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Healthy yogurt fortified with n-3 fatty acids from vegetable sources

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ABSTRACT

The concentration of n-3 polyunsaturated fatty acids (PUFA) in yogurt was increased using 5 different vegetable oils obtained from flaxseed, *Camelina sativa*, raspberry, blackcurrant, and *Echium plantagineum*. The vegetable oils were added to partially skim milk before lactic fermentation at a concentration adequate enough to cover at least 10% of the recommended daily intake of 2 g/d of α-linolenic acid according to EC regulation no. 432/2012. Microbiological (lactobacilli and streptococci, yeast, and molds), chemical (pH, syneresis, proximate composition, fatty acids, oxidation stability), and sensory evaluations were assessed for all of the fortified yogurts after 0, 7, 14, and 21 d of storage at 4°C. Sensory evaluations were conducted at 21 d of storage at 4°C. Among the yogurts produced, those that were supplemented with flaxseed and blackcurrant oils exhibited the highest α-linolenic acid content (more than 200 mg/100 g of yogurt) at the end of storage. The addition of oil did not influence the growth of lactic acid bacteria that were higher than 10^7 cfu/g at 21 d of storage. All of the yogurts were accepted by consumers, except for those supplemented with raspberry and *E. plantagineum* oils due to the presence of off flavors.
Key words: yogurt, vegetable oil, n-3 α-linolenic acid, healthy benefit, consumer acceptability

INTRODUCTION

In recent years, the positive role of certain bioactive food nutrients on human health has drawn the interest of the consumer (Goyal et al., 2014). Although many of the foods normally present in our daily diet are naturally rich in bioactive compounds, the market for fortified foods, namely, foods supplemented with ingredients that improve the quality of health, is continuously growing. Among bioactive ingredients, n-6 and n-3 PUFA serve as the primary components of biological structures in the cell membranes of higher mammals (Hulbert et al., 2005) and are also well recognized as essential elements in the human diet (Vella et al., 2013; Ganesanet al., 2014). Among these n-3 PUFA, eicosapentaenoic acid (**EPA**), docosahexaenoic acid (**DHA**), and α-linolenic acid (**ALA**) are the most important (Lane et al., 2014). Eicosapentaenoic acid and DHA are mainly found in marine sources such as fish, fish oils, and algae (El Abed et al., 2008; Iafelice et al., 2008; Bermúdez-Aguirre and Barbosa-Cánovas, 2011), whereas ALA is commonly found in vegetable sources such as flaxseed, walnut, and echium seed oils (DeFilippis and Sperling, 2006; Iafelice et al., 2008; Bermùdez-Aguirre and Barbosa-Cánovas, 2012). All of these n-3 PUFA, generally known as healthful fats, possess several physiological benefits. In fact, their consumption contributes to the maintenance of normal levels of blood triglycerides and blood pressure, reduced risk of cardiovascular disease, protection against some types of cancer and tumors, and increased beneficial effects on the brain, retina, and nervous system (Arterburn et al., 2007; Harris et al., 2008; Gogus and Smith, 2010).

Our bodies require the regular intake of ALA, EPA, and DHA to stay healthy. Worldwide, the current global n-3 PUFA intake level is not sufficient (Sioen et al., 2009), considering that to achieve good physical conditions, the daily EPA or DHA and ALA consumption levels recommended are 250 mg and 2 g, respectively (European Council, 2006; EFSA, 2009; European Union, 2012).

In view of the interesting health benefits associated with n-3 consumption that were discovered in the last few years (Welch et al., 2010), foods such as infant formula, some dairy, meat (Özer and Kirmaci, 2010; Escobar et al., 2011), and bakery products as well as juices (Ganesan et al., 2014) have been referred to as vehicles of fortification mostly for EPA and DHA. Because the characteristic fishy flavor of the marine sources of n-3 presents a strong limitation on the many food applications, the possible use of oils coming from vegetables rich in n-3 could represent a good alternative for food fortification. Based on the literature, many vegetables represent a suitable source of n-3, such as

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flaxseed, rapeseed, soybean, echium, kiwi, raspberry, and camelina (Piombo et al., 2006; Botelho et al., 2013; Waraich et al., 2013; Ganesan et al., 2014).

Thus, the aim of this study was to develop an innovative n-3 enriched yogurt by direct incorporation of several vegetable oils. The quality of the functional yogurt was evaluated by means of physical, chemical, and microbiological analyses during the 21 d of storage at 4°C. Moreover, the sensory discriminability and the consumer acceptability of the products were investigated.

MATERIALS AND METHODS

Yogurt Manufacture

Ultra-high temperature partially skimmed cow milk acquired in the local market was used for yogurt production. Before the addition of lactic acid bacteria, 5 vegetable oils furnished by AVG s.r.l. (Milan, Italy) with a high content of n-3 ALA fatty acid and obtained by cold pressing flax (**FS**, 71% ALA), *Camelina sativa* (**CAM**, 36% ALA), raspberry (**RAS**, 29% ALA), *Echium plantagineum* (**EC**, 33% ALA), and blackcurrant (**BC**, 14% ALA) seeds were separately added in different milk batches. For each oil, the percentage of addition was defined according to its ALA content to obtain a yogurt with at least 200 mg of ALA per serving size (125 g), corresponding to 10% of the recommended daily intake of ALA (European Union, 2012). To prevent oil from rising to the surface, the oils were mixed with modified vegetable starch Novation Indulge 1720 (Prodotti Gianni S.p.A, Milan, Italy) before their addition into the milk. For all the productions, the addition of starch containing oil was performed in amounts equivalent to 2% concentration in milk. After the addition of the mixture, the milk was then slightly heated for 5 min at 60°C and cooled down to 42°C for starter addition (LYOFAST Y450 B, Clerici-Sacco, Milan, Italy), which contained cultures of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus*. The inoculated milk was aseptically distributed into sterilized plastic pots (125 g), left to stand in an incubator at 42°C ± 1°C to reach pH 4.5, and then stored at 4°C for 21 d. For each oil considered, production yielded 2 batches (replicates), and for each batch, 8 pots (125 g) were obtained. Two batches of yogurt supplemented with starch but without oil were used as the control.

Proximate Analyses and Syneresis Evaluation

The moisture, proteins, fats, pH, ash, and lactose levels were evaluated according to AOAC International (2006). Syneresis was evaluated after fermentation and 7, 14, and 21 d of storage at 4°C. For each sampling time, 10 g of yogurt was centrifuged at 350 × *g* for 30 min at 10°C (González-Martinez et al., 2002). After centrifugation, the drained whey was removed and the tubes were weighed again. Syneresis was expressed as the percentage of drained whey per 100 g of yogurt. Two evaluations of syneresis were performed on each batch.

Peroxide Value, Anisidine Value, and Acidity

To evaluate the oxidative stability of yogurt, the lipids of the yogurt samples (10 g) were extracted according to the Röse-Gottlieb method (AOAC International, 2000; method 905.02) and used to determine the peroxide value, anisidine value, and acidity. The tests were performed using the FoodLab method (CDR s.r.l., Florence, Italy), and the results for the peroxide value, anisidine value, and acidity were expressed as $mEqO_2$/kg of oil, p-anisidine value (**AnV**), and % oleic acid, respectively. Three tests were conducted in duplicate analyses on each pot.

n-3 Quantification

The determination and quantification of n-3 FA were carried out by using gas chromatography analysis. The lipids previously extracted for testing the oxidation stability were methylated as indicated by Ficarra et al. (2010) using as internal standard nonadecanoic acid methyl ester C19:0 (Sigma-Aldrich, Milan, Italy). The n-3 concentration levels were determined using a GC-2010 Shimadzu gas chromatograph (Shimadzu, Milan, Italy) equipped with a flame ionization detector, split-splitless injector, AOC-20i autosampler, and SP-2560 capillary column (100 m × 0.25 mm i.d. × 0.20 μm; Supelco, Milan, Italy). The oven temperature was programmed starting from 140°C for a 20-min hold, and then set to increase to 240°C at a rate of 4°C/min and held for 20 min. The injector temperature and the detector were set at 250°C. Each n-3 FA was identified and quantified by comparing the retention times with the fatty acid methyl standards (Sigma-Aldrich). The fatty acid concentrations were expressed as milligrams of FA/100 g of sample calculated according to AOAC International(2000; method963.22). All of the analyses were carried out in duplicate.

Microbiological Analysis

Microbiological analyses were performed after fermentation and 7, 14, and 21 d of storage at 4°C. For lactobacilli and streptococci yeast and mold counts, 10