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Functional Yogurt Added with Aqueous Extract of Canary Seed (*Phalaris canariensis L.*): Effect on the Physicochemical, Microbiological, Sensory and Rheological Characteristics

Estrada-Fernández AG and Mendoza-Mendoza-Bethsua*

Cuerpo Académico de Industrias Alimentarias, Tecnológico Nacional de México, Instituto Tecnológico Superior del Oriente del Estado de Hidalgo, México

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***Corresponding author:** Mendoza-Mendoza-Bethsua, Cuerpo Académico de Industrias Alimentarias, Tecnológico Nacional de México, Instituto Tecnológico Superior del Oriente del Estado de Hidalgo, Carretera, Apan-Tepeapulco km 3.5, Colonia Las Peñitas, Apan, Hidalgo, C.P. 43900, México

Abstract

Canary seed is used as bird food. Recently it is considered a cereal with nutraceutical potential thanks to the bioactive peptides produced during digestion. The objective of this study was to elaborate a fermented and functional milk drink added with aqueous extract of birdseed (EA). In the first stage, the birdseed was subjected to alkaline heat treatment (92 °C/40 min, Ca (OH)2 1% w/v), to remove the husk from the grain. In the second stage, the aqueous extract of birdseed was obtained and it was mixed in different proportions (75, 50, 25%) with milk (25, 50, 75%) to carry out the fermentation. The final acidity indicated that the YBF75:25 treatment significantly reached the desired value and organoleptic characteristics typical of a yogurt. In the third stage, the bromatological analysis was carried out: moisture (83.5 g/100g YBF), ashes (2.6 g/100g YBF), protein (5.7 g/100g YBF), crude fiber (0.25 g/100g YBF), fat (2 g/100g YBF) and carbohydrates (5.8 g/100g YBF), indicating that the product is low in fat and high in fiber and protein. In the fourth stage, the microbiological analysis and sensory evaluation revealed that the product is suitable for human consumption since the microbial load of the YBF is within the limits established in the standards. Finally, with the results of hardness (31 ± 2.58 g) and adhesiveness (0.1 ± 0.15 mJ) the YBF is considered a drinkable yogurt.

Keywords: Functional yogurt, *Phalaris canariensis*, Bioactivos canary seed milk, Aqueous extract of canary seed, Bioactive compounds

Abbreviations: EA: Aqueous Extract, T: Treatment, YBFXZ: Functional Drinkable Yoghurt, EMB: Eosin and Methylene Blue Agar, PDA: Potato Dextrose Agar

Introduction

Canar seed (*Phalaris canariensis L.*), is a member of the *Poaceae* family, subfamily Pooideae [1]. In the past, canary seed was considered the main component of feed mixes for wild and caged birds, it was not considered an edible cereal for humans due to the harmful effects associated with the siliceous hairs that coat the shells of the seeds, the which have been linked to esophageal cancer. In recent years, varieties based on mutagenesis and traditional breeding [2] have been developed and it has come to be considered a true cereal crop, along with other predominant cereals, such as wheat, oats, barley and rye [1]. It is native to Africa and Europe, although it is cultivated throughout the world, specifically in England, Austria and Germany [3]. At present, it has been considered a promising cereal to be included in the human diet, thanks to its high nutritional, nutraceutical and functional value for industrial purposes in food manufacturing [4]. It is rich in protein, fat, and is also a good source of minerals and vitamins [2], it contains approximately 20% (w/w) protein, 61% (w/w) starch, 8% (w/w) of crude fat and 7% (w/w) of total dietary fiber [1]. Despite the fact that canary seed contains a high content of prolamide and lutenin protein fractions, no immunotoxic proteins have been found related to celiac disease, which makes canary seed be used as an alternative source of gluten-free cereals [1]. Among the possible benefits that birdseed offers to human health,

and has been used in popular medicine, as a remedy for kidney diseases, pancreas, bladder and obesity. They have been shown to have antihypertensive, antidiabetic and anti-inflammatory effects, ability to lower blood lipids and cholesterol [4].

Phalaris canariensis is commonly used as a substitute for milk, which is prepared by immersing the seeds in water for a period of 12 or 14 hours, followed by grinding the seeds with water, the liquid resulting from the milling is called milk of birdseed [5]. The health benefits associated with consuming canary seed milk have been found to be related to the bioactive peptides produced during digestion. These bioactive peptides are specific and active protein fragments released from food proteins by proteolytic enzymes during protein digestion, which favorably affect health. Depending on the composition and sequence of amino acids, bioactive peptides have different types of activity, which include antioxidant, antimicrobial, antihypertensive, radical scavenger, anti-inflammatory, immunomodulatory, anticancer, chelating, antidiabetic, among others [6].

Yogurt is a dairy product for mass consumption with great acceptability due to its benefits for human health and nutrition. It is produced by controlled fermentation of milk, through the combined action of symbiotic cultures of Streptococcus thermophilus and Lactobacillus delbrueckii ssp. bulgaricus, resulting in a product with creamy characteristics, typical aromas and a slightly acid taste [7]. Different types of yogurt are manufactured varying the processing conditions and the composition of the milk, there are yogurts where the structure of the gel is not altered and there are others that are incubated in large fermentation vessels, which undergo agitation in order to obtain a smooth and viscous product [8]. Yogurt contains sufficient amounts of probiotics which can provide health-promoting functions, such as reducing symptoms associated with lactose intolerance, hyperlipidemia, and urinary tract infections, and stimulating the immune system, by improving the gut microbiota and thereby both gut health [9].

The benefits offered by both yogurt and canary seed milk are due to the bioactive compounds, which are generally found in small amounts in food and are considered non-nutritional ingredients but vital for the maintenance of human health. Research on bioactive ingredients for human consumption has increased as consumers have become aware of the benefits associated with health and wellness [10]. The aim of this work was to elaborate a functional fermented drink from different mixtures of milk with aqueous extract of canary seed (EA), to take advantage of the present bioactive compounds, which can be used to obtain benefits for the health of consumers, without altering the sensory characteristics of the food.

Materials and Methods

This research was divided into four stages: obtaining the aqueous extract made in the first stage; the second stage consisted of the fermentation of the yogurt using different concentrations of aqueous extract of birdseed and milk; the third stage was related to the proximal chemical analysis of functional yogurt; and the fourth stage with the microbiological, sensory and texture characterization comparing with commercial brands.

Stage I: Obtaining and conditioning canary seed

Canary seed grains were acquired in the local market of Apan Hidalgo, Mexico. The quality of the grain was verified, determining the number of diseased, damaged, or green grains and contaminants of physical and biological origin that could compromise the quality of the finished product, through a representative sample. The separation of foreign materials and grains that were not in good condition was carried out by means of a sieving.

Alkaline heat treatment

The canary seed grains were immersed in an alkaline solution of calcium hydroxide [Ca (OH)2] at 1 %, ratio 1:5 w/v (canary seed/water), followed by a thermal process or boiling at a temperature of 90 - 95 °C, for a time of 40 ± 2 min. Subsequently, the canary seed grains were washed with sufficient distilled water until neutral pH was obtained, and finally they were dried at 80°C for 12 h in a forced convection oven (Riosaa, model E-51, Mexico City, Mexico) until a constant weight was obtained. Equilibrium was assumed when the difference between two consecutive weightings was less than 1 mg/g. In all cases the moisture content of the grains was 6.0 ± 0.5% by weight.

Obtaining the aqueous extract of canary seed (EA)

The canary seed was ground for four minutes in an industrial blender (Osterizer® model 465-43), with distilled water in a 1:10 w/v ratio. The mixture was filtered, preserving the liquid phase, obtaining an aqueous extract of canary seed (EA) or canary seed milk. This was pasteurized at a temperature of 65 °C for a time of 30 minutes, followed by a thermal shock. It was stored at refrigeration temperature (4°C) until use [4].

Stage II: Preparation of drinkable yogurt with aqueous extract of canary seed

In the process of making functional yoghurt, three formulations were made, with variation in the percentage of cow's milk (75, 50 and 25%) and EA (25, 50 and 75%). The treatments were coded as Functional Drinkable Yogurt YBFXZ, where the subscript "X" denotes the percentage of cow's milk used and the subscript "Z" denotes the percentage of EA of canary seed used to make the yoghurt.

Batches of 15 L of raw milk (132 gL^{-1} of total solids, 37 gL^{-1} of milk fat, 32 gL^{-1} of protein and 16 D) were acquired in the municipality of Apan, Hidalgo, Mexico, and were standardized with skim milk at 32 gL^{-1} of milk fat. The standardized milk was pasteurized in a tub at 72°C for 15 min. The milk was cooled to 36 °C and inoculated with a starter culture of Streptococcus thermophilus,

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Lactobacullis delbrueckii subsp. lactis, Lactobacillus delbrueckii subsp. bulgaricus (CHOOZIT MY 800 LYO 5 DCU, Danisco México S.A. de C.V. Ciudad de México, México) with an incubation rate of 20DCU /100L of milk. The incubation was carried out at a temperature of 42 ± 1 °C for a time of 8 h, in an incubator (Labtech model LSI-3016), without shaking. During this period, the acidity and pH values were monitored in order to determine the best formulation to obtain the desired organoleptic characteristics, until reaching a pH of 4.5. The yoghurts were then stored at 4 °C for 24 h before evaluation [7]. Finally, with the selected treatment, the yoghurt making process was completed by adding a natural strawberry concentrate.

Stage III: Bromatological analysis of functional yoghurt

The nitrogen content of functional yogurt with canary seed extract was determined using the *Kjeldahl* method [11], moisture and ash were determined by weight difference [12]. Fat content was determined by the Gerber method [13], crude fiber [14], and carbohydrate by weight difference. All samples were carried out in triplicate.

Stage IV: Microbiological analysis

Culture media were used, for Gram negative Enterobacteriaceae [15], Standard Methods Agar (ST) for the count of aerobic mesophilic bacteria [16] for the determination of molds and yeasts (BD Bioxon) [17].

Sensory Analysis

The sensory evaluation of the yogurts stored at 4°C for 7 days, on the appearance, taste, texture and total acceptance was carried out by 60 untrained panelists. All yoghurts were served in plastic cups at 12 ± 2°C. Each panelist was given a representative sample of 20 mL of yoghurt made with aqueous extract of canary seed. The panelists, using a hedonic test from 1 (I like it a lot) to 5 (I dislike it a lot), determined the level of satisfaction of the product. Cold water was served to rinse the mouth between the yogurt samples [9].

Texture Analysis

The analysis was performed in a Brookfield CT-3 texture measuring equipment with a 38 mm diameter TA4/1000 cylindrical probe at a speed of 1 mm/s and a force of 4.5 g, following the recommendations provided in the application section of the equipment. A comparative analysis of functional yogurt added with canary seed aqueous extract and two commercial brands of yogurt was carried out.

Statistical Analysis

Each analysis was carried out in triplicate, reporting the mean and standard deviation. To determine statistically significant differences, a one-way ANOVA was applied, followed by a Tukey mean comparison with 95% ($p < 0.05$) reliability in the Simplot 12.0 statistical program.

Results and Discussion

Stage I: Obtaining aqueous extract of canary seed/ canary seed milk

Functional foods have a growing trend among consumers today, because they not only eat foods to satisfy their hunger, They eat specific foods to maintain or improve their health. Although there is no official definition of functional food, the general idea is that its consumption provides exceptional nutritional benefits for health beyond basic nutrition. Some food products designated as superfoods offer more than one health-promoting property, and recent trends include oats, hemp seeds, almonds, kale, acai berries, blueberries, green tea, and more. In recent years, research has focused on discovering the use of new bioactive compounds, including proteins from cereals, nuts and legumes for the generation of bioactive peptides with health benefits [6]. Valverde et al. [4], analyzed protein fractions of canary seed flour as a milk substitute (prepared by soaking the seeds in water for a time of 12 and 24 hours). They evaluated the antioxidant and antihypertensive activity of the peptides obtained during in vitro digestion. They observed that prolamins were the main protein fraction, followed by glutelins. Canary grass prolamins were also the compounds responsible for antioxidant and antihypertensive activity. Derived from these results and other studies, canary seed can be considered an accessible and inexpensive source to prepare milk substitutes with a high content of bioactive peptides with functional properties.

Stage II: Preparation of drinkable yoghurt with canary seed aqueous extract.

Drinking yoghurt is prepared from a yoghurt mixture with reduced milk solids, which gives it a low viscosity. The manufacturing process is similar to whipped yogurt, except for the breaking of the coagulum after fermentation, which is achieved by high stirring speed [18]. As a result of the lactic acid production process by the inoculated lactic acid bacteria, the pH of the medium formed by the different portions of cow's milk and canary seed EA decreases, this behavior is observed in figure 1a and the acidity increases (Figure 1b) in the three treatments ($YBF_{25:75}$, $YBF_{50:50}$, and $YBF_{75:25}$). However, the final pH values reported in Table 1, present a statistically significant change ($p < 0.05$) only in YBF75:25 (4.25 ± 0.045). With respect to final acidity values, all treatments are statistically different, with the highest being YBF75:25 (5.55 ± 0.03). These results indicate that the YBF75:25 treatment is the one that presents the best characteristics to carry out the fermentation in the yogurt elaboration process, reaching the characteristic pH and acidity values, as well as the desired organoleptic properties for this type of product.

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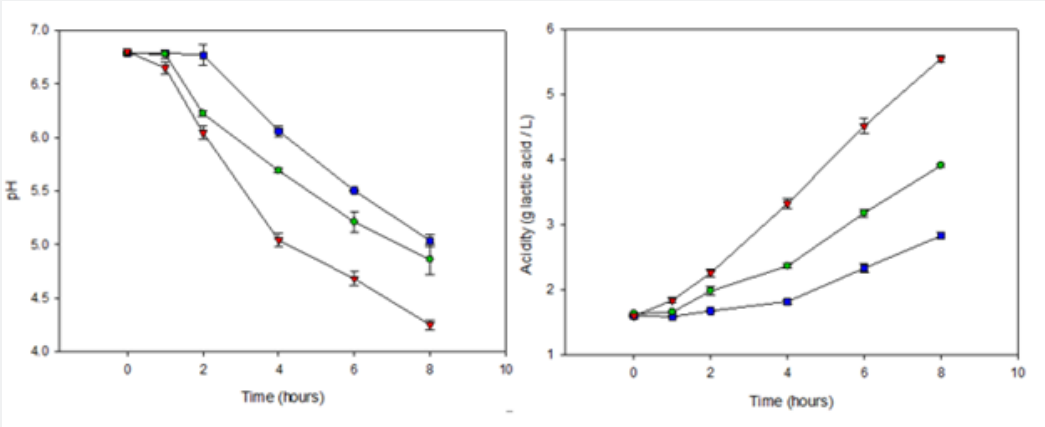

Figure 1: Change in pH values (a) and acidity (b) in the preparation of functional yoghurt added with aqueous extract of canary seed. Where; YBF 25:75 ; YBF 50:50; YBF 75:25.

Table 1: Results of pH and acidity in the different treatments for the elaboration of functional yoghurt fortified with canary seed aqueous extract.

Treatment	Determination	
	pH	Acidity (g lactic acid/L)
YBF25:75	5.03± 0.055 a	2.82± 0.047 a
YBF50:50	4.86± 0.144 a	3.91± 0.03 b
YBF75:25	4.25± 0.045 b	5.55± 0.05 c

In lactic fermentation, glucose obtained from the hydrolysis of lactose (the sugar contained in milk) is used as a substrate, giving rise to lactic acid and a series of compounds that contribute to the aroma. Based on the results obtained on acidity and pH, it can be inferred that the low content of cow's milk in the $YBF_{25:75}$, $YBF_{50:50}$ treatments caused a decrease in the concentration of lactose, so that the microbial proliferation was also low and the production of the substances slow or null. The pH value of the $YBF_{75:25}$ treatment agrees with that reported by González-Cuello et al. [19] with a pH of 4.2 and Parra-Huertas et al. [20] who report a pH value of 4.1, in yoghurt with microencapsulation of probiotics and yogurt added with carambola concentrate respectively. Díaz-Jiménez et al. [21], determined that the pH value in freshly prepared yoghurt and during storage, presented values above 4.0, the difference presented in the systems responds mainly to two aspects, to the influenza that is exerting the fiber in the pH of the yoghurt and the production of lactic acid by the lactic bacteria of each treatment. As for the acidity of yoghurt, it is closely and inversely related to the decrease in PH.

$YBF_{X;Z}$, where the subscript X denotes the percentage of cow's milk used and the subscript Z denotes the percentage of canary seed EA used to make yoghurt. The averages of three determinations and standard deviation are presented. Different letters in the same column represent statistically significant differences ($p < 0.05$), one-way analysis of variance and Tukey's comparison of means.

Stage III: Bromatological analysis of functional yoghurt

According to the results obtained in stage II, the treatment that showed the best characteristics for fermentation was $YBF_{75:25}$, with this ratio of cow's milk and canary seed aqueous extract, the functional yoghurt added with canary seed extract was made. The raw material used was established according to what is established in [23], which indicates that a yoghurt may contain 50% (w/w) of non-dairy ingredients. These ingredients can be sweeteners, fruits and vegetables, as well as juices, purees, pastes, preparations and preservatives derived from them, cereals, honey, chocolate, nuts, coffee, spices and other natural and innocuous flavoring foods and/or flavors.

The results of the proximate chemical composition of yoghurt are presented in Table 2. According to [22], yoghurt should contain a minimum limit of 2.9 % (w/w) protein and 15% (w/w) maximum fat. Therefore, the yoghurt produced with the $YBF_{75:25}$ treatment presents these physicochemical specifications within the established parameters. The protein content of the functional yoghurt added with canary seed aqueous extract was 5.7% (w/w) and fiber content 0.25% (w/w), which is higher in comparison with commercial yoghurts, which contain about 3.5% (w/w) of protein and do not provide any percentage of fiber content. The aqueous extract of canary seed is considered a drink of vegetable origin with high protein content, rich in lipase, an enzyme that is directly involved in the process of burning and assimilation of fats by the body. For this reason, canary seed extract is a good ally for eliminating cholesterol and controlling blood sugar levels [23]. The fat content in the functional yoghurt added with canary seed aqueous extract was 2 % (w/w), this result is similar to that reported in various investigations. Andrade et al. [23] mentioned that a yoghurt made from buffalo milk with wheat bran added had a fat content of 2.7% (w/w). Gómez-Ramírez et al. [24] report fat values between 2.5 -2.7 % in yoghurt added with DHA.

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Table 2: Proximal chemical composition of functional yoghurt made with cow's milk added with aqueous extract of canary seed (YBF75:25).

Component	Per 100 g of yoghurt
Moisture	83.50 ± 0.50
Ash	2.65 ± 0.27
Protein	5.70 ± 0.25
Fat	2.00 ± 0.10
Crude fiber	0.25 ± 0.50
Carbohydrates	5.80 ± 0.45
Caloric content	64 kcal

Stage IV: Microbiological, sensory and texture analysis

The concentration of coliform microorganisms (10 CFU/mL), fungi and yeasts (130 CFU/mL), as well as aerobic mesophiles (100 CFU/mL), is within the permissible limits established in [24], therefore YBF is considered fit for human consumption.

The results of the sensory analysis indicated that the acceptability of the functional yoghurt added with aqueous extract of canary seed presented 98% approval by the judges. The comments issued by the judges indicated that the $YBF_{75:25}$ presents areas of opportunity, for example: slight increase in acidity, little perception of strawberry flavor and lack of consistency. Regarding the flavor, it is important to mention that no artificial flavorings were added to the product, only a natural strawberry concentrate, on the other hand, it is important to note that in the evaluation of the texture, touch, sight and hearing intervene for what it is a property difficult to measure and interpret even with trained judges, so it is advisable to use objective methods to measure it [25].

Table 3 presents the results obtained from the texture analysis. The $YBF_{75:25}$ was compared with two commercial brands of drinkable yoghurt, presenting hardness properties similar to brand 2 yoghurt (31 ± 2.58 and 32 ± 6.19 g, respectively). On the other hand, it was observed that brand 1 yoghurt has a higher hardness (45 ± 3.46 g) which is due to the use of thickeners derived from starch, pectins, milk solids and among other additives that allow the increase of the hardness. The hardness values obtained in the present study are higher than those reported by Salazar-Chacon [26], who found hardness values between 10.9-15.9 g for liquid or drinkable yogurt with mucilage as a thickener, likewise Trujillo & Álvarez [27], indicated values between 15 and 23g for low sugar yogurt. The physical properties of coagulated dairy products are affected by their structural components, the arrangement of the structural network determines the rheological and textural characteristics of yogurt and cheese and is influenced by factors such as composition and manufacturing processes [28].

Table 3: Texture analysis of functional yoghurt added with canary seed aqueous extract (YBF75:25) and two commercial yoghurt products.

Sample	Yoghurt presentation	Hardness (g)	Adhesion (mJ)
CYP 1	Strawberry flavor yogurt shake	45 ± 3.46 a	0.21 ± 0.20 a
CYP2	Strawberry flavor drinkable yogurt	32 ± 6.19 b	0.25 ± 0.25 a
YBF75:25	Strawberry flavored drink with natural fruit	31 ± 2.58 b	0.10 ± 0.15 a

The average of three determinations and their standard deviation are shown. Equal letters in columns, there are no statistically significant differences ($p > 0.05$). Where CYP: commercial yoghurt product and YBF: Functional drinking yoghurt.

Conclusion

A functional yoghurt was made with canary seed aqueous extract, using 25% aqueous extract and 75% whole cow's milk, which, according to the characterization, has a high protein and fiber content and is low in fat, compared to products currently on the market. The sensory evaluation showed that the product is liked by the consumer, since 98% of the judges stated that they liked the product. The results obtained in the sensory analysis are supported by the texture test. This shows that the hardness and adhesiveness of functional yogurt added with aqueous extract of birdseed is comparable with commercial yogurts. The results obtained in this research are necessary in meeting the current needs of the consumer, offering functional foods that, in addition to nourishing, provide additional health benefits. On the other hand, an alternative use is offered for birdseed, giving added value.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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