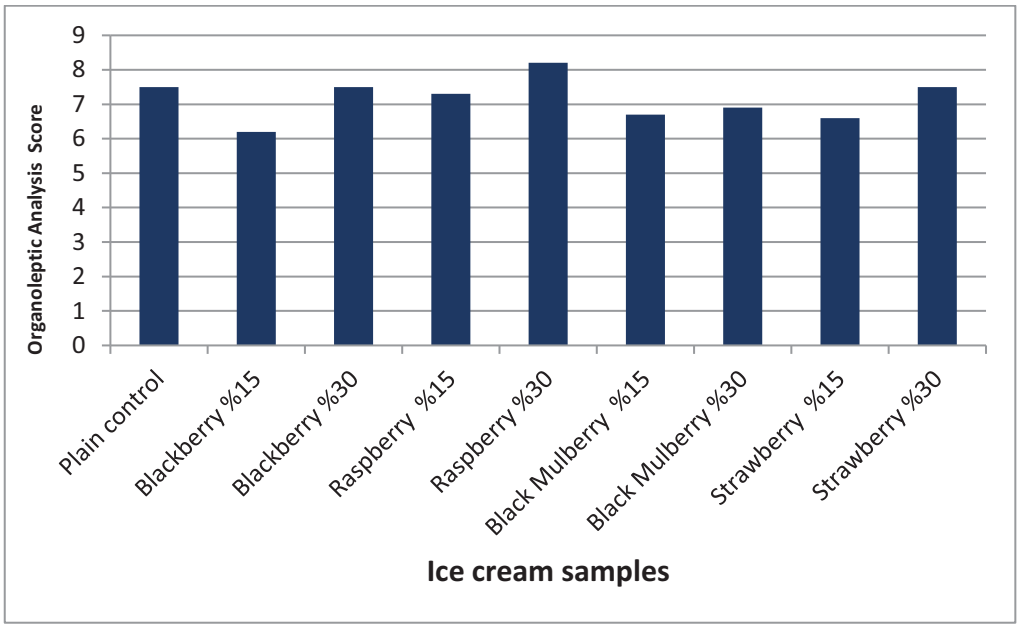

FIGURE 6
Differences of taste and flavor of samples of yoghurt ice cream added fruits (Maximum score was 9).

Results of Sensory. The Organoleptic analysis of yoghurt ice cream added with different types of fruit (Maximum score was 9) was summarized in Table3. As can be seen from Table 3, panelists preferred as high at significant level ($p < 0.01$) the color of yoghurt ice cream samples added black mulberry to others and also for the color of the ice creams added more fruits was a preference ($p < 0.01$) which was also reported by [6]. Panelists liked the structure and consistency of samples plain and added strawberry according to others. Panelists preferred the taste and flavor of samples plain and added raspberry according to others (Fig.6). Panelists found that the feeling in the mouth scores of samples added blackberry, raspberry and strawberry was higher than that of others. The structure and consistency scores of samples produced with black mulberry and strawberry addition were higher than other samples. The increment of strawberry jam ratios in ice cream resulted in the increment of the structure and consistency scores given by panelists. The differences of general acceptability of samples was not important ($p > 0.05$) (Table 3). The general acceptability of samples added 30 % fruits were higher ($p < 0.01$) than that of 15%. The panelists preferred ice cream samples added 10% and 15% vegetable marrow according to 20% vegetable marrow [8].

CONCLUSIONS

This study reported that addition of different fruits to the yoghurt ice cream was increased the final quality of yogurt ice cream. The addition of 30% fruits resulted in higher quality values compared to the 15 % fruit addition. Panelists mostly preferred the taste of raspberry samples at 30 % ratio. Blackberry, raspberry, black mulberry and strawberry at the concentration of minimum 30% can be added to ice-cream mix.

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