Agronomy Research **17**(S2), 1313–1323, 2019
https://doi.org/10.15159/AR.19.086

Development of formulation and technology of yogurt with prolonged shelf life enriched with biologically active substances from fennel seed extract

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Abstract. Spray drying is most common drying technology in food industry and can be used as an alternative to freeze drying method for the production of extracts in powder form. Fennel seeds are used to treat diabetes, bronchitis and chronic cough. They possess antibacterial, antifungal, antithrombotic, anti-inflammatory, hepatoprotective and antidiabetic activities. The aim of this study was to obtain fennel extract in dry form and investigate the influence of dry fennel extract incorporation on the possibility of yogurt production. The effect of inlet temperatures on wettability, solubility, moisture content and water activity of spray-dried fennel seed extract obtained by decoction technique was investigated. The inlet temperature 165 °C was preferred. Lactic acid accumulation during fermentation occurs faster in the sample with fennel powder. Based on the results of rheological, organoleptic, physico-chemical properties, water-holding capacity and shelf life of the finished product, the recommended doses of dried fennel powder for yogurt manufacture is not more than 1%.

Key words: decoction, fennel extract, natural food preservation, spray drying.

INTRUDUCTION

Natural foods that promote well-being and reduce the risk of various diseases cause an increased interest among consumers. The consumers are more attracted by products containing natural antioxidants, rather than synthetic ones, whose use is limited due to their toxicity and carcinogenic effect. Dairy products themselves are among the most interesting and promising products in terms of antioxidant activity, due to the presence of caseins and whey proteins, as well as traces of various antioxidants (Niero et al., 2016). Medicinal plants, rich in natural antioxidants and phenols, are increasingly used in the manufacture of dairy products to improve their nutritional and therapeutic properties. Fermented milk products are among them (Alenisan, M.A. et al., 2017).

There are lots of studies proving the undeniable benefits of yogurt. Yogurt is considered as a nutrient-dense food that contains essential nutrients such as protein, vitamins and minerals necessary for growth. Yogurt consumption reduces the risk of diseases that cause an increase in systolic and diastolic arterial (Dong et al., 2013), reduces the concentration of certain cariogenic bacteria in the mouth (mainly streptococci and lactobacilli), and also increases the concentration of calcium and phosphorus in plaque (Ravishankar et al., 2012). Studies conducted by Dougkas et al. (2012) have shown that consuming yogurt reduces hunger and calorie intake by the next meal by 11%. The results of seven studies involving 254,892 people showed that consuming 200 grams of yogurt per day reduces the risk of diabetes by 22% (Aune et al., 2013). According to the research of Burgain et al. (2012), yogurt consumption is recommended for people suffering from lactose intolerance, which reduces the risk of calcium and vitamin D deficiency in the diet. In view of the above mentioned consumption of dairy products such as yogurt helps to improve the overall quality of the diet.

Spray drying is the most widely used technology in the food industry and the pharmaceutical industry. This process is continuous, ensuring a constant drying temperature and product flow rate (Cosmin et al., 2013). It allows to turn a liquid into a powder with the exact characteristics (particle size, morphology, physical and chemical stability) and it is a relatively economically viable process. The spray drying process makes it possible to preserve the content of polyphenols in the extracts to the greatest extent possible and can be used as an alternative to freeze drying for the production of extracts in the form of powder (Michalska et al., 2018).

There is a growing interest, both in industry and in research to aromatic and medicinal plants due to their antioxidant properties and content of many active substances, such as tocopherols, flavonoids, terpenoids, etc. ((El-Belghiti). Herbal extracts are widely used in the dairy products as a source of antioxidants (Shori & Baba, 2011a; Shori & Baba, 2011b; O'Sullivan et al., 2014; El-Said, 2014).

Fennel (*Foenicnicum vulgare*) is a plant belonging to the Apiaceae family, and thanks to its edible and very fragrant leaves and seeds has a long history of use.

Fennel seeds contain polyphenols and flavanoids and are used for medicinal purposes and as a flavor component. Fennel is used for diabetes, bronchitis, chronic cough, and kidney stone treatments (Angelov & Boyadzhieva, 2016).

The results obtained by Oktay et al. (2003) indicate that the fennel (*F. vulgare*) seed is a potential source of natural antioxidant. Fennel decoction, also employed as a carminative. In the Indian subcontinent, fennel seeds are eaten raw, sometimes with some sweetener to improve eyesight. Extracts of fennel seeds have been shown in animal studies to have a potential use in the treatment of glaucoma and a potential drug for the treatment of hypertension. It has been used as a galactagogue improving the milk supply of a breast feeding mother (Manzoor et al., 2016). Fennel is known in folk medicine for its diuretic and digestive aid. Phenolic compounds of fennel contribute to the prevention of chronic diseases such as cancer, cardiovascular diseases. Fennel components have antibacterial, antifungal, antithrombotic, anti-inflammatory, hepatoprotective effects (Ferioli et al., 2017).

The aim of this study is to create an optimal formulation and technology for yogurt manufacture with the addition of fennel extract, which would have not only satisfactory organoleptic properties, but could also contribute to maintaining high quality in the lack of milk fat.

MATERIALS AND METHODS

Fennel decoction preparation

Fennel seeds were purchased from the drugstore in St. Petersburg, Russia. The samples were reduced to powder (328 ± 8 μm) and added (120 g) to 1 L of distilled water. The mixture was boiled for 4 min, left to stand for 15 min and then filtered using 3 layers of cheese cloth to avoid the presence of any suspended particles. The fennel extracts were concentrated in a rotary evaporator (BUCHI, B-491) until 14% total solids and kept at 4 °C until spray drying.

Spray drying process

In the present study, the fennel decoction was spray dried using a lab–scale spray dryer Eyela SD-1000 (Tokyo Rikakikai Co. Ltd, Japan) using two nozzles of 0.4 mm in diameter. The emulsion was fed into the main chamber with the aid of peristaltic pump. The fennel decoction was mixed continuously by a magnetic stirrer during the entire process of spray drying. The inlet temperature varied from 120 °C to 165 °C with the increment of 15 °C. The atomization pressure was fixed as 90 kPa, while the drying air flow rate varied from 0.45–0.50 m^3 min^{-3}. The outlet temperature was kept constant and controlled by the flow rate of the feed product. For each spray-drying experiment, 1,000 mL of feed was prepared. The powder obtained from spray drying, both in the chamber and the cyclone, was stored in airtight containers before analysis.

Yogurt production

For the yogurt production skimmed milk powder (commercial brand Central Lechera Asturiana, Corporación Alimentaria Peñasanta, S.A., Spain) was reconstituted at 11% w/v solids-non-fat to be further completed with fennel powder. The skimmed milk powder reconstitution was carried out using distilled water at a temperature 45 °C. Milk was fortified with dry fennel powder in the amount ranged from 1% to 2% of milk weight with the increment of 1% and pasteurized at 85–87 °C for 10 min. Control sample was made without fennel powder. Samples were inoculated with 5% of reactivated commercial yogurt starter culture (containing *Lactobacillus* subsp. *bulgaricus* and *Streptococcus thermophilus*) at 43 °C. Fermentations were stopped by rapid cooling and the samples were placed in a cold storage at 5 ± 1 °C. All the yogurts were prepared in triplicate.

The samples were analyzed immediately after cooling and during the shelf life at 5 ± 1 °C. Solids-non-fat contents were measured using milk analyzer 'Klever-2M' (LLC Scientific and production Enterprise 'BIOMER', Russia). pH values were measured using pH-meter pH-410 with combined glass electrode (Scientific Production Association 'TECHNOKOM', Russia). Titratable acidity was measured according to AOAC method 947.05 (AOAC, 2007), using NaOH 0.1 N and phenolphthalein solution as an indicator. Acidity was expressed as % lactic acid.

Antioxidant activity was determined according to the method of Najgebauer-Lejko et al. (2011) using DPPH reagent (DPPH) 1, 1-diphenyl-2-picrylhydrazyl. First, an aqueous extract of yogurt was obtained according to Shori & Baba (2011a). Samples of yogurt with fennel extract or without fennel extract (10 g) were mixed with 2.5 mL of distilled water and acidified with 0.1 HCl to pH 4.0. Acidified yogurts were incubated for 10 min in a water bath at a temperature of 45 °C, followed by centrifugation

(5,000 rpm, 10 minutes, laboratory centrifuge Sigma). The pH value of the resulting supernatant was adjusted to 7.0 by the addition of 0.1 M NaOH and centrifuged (5,000 rpm, 10 min., Sigma laboratory centrifuge). To determine the antioxidant activity, samples of yogurt extracts were mixed with 3.0 mL 0.1 mM DPPH solution (39.4 mg DPPH in 1 liter of methanol) and incubated for 2 hours at room temperature in a dark place. Absorbency (Abs) was determined using a Shimadzu UV-1800 spectrophotometer at a wavelength of 515 nm. Control was a mixture of methanol and DPPH reagent. Antioxidant activity was calculated using the formula as follows:

$$Antioxidant\ activity, \% = 1 - \left(\frac{Abs\ treatment\ sample}{Abs\ control\ sample}\right) \cdot 100$$

Wetting time was measured according to Pabari & Ramtoola (2012) with some modifications. A Whatman No. 1 filter paper disk folded once diametrically was placed in a Petri dish of 8.5 cm in diameter. A small volume (8 mL) of distillated water was added to the filter. Then, the compact was carefully placed on the wetted tissue paper at $t = 0$ (initial time) and the time for complete wetting of the sample was measured.

Solubility of fennel powder was determined by Eastman & Moore's (1984) method with some modifications. Sample of fennel powder (1 g) was mixed thoroughly with 100 mL of distilled water. This solution was transferred to some experimental tubes and centrifuged at 3,000 rpm for 5 min and allowed to settle for 30 min. An aliquot of 25 mL of the supernatant was transferred to preweighed porcelain cup and immediately oven-dried at 105 °C for 5 h. The solubility (%) was calculated as the weight difference.

The moisture content was determined gravimetrically by estimating the powder's weight loss after oven drying at 105 °C until a constant weight (AOAC, 2007).

The water activity of the powders was measured using a water activity meter Aqualab CX-2 (Aqualab Series 4TE, Decagon Devices, Inc., Pullman, Washington, USA).

The forced syneresis was estimated by the amount of separated whey during the centrifugation (1,500 rpm) of 10 g of control yogurt samples or samples with different amount of fennel powder. Each sample was weight into centrifuge tubes. The tubes were centrifuged and the amount of separated whey was measured every 5 min during 30 min. The liquid expelled from the yogurt gel was denoted as expressible whey.

Lactic acid bacteria were determined according to GOST 10444.11-2103. Moulds and yeasts were determined according to GOST 10444.12-12013.

The suitable level of fennel powder determined using a 5-point Just About Right (JAR) scale (Rothman & Parker, 2009), where 1 = not enough attribute, 3 = just right and 5 = too much of the attribute. Each consumer testing consisted of 67 panelists who were mainly 18 to 45 years of age (> 70% of total populace) with > 70% being frequent yogurt consumers, including university staff and students. Samples were presented in 3 digit-coded 2-oz soufflé cups with lids.

Rheological measurements were carried out in triplicate with the aid of Rheotest 2 type rotating viscometer (VEB-MEDINGEN, Germany). For this study, the coaxial cylinder device S2 was used. The shear rate varied from 0.3333 to 437.4 s^{-1}. The rheological measurements were performed at controlled temperature of 20.0 ± 0.5 °C. The shear stress was calculated at increasing shear rates (upward flow curve) followed by decreasing shear rates (downward flow curve). The areas under the upward and downward flow curves were estimates. Apparent viscosity η, Pa s, was calculated using the following formula:

$$\eta = \frac{\tau}{\gamma} \quad (1)$$

where γ – shear rate, s^{-1}; τ – shear stress, Pa.

The storage time was estimated according to MUK 4.2.1847-04. According to recommendations the product research dates should be longer than the expected shelf life for the time determined by the so-called reserve ratio. The reserve ratio for perishable products with a shelf life of up to 30 days is in 1.3. MUK 4.2.1847-04 also establishes the frequency of microbiological analyzes. To determine the shelf life of the finished product, the control and treatment samples of yogurt were packed in glass containers and stored at 4 ± 2 °C.

The experiments were replicated three times. Significant differences were determined at $\alpha = 0.05$. Statistical processing of data was carried out using Microsoft Office Excel 2010 and Mathcad 15.0

RESULTS AND DISCUSSIONS

The data in Table 1 show the influence of inlet temperature on quality parameters of fennel powder. It can be seen from the Table the moisture content of the powders increased with increasing inlet temperature. Apparently, an increase in the inlet air temperature results in a rapid formation of dried layer on the droplet surface and causes the formation of impermeable films on the particle surface, followed by the formation of a crust on the drop surface. Moisture content of fennel powders varied from 3.34 to 4.33% and this amount of moisture is sufficient to assure microbiological safety of food powder.

Table 1. The influence of inlet temperature on quality parameters of fennel powder

No.	Inlet temperature, °C	Outlet temperature, °C	Wettability, s	Solubility, %	Moisture content, %	Water activity, a_w (25 °C)
1	120	79–82	430 ± 7	93.94 ± 0.98	3.34 ± 0.06	0.28 ± 0.01
2	135	79–82	349 ± 9	94.23 ± 0.96	3.69 ± 0.05	0.26 ± 0.01
3	150	79–82	280 ± 8	96.12 ± 1.01	4.14 ± 0.05	0.22 ± 0.01
4	165	79–82	117 ± 9	98.56 ± 0.99	4.33 ± 0.06	0.19 ± 0.01

Wettability can be defined as the ability of a powder bulk to be penetrated by a liquid caused by capillary forces (Hogekamp & Schubert, 2003). Powders produced with an inlet temperature of 165 °C showed the lowest wetting time (117 s), whereas the highest value (430 s) was shown by sample with an inlet temperature of 120 °C. Therefore, the presented data show that an increase in the inlet temperature decreased the wetting time and can reduce the incidence of powder clumping upon contact with water.

The results also showed that the solubility of the powders increased with the inlet temperature increase. At the highest temperature (165 °C) fennel powder showed the highest solubility (98.56%), whereas at the lowest temperature (120 °C), fennel powder showed the lowest solubility.

All powders obtained showed water activity values below 0.3. The average value of water activity varied from 0.19 to 0.28, therefore, the obtained dry extracts are biochemically and microbiologically stable.

As a result of the analysis of the data presented in Table 1, it was concluded that, based on the combination of the studied parameters, the most suitable for further use and research is fennel powder obtained at a drying temperature of 165 °C.

The inclusion of fennel powder into milk prior to bacterial fermentation influence on titratable activity, pH and lactic acid content compared to milk alone (Table 2). Yogurt fermentation was monitored every hour up to 6 h. The pH of yogurt after 6 h fermentation was in the range of 4.82 to 4.61. Titratable acidity, lactic acid content varied from 80 to 104 °T and 0.720 to 0.936% LA, respectively and was found to vary with the fennel powder concentrations.

The presented data showed that with an increase of the concentration of the applied powder, the acidity during the fermentation process increases faster and the lactic acid accumulates to higher level.

The forced syneresis values of yogurts were affected fennel powder concentration and the changes are shown in (Fig. 1). As seen in Fig. 1, the addition of fennel powder caused a decrease of the amount of whey expelled. All yogurts with fennel showed a lower syneresis percentage compared to plain yogurt. Yogurt with 2% (w/v) fennel powder showed the highest water holding capacity, while the control sample had the lowest water holding capacity.

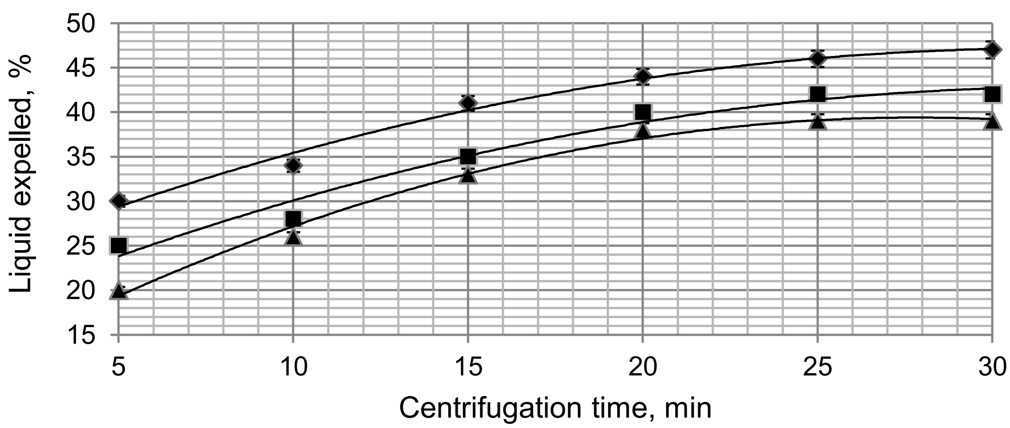

Figure 1. Estimation of the water holding capacities of yogurt samples made using: ◆ – control sample; ■ – treatment sample (1% of fennel powder); ▲ – treatment sample (2% of fennel powder).

Table 2. The effect of the fennel extract concentration on the dynamic of acid accumulation

Fermentation time, h	Control sample			Sample with 1% of fennel powder			Sample with 2% of fennel powder		
	°T	%LA	pH	°T	%LA	pH	°T	%LA	pH
0 (before fermentation)	23 ± 2	0.207 ± 0.01	6.25 ± 0.02	21 ± 1	0.189 ± 0.01	6.27 ± 0.05	22 ± 1	0.198 ± 0.01	6.29 ± 0.04
1	24 ± 2	0.216 ± 0.01	6.15 ± 0.04	23 ± 1	0.207 ± 0.01	6.13 ± 0.04	26 ± 2	0.234 ± 0.01	6.09 ± 0.03
2	33 ± 3	0.297 ± 0.01	5.96 ± 0.02	37 ± 1	0.333 ± 0.01	5.83 ± 0.06	39 ± 2	0.351 ± 0.01	5.76 ± 0.06
3	48 ± 2	0.432 ± 0.01	5.61 ± 0.04	54 ± 2	0.486 ± 0.01	5.46 ± 0.05	56 ± 3	0.504 ± 0.01	5.37 ± 0.06
4	62 ± 2	0.558 ± 0.01	5.35 ± 0.05	69 ± 2	0.621 ± 0.01	5.15 ± 0.04	76 ± 3	0.684 ± 0.01	4.82 ± 0.03
5	69 ± 2	0.621 ± 0.01	5.05 ± 0.04	78 ± 1	0.702 ± 0.01	4.95 ± 0.04	94 ± 2	0.846 ± 0.01	4.72 ± 0.04
6	80 ± 1	0.720 ± 0.01	4.82 ± 0.04	94 ± 2	0.846 ± 0.01	4.72 ± 0.05	104 ± 3	0.936 ± 0.01	4.61 ± 0.04

Table 3. Quality indicators of control and treatment samples of yogurt

Indicators	Storage time, days								
	Control sample				Treatment sample with 1% of fennel powder				
	0	5	10	13	0	5	10	15	20
Titratable acidity, °T	80 ± 1	92 ± 1	103 ± 2	116 ± 2	104 ± 2	112 ± 3	124 ± 2	134 ± 2	144 ± 2
DPPH radical scavenging activity, %	32.8 ± 1.1	30.5 ± 1.2	27.9 ± 0.9	25.6 ± 1.0	51.4 ± 2.1	48.3 ± 1.6	45.6 ± 1.4	43.2 ± 1.7	41.4 ± 1.2
Lactic acid bacteria CFU mL^{-1}	4.2 x 10^9	4.9 x 10^9	6.2 x 10^8	1.1 x 10^7	4.9 x 10^9	5.3 x 10^9	6.8 x 10^8	4.1 x 10^7	1.2 x 10^7
Yeasts, CFU mL^{-1}	<10	<10	16	28	<10	<10	<10	16	26
Molds, CFU mL^{-1}	<10	<10	<10	31	<10	<10	<10	15	30

Fig. 2 shows the flow curves for yogurt of different fennel powder concentration. Data presented showed that the degree of thixotropy in yogurt samples decreased with the fennel powder content.

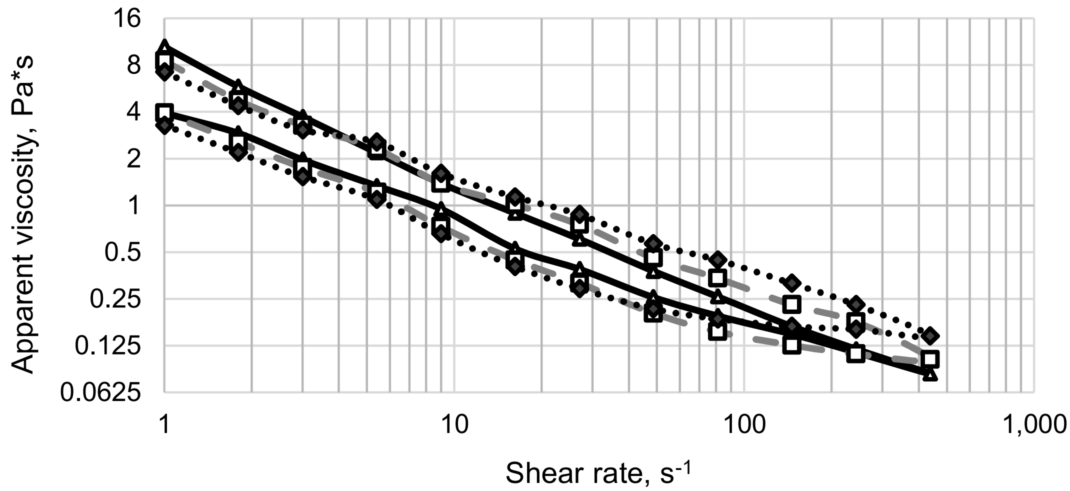

Figure 2. Hysteresis loop of the yogurt samples of different fennel powder content (– 0%; – 1% (w/v); – 2% (w/v)).

Control sample without fennel powder showed more resistant to action of shear forces. This can be attributed to the increased strength of interparticle interactions in the plain yogurt, mainly caused by self-aggregation of proteins. Analyzing the area between the upward and downward flow curves of each analysis sample, it can be seen that the rheological properties of the sample with 1% of fennel powder is the closest in degree of thixotropy to the control sample, whereas the hysteresis loop area of the sample with 2% of the extract was the largest. The presence of hysteresis loops, i.e., the difference existing between the forward and backward measurements, reveals that all yogurt samples would exhibit a thixotropic behavior, but sample with 2% of fennel powder has decreased the ability for rebuilding. The sample assessment, considering the attribute of fennel concentration is shown in Fig. 3.

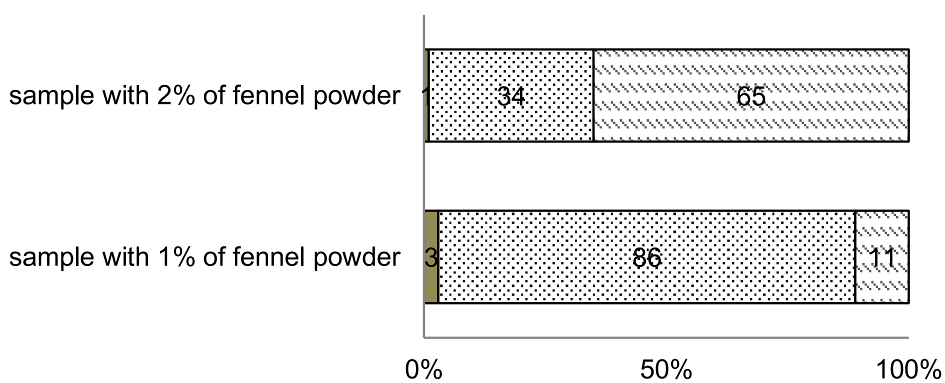

Figure 3. 'Just Right' ratings: – 'Not enough'; – 'Just right'; – 'Too much'.

From the graphics above, the yogurt sample with 2% of fennel powder was considered as having too much fennel (65%). Overall, 86% of the assessors scored 1% of fennel powder 'Just right,' whereas only 34% scored 2% of fennel powder 'Just right'. Based on the obtained data, the most optimal concentration of fennel powder is 1% (w/v).

Based on the results of preliminary organoleptic evaluation, titratable acidity and pH the expected shelf life of control and treatment sample was 10 and 15 days, respectively. According to MUK 4.2.1847-04 the frequency of analysis was established (Table 3). Changes in titratable acidity, microbiological indicators and antioxidant activity during estimated shelf life are presented in Table 3. Analyzing the data presented, it can be concluded that quality indicators during the shelf life of the samples do not exceed the established parameters for yogurt. The results of the present study showed that the antioxidant activity of yogurts was enhanced by the presence of natural extracts, such fennel powder. The results presented in Table 3 revealed that the recommended period of storage for fortified yogurt is 15 days at 4 ± 2 °C.

CONCLUSIONS

The incorporation of fennel extract into yogurt allowed to create a product with unique organoleptic characteristics and health benefits. The present investigation concludes that the inlet temperature have significant effects on physicochemical properties. The spray-dried powders produced using higher inlet air temperatures showed higher moisture contents, solubility, lower water activity and requires less time for wetting. The results obtained indicate that good quality powders can be produced by spray drying if the inlet temperature of 165 °C is used. The addition of fennel powder accelerates acid accumulation and increases water holding capacity of yogurt gel in comparison with plain yogurt.

The incorporation of fennel powder appeared to extend the shelf life of yogurt from 10 to 15 days and guarantees the quality preservation of the product at a storage temperature of 4 ± 2 °C. The recommended dose of the fennel powder is 1% of mixture weight. This fennel powder positively affected the antioxidant activity of yogurt during the refrigerated storage. Since fennel powder is a natural product with therapeutic and antioxidant properties it is recommended as a novel ingredient to enhance yogurt's properties.

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