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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 vogurts 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 biologi-

cal 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 vogurt 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 $42^{\circ}C \pm 1^{\circ}C$ 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 vogurt 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 $350 \times g$ 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 mEqO₂/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 \times 0.25 mm i.d. \times 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; method 963.22). 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 g of yogurt was suspended in 90 mL of Ringer solution (Oxoid, Milan, Italy). Serial dilutions were made and poured into the de Man, Rogosa, and Sharpe agar (Biolife, Milan, Italy) for lactobacilli, M17 agar (Biolife) for streptococci, and spread into malt extract agar (Biolife) for yeast and mold and incubated at $37 \pm 2^{\circ}$ C for 24 to 48 h. All of the analyses were performed in duplicate.

Consumer Test

Sensory evaluations were conducted to assess the degree of distinctiveness of the new developed products and to evaluate the consumer acceptability of samples. Seventy-two regular yogurt consumers (43% male, 57% female; 18–40 yr, mean age 20 yr) voluntarily participated in the test. Evaluations were conducted in individual booths under white light. The experimenters verbally introduced the consumers to the computerized data collection procedure (FIZZ Acquisition software, version 2.46A, Biosystèmes, Courtenon, France).

Five samples were assessed, including 4 n-3 enriched yogurts (FS, CAM, RAS, EC) and a control sample (control). The yogurt enriched with BC oil was not examined due to its objectionable odor. Sensory evaluation were conducted at 21 d of storage at 4°C, the most proximate to the expiration date and therefore the most potentially critical one. The yogurt samples (10 g) were served at room temperature ($25 \pm 1^{\circ}$ C), under blind testing conditions, in opaque white plastic cups (38 mL) sealed with a clear plastic lid and identified by random 3-digit codes.

The general instructions required the subjects to thoroughly stir each sample with a white plastic teaspoon before tasting and to rinse their mouth with water before the beginning of the test and between samples. The evaluation was divided in 2 sessions: the first session consisted of a series of triangle tests and the second part consisted of a liking test. A 15-min break was enforced between the 2 sessions.

In the first session, the 3 triangle tests were performed with a balanced design (Meilgaard et al., 2006). Samples were presented in triads (3 samples at one time). In each triad, a prototype was compared with the control sample to assess whether the new functional yogurt was perceived as significantly different. For this test, the EC sample was not considered because based on preliminary sensory evaluations, a measurable difference from the control was observed. The triads were served in trays that held a total of 9 samples. For each triad, the subjects were asked to taste the yogurts and to mark the odd sample. Participants were instructed to give an answer even if they were not sure. To preliminarily explore the potential differences between samples, the participants were asked to provide few words to describe the odd sample considering its sensory characteristics. For the sample chosen as the odd one, the participants were asked to provide a few sensory attributes responsible for the perceived difference. The consumers were also explicitly told to avoid personal judgements. A rest period of 5 min was enforced between triads.

During the second session, a second set of 5 samples (FS, CAM, RAS, BC, EC, and control) monadically presented was provided. The subjects were instructed to taste the samples according to the presentation order and to express their liking on a 9-point hedonic scale ranging from dislike extremely (1) to like extremely (9) (Peryam and Pilgrim, 1957). The presentation order of the yogurt samples was randomized and balanced across all subjects. The combination of a timer on the screen and the monadic presentation enforced a rest period of 60 s between samples. A rest period of 60 s was enforced between samples. The evaluations had a total duration of approximately 45 to 50 min.

Statistical Analysis

A one-way ANOVA with Duncan's test (P < 0.05) as a multiple range test was used to highlight the significant differences between all of the treatments in terms of physical, chemical, and microbiological parameters. All calculations were performed with the Statistica for Windows statistical software package (Release 7.0, StatSoft Inc., Tulsa, OK). Differences in sensory triangle tests were estimate by binomial distribution (Meilgaard et al., 2006). Just the sensory descriptors provided by consumers who correctly identified the odd sample within each triangle test were considered to describe samples. The vocabulary was standardized. Comparative terms (more than, less than, and so on) referring to the control samples were converted and referred to the enriched prototypes [e.g., "sample 155 (control) is less thick" it was considered as "FS sample (fortified sample) is more thick (than control)". Descriptors were grouped according sensory modality into 4 categories: appearance, taste, flavor, and texture. Liking data were submitted to a 2-way mixed ANOVA model (fixed factor: sample; random factor: subject) by performing Fisher's least significance difference (LSD; P < 0.05). To better explore a consumer's preference for certain prototypes, a subject segmentation was performed by conducting a hierarchical cluster analysis on the liking data using the XLStat 2012.6 software (Addinsoft, Paris, France). The liking data of each obtained cluster were separately submitted to a 2-way ANOVA model (fixed factor: sample; random factor: subject) by performing Fisher's LSD (P < 0.05). The ANOVA analyses were conducted using the SYSTAT

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Table 1. Proximate composition	$(\text{mean} \pm \text{SD})$ of nonfortified	(control) and fortified n-3 yogurts and the resu	lts of the ANOVA

Item	Lactose (% wt/wt)	Protein (% wt/wt)	$_{(\% wt/wt)}^{Fat}$	Moisture (% wt/wt)	$^{\rm Ash}_{(\% \rm wt/wt)}$	Energetic value (kcal/100 g)
Control	3.12 ± 0.01	3.57 ± 0.01	$1.65 \pm 0.02^{\rm a}$	88.01 ± 0.02	0.76 ± 0.01	$55.00 \pm 0.01^{\rm a}$
Raspberry	3.10 ± 0.04	3.30 ± 0.01	$2.01 \pm 0.01^{ m b}$	87.38 ± 0.01	0.74 ± 0.01	$59.00 \pm 0.03^{\rm a}$
Flaxseed	2.72 ± 0.05	3.43 ± 0.01	$3.18 \pm 0.01^{ m b}$	87.68 ± 0.01	0.73 ± 0.03	$64.00 \pm 0.01^{\rm a}$
Camelina sativa	2.99 ± 0.03	3.40 ± 0.03	$2.00\pm0.02^{\rm b}$	87.42 ± 0.01	0.76 ± 0.02	$59.00 \pm 0.02^{\rm a}$
Echium plantagineum	2.65 ± 0.01	3.45 ± 0.01	$2.54 \pm 0.01^{ m b}$	87.31 ± 0.01	0.73 ± 0.01	$62.00 \pm 0.01^{\rm a}$
Blackcurrant	2.97 ± 0.02	3.32 ± 0.01	$4.92 \pm 0.03^{ m c}$	85.11 ± 0.02	0.75 ± 0.01	$84.00 \pm 0.01^{ m b}$
Statistical significance	NS	NS	**	NS	NS	*

^{a-c}Different letters in the same column indicate significant differences (Duncan test, P < 0.05).

*P < 0.05, **P < 0.01.

version 13.1 software (Systat Software Inc., San José, CA). An internal preference map was obtained by conducting a principal component analysis on the liking ratings provided by the 72 subjects, considering the subjects as variables and including the products and the mean liking values of clusters as dummy variables (The Unscrambler X vers. 10.3, Camo Software AS, Oslo, Norway).

Similar values of syneresis were observed for all yogurt samples at time 0, in particular CAM and RAS (24%), control, FS, EC (25%), and BC (26%; Table 2). During storage, the syneresis values tend to significantly decrease to a value of 5% over the course of 21 d for the control yogurt and to values ranging between 3 and 7% for the fortified yogurt. It is well known that the addition of modified starch decreases the amount of water released from the yogurt (Radi et al., 2009).

RESULTS AND DISCUSSION

Proximate Analyses and Syneresis Evaluation

Table 1 shows the proximate composition of the yogurt samples. Fortified n-3 yogurts compared with control yogurt showed changes mostly related to fat content due to the addition of oil. In particular, significant variation in the fat content, and therefore the energy value, was observed in the BC yogurt compared with the other products (P < 0.05). No significant changes were otherwise observed in the protein and lactose content as well as moisture and ash.

Syneresis or spontaneous whey separation on the surface of set yogurt is considered a defect (Amatayakul et al., 2006), and the addition of starch in yogurt could have effects on the thickening and gelling properties of the product (Decourcelle et al., 2004; Oh et al., 2006).

Oxidation Stability

Lipid oxidation gives rise to the formation of undesirable off flavors and unhealthy compounds such as free radicals and reactive aldehydes (Jacobsen, 2010), which are implicated in the decreased shelf-life, consumer acceptability, functionality, nutritional value, and safety of food (Arab-Tehrany et al., 2012). To determine the oxidative stability in terms of the level of peroxides (**PV**), AnV and acidity were then measured in the pure vegetable oils used for fortification (Table 3) and in all fortified yogurts at time 0 and at 21 d (Table 3). The peroxide value in the control yogurt after the fermentation (time 0) was 7.98 mEqO₂/kg. At the same time, the values of the fortified yogurts made with RAS (9.24 mEqO₂/kg), FS (11.90 mEqO₂/kg), CAM (4.68 mEqO₂/kg), EC (5.81 mEqO₂/kg), and BC oils (11.40

Table 2. Syneresis value (%; mean \pm SD) of nonfortified (control) and fortified yogurts made with vegetable oils and the results of the ANOVA

		Days					
Item	0	7	14	21			
Control	25.60 ± 0.05	$17.90 \pm 0.15^{\rm b}$	$12.70 \pm 0.50^{\rm b}$	5.10 ± 0.01^{a}			
Raspberry	24.18 ± 0.02	$14.93 \pm 0.02^{\rm a}$	$9.39 \pm 0.01^{ m a}$	$3.48 \pm 0.02^{\rm a}$			
Flaxseed	25.12 ± 0.01	$18.27 \pm 0.01^{ m b}$	$9.88 \pm 0.30^{\rm a}$	$4.25 \pm 0.05^{\rm a}$			
Camelina sativa	24.17 ± 0.01	$16.70 \pm 0.20^{\rm a}$	$9.00 \pm 0.14^{\rm a}$	$4.88 \pm 0.01^{ m a}$			
Echium plantagineum	25.37 ± 0.05	$17.78 \pm 0.01^{ m b}$	$10.57 \pm 0.02^{\rm a}$	$3.46\pm0.03^{\rm a}$			
Blackcurrant	26.28 ± 0.03	$14.66 \pm 0.05^{\rm a}$	$9.93 \pm 0.20^{ m a}$	$6.73\pm0.02^{\rm b}$			
Statistical significance	NS	*	**	**			

^{a,b}Different letters in the same column indicate significant differences (Duncan test, P < 0.05).

*P < 0.05, **P < 0.01.

mEqO₂/kg) were significantly higher compared with the control (P < 0.05). After 21 d of storage, the PV similarly increased in all of the samples with no significant differences (P > 0.05). The results obtained in the pure vegetable oils were within acceptable limits according to Codex STAN 210–1999 (Codex Alimentarius Commission, 1999), reporting values up to 15 mEqO₂/ kg and values up to 10 mEqO₂/kg of oil for cold-pressed and virgin oils and refined oils, respectively. Specific limits are not available for PV of dairy products, so we can assume a very low level of oxidation for all the fortified yogurts during storage at 4°C for up to 21 d.

The AnV measurements highlighted the significant differences (P < 0.05) among the oils with the highest values for EC and BC products (Table 3). At time 0, similarities were observed between the control (0.65) and RAS (1.25) yogurts and between the FS (1.05) and CAM (0.30) yogurts, whereas the yogurt fortified with EC and BC oils showed significantly higher values, which were probably due to the high values detected in the pure vegetable oils (Table 3). During the 21 d of storage, the data showed significant increases, particularly for the control (+77%) and the yogurt made with CAM (+917%) and BC (+6%) oils.

However, the AnV values were lower than PV, which highlighted that decomposition into the secondary oxidation products did not occur (Frankel, 1998).

The acidity values, which were expressed as the percentage of oleic acid, showed low values both for the pure vegetable oils and for all yogurt samples, with a maximum of 0.53% for EC yogurt at 21 d. This value is lower than the limit of 3%, which was reported as the lowest acceptable level for acidity content (Gracey et al., 1999).

n-3 Quantification

The n-3 PUFA content of yogurts fortified with vegetable oils and stored for 21 d at 4°C are shown in Table 4. The n-3 PUFA concentration significantly increased (P < 0.05) in all of the fortified yogurts compared with the control yogurt at time 0 (8.52 mg/100 g). In particular, α -linolenic C18:3n-3 (ALA)was the most abundant PUFA in the FS, EC, and BC yogurts. During the first 14 d of storage, a significant drop (P < 0.05) in the ALA concentration, more than 40%, was highlighted for all fortified yogurts. The smallest decrease were observed for CAM (from 188.31 to 182.11 mg/100 g) and BC (from 423.73 to 488.464 mg/100 g).

It is well known that n-3 PUFA are highly susceptible to lipid oxidation (Let et al., 2005; Jacobsen, 2010); therefore, a possible explanation for the observed decrease in n-3 PUFA content could be attributed to

Control IV) $Control = 100000000000000000000000000000000000$	Table 3. Oxidation values (mean \pm SD) for vegetable oils and yogurts at time 0 and after 21 d of storage and the results of the ANOVA	\pm SD) for vegetable α	oils and yogurts at tim	e 0 and after 21 d of	storage and the resu	lts of the ANOVA		
$ \begin{array}{c} 2^{2}(\mathrm{kg}) \\ \mathrm{e} \left(\mathrm{AnV} \right) \\ \mathrm{acid} \\ \mathrm{acid} \\ 2^{2}(\mathrm{kg}) \\ \mathrm{acid} \\ 2^{2}(\mathrm{kg}) \\ \mathrm{acid} \\ 2^{2}(\mathrm{kg}) \\ \mathrm{acid} \\ 2^{2}(\mathrm{kg}) \\ \mathrm{acid} \\ \mathrm{acid} \\ \mathrm{acid} \\ \mathrm{b} \\ \mathrm{c} \left(\mathrm{AnV} \right) \\ \mathrm{b} \\ \mathrm{c} \left(\mathrm{AnV} \right) \\ \mathrm{b} \\ \mathrm{c} \left(\mathrm{cAnV} \right) \\ \mathrm{c} \left(\mathrm{cAnV} \right) \\ \mathrm{b} \\ \mathrm{c} \left(\mathrm{cAnV} \right) \\ \mathrm{c} \left($	Item	Control	Raspberry	Flaxseed	$Camelina\ sativa$	$Echium \ plantagineum$	Blackcurrant	Statistical significance
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	Vegetable oil			-			1 0 - 1 1 1 1 1	
e (AnV) 5.00 ± 0.14 5.25 ± 0.64 5.15 ± 0.49 9.65 ± 0.07 acid) 0.22 ± 0.01 0.14 ± 0.01 0.01 ± 0.00 0.08 ± 0.00 $2^{2}/\text{kg}$ $7.98 \pm 0.16^{\text{abc}}$ $9.24 \pm 0.80^{\text{bed}}$ $11.90 \pm 1.34^{\text{d}}$ $4.68 \pm 0.73^{\text{a}}$ $5.81 \pm 0.54^{\text{ab}}$ $e(\text{AnV})$ $0.65 \pm 0.07^{\text{a}}$ $1.25 \pm 0.78^{\text{a}}$ $1.05 \pm 0.73^{\text{a}}$ $5.81 \pm 0.54^{\text{ab}}$ $acid$ $0.17 \pm 0.04^{\text{a}}$ $0.33 \pm 0.18^{\text{a}}$ $0.26 \pm 0.09^{\text{a}}$ $0.59 \pm 0.01^{\text{a}}$ $0.26 \pm 0.01^{\text{a}}$ $acid$ $0.17 \pm 0.04^{\text{a}}$ $0.33 \pm 0.18^{\text{a}}$ $0.26 \pm 0.09^{\text{a}}$ $0.59 \pm 0.01^{\text{b}}$ $0.26 \pm 0.01^{\text{a}}$ $2/\text{kg}$ $27.02 \pm 0.04^{\text{a}}$ $0.23 \pm 0.07^{\text{a}}$ $0.24 \pm 0.07^{\text{b}}$ $0.53 \pm 0.03^{\text{b}}$ $0.51 \pm 0.07^{\text{c}}$ $0.53 \pm 0.09^{\text{b}}$ $0.53 \pm 0.09^{\text{b}}$ $0.53 \pm 0.09^{\text{b}}$ $0.53 \pm 0.09^{\text{b}}$ $0.53 \pm 0.00^{\text{b}}$ $0.53 \pm 0.00^{\text{b}}$ $0.53 \pm 0.00^{\text{b}}$ $0.53 \pm 0.09^{\text{b}}$ $0.53 \pm 0.09^{\text{b}}$ $0.53 \pm 0.09^{\text{b}}$	Peroxide (mEqO ₂ /kg)		1.99 ± 0.07	3.47 ± 0.01	1.19 ± 0.01	12.00 ± 0.59	1.91 ± 0.07	
acid) acid) 0.22 ± 0.01 0.14 ± 0.01 0.01 ± 0.00 0.08 ± 0.00 $2_2/kg)$ 7.98 ± 0.16^{abc} 9.24 ± 0.89^{bcd} 11.90 ± 1.34^d 4.68 ± 0.73^a 5.81 ± 0.54^{ab} $e(AnV)$ 0.65 ± 0.07^a 1.25 ± 0.78^a 1.05 ± 0.78^a 0.30 ± 0.01^a 2.60 ± 1.14^b $acid)$ 0.17 ± 0.04^a 0.33 ± 0.18^a 0.26 ± 0.09^a 0.59 ± 0.01^b 0.26 ± 0.01^a $2_2/kg)$ 27.02 ± 0.04 23.20 ± 2.66 28.30 ± 8.61 29.30 ± 7.38 35.10 ± 5.76 $e(AnV)$ 1.15 ± 0.07^a 1.65 ± 0.07^b 0.24 ± 0.01^a 0.30 ± 0.07^c 3.35 ± 0.35^c $acid)$ 0.28 ± 0.04^a 0.23 ± 0.01^a 0.24 ± 0.01^a 0.30 ± 0.05^a 0.53 ± 0.07^c	p-Anisidine value (AnV)		5.00 ± 0.14	5.25 ± 0.64	5.15 ± 0.49	9.65 ± 0.07	6.90 ± 0.57	
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	Acidity (% oleic acid)		0.22 ± 0.01	0.14 ± 0.01	0.01 ± 0.00	0.08 ± 0.00	0.13 ± 0.00	
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	Yogurt at d 0							
(a (AnV) $0.65 \pm 0.07^{\text{a}}$ $1.25 \pm 0.78^{\text{a}}$ $1.05 \pm 0.78^{\text{a}}$ $0.30 \pm 0.01^{\text{a}}$ $2.60 \pm 1.14^{\text{b}}$ acid) $0.17 \pm 0.04^{\text{a}}$ $0.33 \pm 0.18^{\text{a}}$ $0.26 \pm 0.09^{\text{a}}$ $0.59 \pm 0.01^{\text{b}}$ $0.26 \pm 0.01^{\text{a}}$ $2/kg$ $27.02 \pm 0.04^{\text{a}}$ $0.33 \pm 0.18^{\text{a}}$ $0.26 \pm 0.09^{\text{a}}$ $0.59 \pm 0.01^{\text{b}}$ $0.26 \pm 0.01^{\text{a}}$ $2/kg$ $27.02 \pm 0.04^{\text{a}}$ $23.20 \pm 2.66^{\text{b}}$ $28.30 \pm 8.61^{\text{b}}$ $29.30 \pm 7.38^{\text{c}}$ $35.10 \pm 5.76^{\text{c}}$ $e(AnV)$ $1.15 \pm 0.07^{\text{a}}$ $1.65 \pm 0.07^{\text{b}}$ $1.65 \pm 0.07^{\text{b}}$ $0.24 \pm 0.07^{\text{c}}$ $3.35 \pm 0.35^{\text{c}}$ $0.53 \pm 0.09^{\text{b}}$ acid) $0.28 \pm 0.04^{\text{a}}$ $0.23 \pm 0.01^{\text{a}}$ $0.24 \pm 0.01^{\text{a}}$ $0.30 \pm 0.05^{\text{a}}$ $0.53 \pm 0.09^{\text{b}}$	$Peroxide (mEqO_2/kg)$	$7.98\pm0.16^{ m abc}$	$9.24\pm0.89^{ m bcd}$	$11.90\pm1.34^{ m d}$	$4.68\pm0.73^{\mathrm{a}}$	$5.81\pm0.54^{ m ab}$	$11.40\pm0.98^{ m cd}$	***
acid) $(0.17 \pm 0.04^{\text{a}} = 0.33 \pm 0.18^{\text{a}} = 0.26 \pm 0.09^{\text{a}} = 0.59 \pm 0.01^{\text{b}} = 0.26 \pm 0.01^{\text{a}}$ $(0.26 \pm 0.01^{\text{a}} = 0.26 \pm 0.01^{\text{a}}$ $(0.26 \pm 0.01^{\text{a}} = 0.26 \pm 0.01^{\text{a}}$ $(0.26 \pm 0.01^{\text{b}} = 0.26 \pm 0.01^{\text{b}}$ $(0.28 \pm 0.07^{\text{b}} = 1.65 \pm 0.07^{\text{b}}$ $(0.28 \pm 0.04^{\text{a}} = 0.23 \pm 0.01^{\text{a}}$ $(0.23 \pm 0.01^{\text{a}} = 0.24 \pm 0.01^{\text{a}}$ $(0.24 \pm 0.01^{\text{a}} = 0.05^{\text{a}} = 0.05^{\text{a}}$ $(0.23 \pm 0.00^{\text{b}} = 0.01^{\text{a}}$ $(0.24 \pm 0.01^{\text{a}} = 0.05^{\text{a}} = 0.05^{\text{a}}$ $(0.23 \pm 0.00^{\text{b}} = 0.24 \pm 0.01^{\text{a}}$ $(0.28 \pm 0.04^{\text{a}} = 0.04^{\text{a}} = 0.23 \pm 0.01^{\text{a}}$ $(0.28 \pm 0.04^{\text{a}} = 0.04^{\text{a}} = 0.23 \pm 0.01^{\text{a}}$ $(0.28 \pm 0.04^{\text{a}} = 0.04^{\text{a}} = 0.01^{\text{a}} = 0.24 \pm 0.01^{\text{a}}$ $(0.28 \pm 0.05^{\text{a}} = 0.05^{\text{a}} = 0.00^{\text{a}}$	p-Anisidine value (AnV)	$0.65\pm0.07^{ m a}$	$1.25\pm0.78^{ m a}$	$1.05\pm0.78^{\mathrm{a}}$	$0.30\pm0.01^{ m a}$	$2.60\pm1.14^{ m b}$	$6.60\pm0.28^{ m c}$	***
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	Acidity (% oleic acid)	$0.17\pm0.04^{ m a}$	$0.33\pm0.18^{\mathrm{a}}$	$0.26\pm0.09^{ m a}$	$0.59\pm0.01^{ m b}$	$0.26\pm0.01^{\rm a}$	$0.22\pm0.03^{ m a}$	*
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	Yogurt at d 21							
V) 1.15 ± 0.07^{a} 1.65 ± 0.07^{b} 1.65 ± 0.07^{b} 3.05 ± 0.07^{c} 3.35 ± 0.35^{c} 0.28 ± 0.04^{a} 0.23 ± 0.01^{a} 0.24 ± 0.01^{a} 0.30 ± 0.05^{a} 0.53 ± 0.09^{b}	$Peroxide (mEqO_2/kg)$	27.02 ± 0.04	23.20 ± 2.66	28.30 ± 8.61	29.30 ± 7.38	35.10 ± 5.76	21.90 ± 0.64	NS
0.28 ± 0.04^{a} 0.23 ± 0.01^{a} 0.24 ± 0.01^{a} 0.30 ± 0.05^{a} 0.53 ± 0.09^{b}	p-Anisidine value (AnV)	$1.15\pm0.07^{\mathrm{a}}$	$1.65\pm0.07^{ m b}$	$1.65\pm0.07^{ m b}$	$3.05\pm0.07^{ m c}$	$3.35\pm0.35^{\circ}$	$7.00\pm0.01^{ m d}$	***
	Acidity (% oleic acid)	$0.28\pm0.04^{\rm a}$	$0.23\pm0.01^{\rm a}$	$0.24\pm0.01^{\rm a}$	$0.30\pm0.05^{\rm a}$	$0.53\pm0.09^{ m b}$	$0.25\pm0.01^{\rm a}$	*

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 $^{*}P < 0.05; \ ^{**}P < 0.01; \ ^{***}P < 0.001$

Item	Days	Control	Raspherry	Flaxseed	sativa	plantagineum	Blackcurrant	significance
o-Linolenic C18:3n-3	0 2 -	$6.64\pm0.13^{ m A}\ 6.28\pm0.34^{ m A}$	$206.01 \pm 41.81^{ m B}$ $133.14 \pm 51.55^{ m B}$	$732.23 \pm 7.08^{\mathrm{c.E}}$ $423.22 \pm 18.77^{\mathrm{b,C}}$	$\begin{array}{c} 188.31 \pm 7.14^{\rm B} \\ 161.11 \pm 45.37^{\rm B} \\ 160.71 \pm 45.37^{\rm B} \end{array}$	$560.00 \pm 47.33^{\mathrm{b,D}}$ $390.19 \pm 2.05^{\mathrm{a,B}}$	$\begin{array}{c} 423.73 \pm 29.31^{\rm a,C} \\ 384.88 \pm 21.29^{\rm a,C} \\ 364.86 \pm 21.29^{\rm a,C} \\ \end{array}$	* * * * * * * * *
Statistical significance	$\frac{14}{21}$	$\begin{array}{c} 0.04 \pm 0.12^{\circ} \ 0.04^{ m A} \ 0.04^{ m A} \ { m NS} \end{array}$	$101.80 \pm 10.44^{\circ}$ $110.39 \pm 43.84^{ m B}$ NS	301.05 ± 15.08 302.44 ± 33.59 ***	$132.78 \pm 12.64^{\circ}$ 182.11 ± 63.89^{AB} NS	185.47 ± 25.67 168.33 $\pm 60.16^{a,B}$ **	477.00 ± 19.26^{3p} $488.46 \pm 14.83^{b,D}$ *	<
Eicosaurienoic CZ0:511-3	0 7 14	$\begin{array}{c} 0.05 \pm 0.08^{\mathrm{ab,A}} \\ 0.00 \pm 0.00^{\mathrm{ab,A}} \\ 0.10 \pm 0.00^{\mathrm{ab,A}} \end{array}$	$\begin{array}{c} 0.51 \pm 0.10^{\mathrm{AB}} \ 0.54 \pm 0.22^{\mathrm{A}} \ 0.22 \pm 0.04^{\mathrm{A}} \ 0.22 \pm 0.04^{\mathrm{A}} \end{array}$		$\begin{array}{c} 7.92 \pm 0.29^{\rm C} \\ 7.37 \pm 0.81^{\rm B} \\ 5.58 \pm 0.54^{\rm C} \end{array}$	$\begin{array}{c} 0.59 \pm 0.05^{\mathrm{b,AB}} \\ 0.41 \pm 0.04^{\mathrm{a,A}} \\ 0.17 \pm 0.10^{\mathrm{c,B}} \end{array}$	$\begin{array}{c} 0.44 \pm 0.63^{ m AB} \\ 0.80 \pm 0.07^{ m A} \\ 0.00 \pm 0.00^{ m A} \end{array}$	* * * * * * * * * * *
Statistical significance Ficosamentaenoic C20:5n-3	21	$0.11 \pm 0.00^{0.13}$ NS	$0.61 \pm 0.74^{ m A}$ NS	$0.57 \pm 0.02^{a,A}$ ***	$7.51 \pm 2.57^{ m p}$ NS	$0.61 \pm 0.05^{ m a, A}$ ***	$0.45\pm0.00^{ m a}$ NS	* *
	$\begin{array}{c} 0\\ 14\\ 21 \end{array}$	$egin{array}{c} 0.62 \pm 0.05^{ m A} \ 0.52 \pm 0.04 \ 0.58 \pm 0.02^{ m A} \ 0.59 \pm 0.04^{ m A} \end{array}$	$\begin{array}{c} 0.39 \pm 0.07^{ m A} \ 0.50 \pm 0.03 \ 0.06 \pm 0.03 \ 0.66 \pm 0.06^{ m A} \ 0.49 \pm 0.17^{ m A} \end{array}$	$egin{array}{c} 1.54 \pm 0.04^{ m b,B}\ 0.83 \pm 0.10^{ m a}\ 0.86 \pm 0.20^{ m a,B}\ 0.92 \pm 0.03^{ m a,B} \end{array}$	$\begin{array}{c} 0.61 \pm 0.27^{ m A} \ 0.44 \pm 0.22 \ 0.55 \pm 0.06^{ m A} \ 0.52 \pm 0.09^{ m A} \end{array}$	$egin{array}{c} 1.37 \pm 0.12^{\mathrm{b,B}}\ 1.01 \pm 0.07^{\mathrm{a}}\ 0.50 \pm 0.21^{\mathrm{a,A}}\ 0.53 \pm 0.11^{\mathrm{a,A}} \end{array}$	$\begin{array}{c} 0.44 \pm 0.00^{\mathrm{a,A}} \\ 0.48 \pm 0.03^{\mathrm{a}} \\ 0.60 \pm 0.05^{\mathrm{a,A}} \\ 0.89 \pm 0.23^{\mathrm{b,B}} \end{array}$	* * * * * * * * * * * *
Statistical significance Docosamentaemoic C22-5n-3		NS	NS		NS		*	
	$\begin{array}{c} 0\\ 14\\ 21 \end{array}$	$\begin{array}{l} 1.21 \pm 0.01^{\mathrm{a,AB}} \\ 0.88 \pm 0.07^{\mathrm{b,A}} \\ 1.10 \pm 0.01^{\mathrm{a,AB}} \\ 1.10 \pm 0.03^{\mathrm{a,A}} \end{array}$	$egin{array}{c} 0.81 \pm 0.05^{ m A}\ 0.93 \pm 0.01^{ m B}\ 1.16 \pm 0.06^{ m AB}\ 1.29 \pm 0.92^{ m AB} \end{array}$	$\begin{array}{c} 1.48 \pm 0.32^{\rm B} \\ 1.53 \pm 0.01^{\rm B} \\ 2.65 \pm 1.19^{\rm B} \\ 1.75 \pm 0.04^{\rm AB} \end{array}$	$\begin{array}{c} 0.93 \pm 0.21^{\mathrm{A}} \ 0.77 \pm 0.35^{\mathrm{AB}} \ 1.10 \pm 0.00^{\mathrm{AB}} \ 0.94 \pm 0.05^{\mathrm{A}} \end{array}$	$egin{array}{c} 2.86 \pm 0.16^{ m C}\ 2.00 \pm 0.13^{ m B}\ 0.93 \pm 1.31^{ m AB}\ 2.11 \pm 0.28^{ m B} \end{array}$	$\begin{array}{c} 0.84 \pm 0.07^{\mathrm{a,A}}\ 0.00 \pm 0.00^{\mathrm{b,A}}\ 0.00 \pm 0.19^{\mathrm{a,A}}\ 0.65 \pm 0.19^{\mathrm{a,A}}\ 1.09 \pm 0.38^{\mathrm{a,A}}\end{array}$	* * * * * * * * *
Statistical significance Sum of n-3		* *	NS	NS	NS	NS	*	
	$\begin{array}{c} 0\\ 14\\ 21 \end{array}$	8.52 ± 0.05 7.68 ± 0.09 7.83 ± 0.03 8.00 ± 0.02	207.72 ± 8.40 135.12 ± 10.36 103.84 ± 2.12 112.78 ± 9.13	$736.29 \pm 1.50 \\ 426.18 \pm 3.78 \\ 305.05 \pm 3.31 \\ 305.67 \pm 6.74 \\ \end{array}$	$\begin{array}{c} 197.77 \pm 1.58 \\ 169.69 \pm 9.35 \\ 140.01 \pm 2.65 \\ 191.08 \pm 13.32 \end{array}$	564.82 ± 9.53 393.61 ± 0.46 187.08 ± 5.46 171.58 ± 12.12	$\begin{array}{r} 425.46 \pm 0.60 \\ 386.15 \pm 4.28 \\ 478.24 \pm 3.90 \\ 490.89 \pm 3.09 \end{array}$	
Sum of n-6	$\begin{array}{c} - \\ 0 \\ 14 \\ 21 \end{array}$	$\begin{array}{c} 31.80 \pm 0.34 \\ 25.50 \pm 0.32 \\ 29.15 \pm 0.09 \\ 29.76 \pm 0.19 \end{array}$	+ + + +	+ + + +	+ + + +	+++++++++++++++++++++++++++++++++++++++	+ + + +	

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the oxidation of fats occurring either initially during fermentation or during cold storage (Jacobsen, 2010).

Between 14 and 21 d, the ALA concentration is generally stable, particularly for the yogurts fortified with FS, EC, and BC oils. At the end of storage, the highest retention in ALA (P < 0.05) was observed for the yogurt fortified with FS and BC oils, where values of 302.44 mg/100 g and 488.46 mg/100 g, respectively, were measured. These high values could be due to the presence of antioxidants, mainly vitamin A and E, in the FS and BC oils (Salobir et al., 2010; Barrett et al., 2011). Others identified the n-3 PUFA as eicosatrienoic C20:3n-3, eicosapentaenoic C20:5n-3, and docosapentaenoic C22:5n-3, but this did not significantly change during the storage of yogurts.

Despite the moderate decrease in the total amount of n-3 PUFA at the end of the storage, the addition of vegetable oil resulted in yogurts with enhanced ALA fortification. In particular, the final ALA content of the yogurt fortified with FS and BC oils in 100 g of product was higher than 10% sufficient to reach at least 20% per serving size (125 g) of the recommended ALA daily intake (EFSA, 2009).

Microbiological Analysis

The addition of oils in milk did not negatively affect the growth of the starter bacteria in the yogurts. In particular, the microbial trend showed analogous growth behavior in all of the yogurts, particularly for streptococci (data not shown). During storage, the counts of streptococci remained at approximately 10^8 cfu/g of yogurt, whereas the lactobacilli started from 10^7 cfu/g at time 0 and ended with a final count of 10^4 cfu/g in all yogurt samples at 21 d. The yeast and mold counts were lower than 10 cfu/g.

Consumer Test

According to the binomial distribution, the minimum number of correct answers to obtain a significant difference (P = 0.05, P = 0.01, P = 0.001) in a triangle test with 72 subjects was 32, 34, and 38, respectively (Meilgaard et al., 2006). The results from the triangle tests indicated significant differences between the control and all of the considered yogurt prototypes, FS, CAM, and RAS (P < 0.01). The number of correct answers obtained was 58, 48, and 58 out of 72, respectively. Therefore, the addition of vegetable oils rich in n-3 PUFA to the yogurt induced significant differences in the sensory properties of the final products. New prototypes were clearly discriminated by consumers.

Comments given by the assessors who properly identified the odd sample within the correspondent triangle test were considered for the analysis of the sensory properties of fortified samples. Comments were intended as the free elicitations of subjects, related to sensory attributes (perceptive sensations) associated with the odd sample. The number of sensory attributes given by a subject in a comment for correctly chosen products varied from 1 to 3. For FS, CAM, and RAS, the number of comments for correctly chosen products was, respectively, 48, 43, and 53. In total, 20, 29, and 18 comments were discarded for FS, CAM, and RAS, respectively. This number was composed of the number of discarded comments because of a wrong answer in the triangle test (14, 24, and 14) and the number of discarded comments, excluded because they were hardly understandable (6, 5, and 4). In particular, this latter category of comments consisted of either emotional terms or personal comments, which could be not unequivocally interpreted by analysts (such as "sample 412 has a different texture" or "sample 897 does not have a satisfying yogurt taste"). The sensory attributes (percentage on total of the elicited attributes) obtained for each fortified yogurt according to the 4 sensory modalities are reported in Figure 1. The n-3 enriched samples were clearly discriminated for texture and were described as more creamy. The sensation of higher creaminess found in samples FS, CAM, and RAS compared with the control sample may be associated with their significant higher fat content because fat content has been proven to increase creaminess in dairy products (Frost et al., 2001). In general, the increased perception of creaminess confirmed that altering the proportion of fat significantly modified the texture of a food matrix, in agreement with other studies (King, 1994; Bermúdez-Aguirre and Barbosa-Cánovas, 2011). When considering taste, the sourcess resulted in a key attribute with a high frequency of elicitation. However, a low agreement was generally observed when defining enriched yogurts as more or less sour than the control sample. The low agreement in defining sourcess could possibly be due to a general confusion among consumers on how to clearly identify sensory stimuli (Stevenson et al., 1999). However, the general tendency was to describe new prototypes as less sour than the control. The FS and CAM tended to be described as sweeter whereas RAS samples had lower agreement among consumers about whether to consider it sweeter than the control. In general, fortified samples tended to be perceived as less sour and sweeter than the control. The combination of these factors (sourness decrease and sweetness increase) suggests the possibility of binary taste interactions, which occurred in food matrices. In particular, the observed results could be explained taking into account that at low intensity/concentration of tastants the sourcess has variable effects on sweetness

(Keast and Breslin, 2002). A bitter taste was elicited a low number of times and only for the FS and RAS samples. The sensory attribute bitter taste has been used in yogurt to describe oxidative flavor deterioration (Hekmat and McMahon, 1997). Comments on flavor (ortho- and retronasal sensations) suggested a discrimination of fortified samples from control. The sample with the highest number of flavor descriptors was FS, among which the following types of flavors were cited: cereal, nuts, vegetable, fruity, and metallic. Vegetable and nutty flavors were elicited also for the CAM sample, whereas wooden and cereal flavors were used to describe RAS. These results suggested (1) that generally positive flavors appear when adding vegetable oils; (2) a clear differentiation of volatile compounds contributed by vegetable oils compared with those typically contributed by animal n-3 oils. Both vegetable and animal oils produce significant effects on the sensory properties of the final products and therefore on their acceptability by consumers. Although the type of oil does not influence the acceptability of the appearance, in particular for color (Bermúdez-Aguirre and BarbosaCánovas, 2011), the type of n-3 significantly affects flavor. In particular, unacceptable fish oil off flavors are frequently found from fish fortification (Jacobsen et al., 1999; Iafelice et al., 2008), whereas a higher acceptability from consumers were given to products fortified with n-3 from vegetable flaxseed, canola, or soybean oil. Similarly, samples prepared with fish oil showed a lower hedonic score for odor if compared with the correspondent prepared with vegetable oil (flaxseed; Bermúdez-Aguirre and Barbosa-Cánovas, 2011). In the same study, even though microencapsulated fish oil was added to prevent any fish odor, panelists detected an undesirable aroma. The susceptibility to oxidative deterioration additionally accelerates the off-flavor formation and limits the use of fish oil for food fortification (Kolanowski et al., 1999). Semi-liquid dairy products (yogurts, creams) were suitable for fortification with fish oil but at very limited levels from 1 up to 5 g/kg(Kolanowski and Weißbrodt, 2007).

The internal preference map, which was built on the liking scale expressed by the 72 subjects, showed a total explained variance of 68% (Figure 2). The consumers

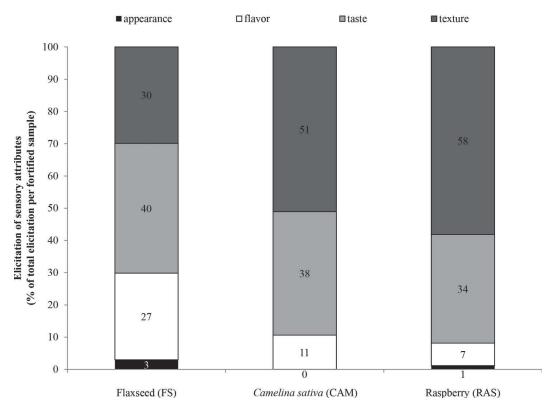


Figure 1. Frequency of the sensory attribute elicitation (% on accepted comments) obtained to describe the 3 enriched yogurts (flaxseed, *Camelina sativa*, and raspberry) after each triangle test: flaxseed vs. control, *C. sativa* vs. control, and raspberry vs. control. Only comments from assessors who correctly identified the odd sample in the correspondent triangle test were considered. The sensory attributes were organized in 4 sensory modalities depicted (appearance, taste, flavor, and texture). The yogurt enriched with blackcurrant oil was not examined in the sensory test because of its evident objectionable odor. The sample *Echium plantagineum* was excluded because of an evident measurable difference from the control, observed in a preliminary sensory evaluation.

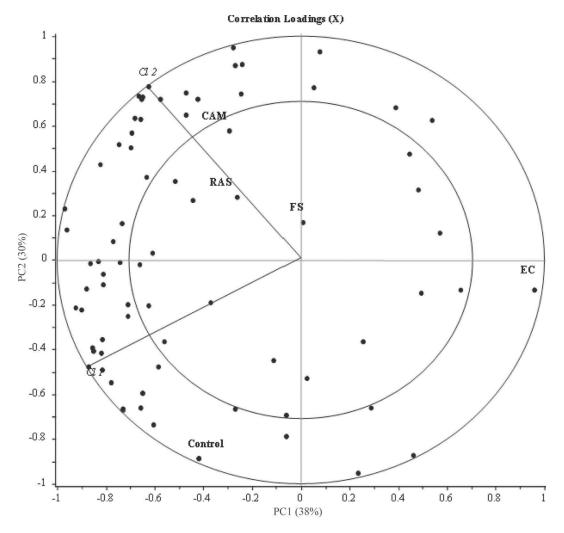


Figure 2. Internal preference map conducted on the liking ratings of 72 subjects (males = 31) and liking of cluster 1 (Cl1; n = 18; males = 9) and cluster 2 (Cl2; n = 54; males = 22) for 5 samples: the nonfortified (control) and 4 fortified yogurts (raspberry, flaxseed, *Camelina sativa*, and *Echium plantagineum*) made with n-3 vegetable oils. The yogurt enriched with blackcurrant oil was not examined in the liking test due to its evident objectionable odor. The map depicts the positioning of assessors considering their expressed overall liking given in the liking test. Liking was expressed on a 9-point hedonic scale ranging from dislike extremely (1) to like extremely (9) (Peryam and Pilgrim, 1957). Cl1 and Cl2 represent the mean liking scores of the 2 clusters. Cluster segmentation was performed by conducting a hierarchical cluster analysis on the overall liking scores given by 72 subjects. PC = principal component.

were mainly concentrated in the left part of the perceptual map, indicating a general agreement among the subjects in preferring the control, RAS, and CAM samples over the EC product. No particular preference was expressed for the FS sample.

The mixed ANOVA model applied to hedonic ratings allowed a deeper investigation of consumers' preferences. The results showed the significant effect of product on the liking scale expressed by the 72 consumers (Table 5). Results generally showed positive hedonic responses by the consumers. In particular, consumers judged new prototypes as "slightly liked" or "liked," except for EC. The liking ratings expressed for the CAM and RAS samples did not significantly differ from those expressed for the control, which was highly liked. The FS reached the acceptability score (considered as the central point of the scale, 5.0 = neither dislike nor like) but showed a significant lower liking compared with RAS and CAM. The EC sample had the lowest score.

Consumer segmentation based on liking data provided 2 clusters of subjects: cluster 1 (Cl1; n = 18; males = 9; 25% of total population) and cluster 2 (Cl2; n = 54; males = 22; 75% of total population). The mean liking ratings calculated for the 2 clusters were superimposed on the internal preference map (Figure 2). Along with PC2, Cl1 clearly tended to prefer the

Table 5. Results of mixed ANOVA models (fixed factor: sample; random factor: subject) separately conducted on the overall liking of 72 subjects and on the liking of Cl1 (n = 18; males = 9) and Cl2 (n = 54; males = 22) for 5 samples: the nonfortified (control) and 4 fortified yogurts (raspberry, flaxseed, *Camelina sativa*, and *Echium plantagineum*) made with n-3 vegetable oils^{1,2}

Subjects	Control	Raspberry	Flaxseed	Camelina sativa	$E chium \ plantagineum$	Statistical significance
Mean $(n = 72)$	$6.46\pm0.22^{\rm A}$	$6.60\pm0.22^{\rm A}$	$5.83 \pm 0.24^{\rm B}$	$6.60\pm0.21^{\rm A}$	$4.68 \pm 0.25^{\circ}$	***
Cl 1 (n = 18)	$7.44 \pm 0.49^{\text{A}}$	$6.22 \pm 0.39^{ m B}$	$4.97 \pm 0.43^{ m C}$	5.81 ± 0.45^{B}	$4.42 \pm 0.40^{\circ}$	***
Cl 2 (n = 54)	$5.47 \pm 0.24^{\rm B}$	$6.97\pm0.27^{\rm A}$	$6.69 \pm 0.28^{\rm A}$	$7.39 \pm 0.24^{\rm A}$	$4.94 \pm 0.18^{\rm B}$	***

 $^{A-C}$ Different letters in the same row indicate significant differences by Fisher's least significance difference (LSD; P < 0.05).

¹Mean values, standard errors of mean, and Fisher's LSD (P < 0.05) are reported. The yogurt enriched with blackcurrant oil was not examined in the liking test due to its evident objectionable odor. Scale from 1 (extremely dislike) to 9 (extremely like; Peryam and Pilgrim, 1957). ²Cluster segmentation was performed by conducting a hierarchical cluster analysis on the overall liking scores given by 72 subjects. ***P < 0.001.

control whereas Cl2 clearly preferred the CAM, BC, and FS samples. The EC sample was strongly disliked by both clusters.

The ANOVA model separately applied to the cluster data revealed a significant effect of product on liking both for Cl1 (F = 29.00, P < 0.01) and Cl2 (F =16.86, P < 0.01). The Cl1 significantly preferred the control sample, which was considered highly likeable by this segment (Table 5). The CAM and RAS were not significantly differentiated and resulted in being slightly liked. Samples FS and EC were significantly less liked; however, they reached the acceptability level (equal to 5, corresponding to the central value of the 9-point scale used). The Cl2 gave extremely high liking scores to sample FS, CAM, and RAS, with no significant differences among them. For Cl2, the most numerous cluster, the enrichment with n-3 in the case of FS, CAM, and RAS clearly increased the palatability of the base yogurt used for addition. In recent studies on vegetable oils, if new prototypes obtain a comparable liking score with the control, this is considered a satisfying result (Umesha et al., 2015). Therefore, acceptability exceeding the standard (Cl2) is a very positive result. In general, our study confirms that vegetable n-3 oils are an interesting ingredient not only from a nutritional point of view but also considering the hedonic performance On the contrary, EC and the control did not significantly differ in liking score and only reached the acceptability level, with significantly lower liking scores.

CONCLUSIONS

Yogurt fortified with n-3 PUFA was successfully produced, obtaining a product that was enhanced in ALA and microbially, physically, and oxidatively stable over 21 d. Moreover, many of the fortified yogurts were sensorially appreciated, in particular those produced with FS, CAM, and RAS oils. These preliminary results highlighted the possibility of producing yogurts with significantly higher amounts of ALA, providing the consumer with a natural fortified product.

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