Healthy yogurt fortified with n-3 from vegetable sources

UNIVERSITÀ DEGLI STUDI DI TORINO

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B. Dal Bello[*1], L. Torri[†], M. Piochi[†‡], G. Zeppa[*]

[*] Department of Agricultural, Forestry and Food Sciences (DISAFA), Largo Paolo Braccini 2, 10095 Grugliasco, Turin, Italy

[†] University of Gastronomic Sciences, Piazza Vittorio Emanuele 9, 12060 Bra (CN), Italy

[‡] Department of Agricultural, Food and Forestry System Management, University of Florence, via Donizetti 6, 51144 Firenze, Italy

[1]Corresponding author: Barbara Dal Bello

Largo Paolo Braccini 2 10095 Grugliasco, Torino, Italy; Tel.: (+39) 011.670.8847; fax: (+39) 011.670.8549; E-mail address: barbara.dalbello@unito.it (B. Dal Bello)

ABSTRACT

The concentration of omega-3 polyunsaturated fatty acids (omega-3 PUFAs) in yogurt was increased using five different vegetable oils obtained from flaxseed (FS), *Camelina sativa* (CAM), raspberry (RAS), blackcurrant (BC) and *Echium plantagineum* (EC). The vegetable oils were added to partially skimmed milk before lactic fermentation at a concentration adequate enough to cover at least 10 % of the recommended daily intake of 2 g/day of α-linolenic acid (ALA) according to Regulation CE n° 432/2012.

Microbiological (lactobacilli and streptococci, yeast and molds) and 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 days of storage at 4 °C. Sensory evaluations were conducted at 21 days of storage at 4 °C.

Among the yogurts produced, those that were supplemented with FS and BC oils exhibited the highest ALA 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 RAS and EC oils due to the presence of off-flavors.

Key words: yogurt, vegetable oil, omega-3 ALA, healthy benefit, consumer acceptability

Abbreviation key: ALA = alpha-linolenic acid; **BC** = blackcurrant; **CAM** = *Camelina sativa*; **DPA** = docosapentaenoic acid; **EC** = *Echium plantagineum*; **EPA** = eicosapentaenoic acid; **ETE** = eicosatrienoic acid; **FS** = flaxseed; **PUFA** = polyunsaturated fatty acid; **RAS** = raspberry; **DHA** = docohexaenoic acid.

INTRODUCTION

In recent years, the positive role of certain bioactive food nutrients on human health has notably 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, omega-6 and omega-3 polyunsaturated fatty acids (**PUFA**s) 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 (Ganesanet al., 2014; Vella et al., 2013). Among these omega-3 PUFAs, eicosapentaenoic acid (**EPA**), docohexaenoic acid (**DHA**) and α-linolenic acid (**ALA**) are the most important (Lane et al., 2014). EPA and DHA are mainly found in marine sources such as fish, fish oils and algae (Bermùdez-Aguirre and Barbosa-Cánovas, 2011; Iafelice et al., 2008; El Abed et al., 2008) while ALA is commonly found in vegetable sources such as flaxseed, walnut and echium seed oils (Bermùdez-Aguirre and Barbosa-Cánovas, 2012; DeFilippis and Sperling, 2006; Iafelice et al., 2008). All of these omega-3 PUFAs, 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; Gogus and Smith, 2010; Harris et al., 2008).

Our bodies require the regular intake of **ALA**, EPA, and DHA to stay healthy. Worldwide, the current global omega-3 PUFAs 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 (EFSA, 2009; Regulation EC n° 1924/2006 and Regulation EU n° 432/2012).

In view of the interesting health benefits associated with omega-3 consumption that were discovered in the last few years (Welch et al., 2010), foods such as infant formula, some dairy, meat (Escobar et al., 2011; Özer and Kirmaci, 2010), 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 flavour of the marine sources of omega-3 present a strong limitation on the many food applications, the possible use of oils coming from vegetables rich in omega-3 could represent a good alternative for food fortification. Based on the literature, many vegetables represent a suitable source of omega-3, such as flaxseed, rapeseed, soybean, echium, kiwi, raspberry, and camelina (Botelho et al., 2013; Ganesan et al., 2014; Piombo et al., 2006; Waraich et al., 2013).

Thus, the aim of this study was to develop an innovative omega-3 enriched yogurt by direct incorporation of several vegetables oils. The quality of the functional yogurt was evaluated by means of physical, chemical and microbiological analyses during the 21 days 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, five vegetable oils furnished by AVG s.r.l. (Milan, Italy) with a high content of omega-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 (Regulation EU n° 432/2012). 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 minutes 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 round yielded two batches and for each batch were obtained eight pots (125 g). Two batches of yogurt supplemented with starch but without oil was used as the Control.

Proximate Analyses and Syneresis Evaluation

The moisture, proteins, fats, pH, ash and lactose levels were evaluated according to AOAC (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 were centrifuged at 350 x *g* for 30 min (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 AOAC 905.02-Rose-Gottlieb method (AOAC, 2000a) 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, AnV and % oleic acid, respectively.

Three tests were conducted in duplicate analyses on each pot.

Omega-3 Quantification

The determination and quantification of omega-3 fatty acids 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). Omega-3 concentration levels were determined using a GC-2010 Shimadzu gas chromatograph (Shimadzu, Milan, Italy) equipped with a flame ionization detector, a split-splitless injector, a AOC-20i autosampler and an SP-2560 capillary column (100 m x 0.25 mm id x 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 omega-3 fatty acid was identified and quantified by comparing the retention times with the fatty acid methyl standards (Sigma-Aldrich). The fatty acid concentrations were expressed as mg fatty acid/100 g of sample calculated according to the AOAC 963.22 method (AOAC, 2000b). 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 were suspended in 90 mL of Ringer solution (Oxoid, Milan, Italy). Serial dilutions were made and poured into the de Man, Rogosa, and Sharpe agar (MRS; Biolife, Milan, Italy) for lactobacilli, M17 agar (Biolife) for streptococci

and spread into malt extract agar (MA; Biolife) for yeast and mold and incubated at 37±2 °C for 24-48 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 years, mean age 20) 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 four omega-3 enriched yogurts (FS, CAM, RAS, EC) and a control sample (Control). The yogurt enriched with blackcurrant (BC) oil was not examined due to its objectionable odor. Sensory evaluation were conducted at 21 days of storage at 4 °C, the most proximate to the expiry date and therefore the most potentially critical one. The yogurt samples (10 g) were served at room temperature (25±1°C), under blind testing conditions, in opaque white plastic cups (38 ml) sealed with a clear plastic lid and identified by random three-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 two sessions: the first session was comprised of a series of triangle tests and the second part consisted of a liking test. A 15 min break was enforced between the two sessions.

In the first session, the three triangle tests were performed with a balanced design (Meilgaard et al., 2006). Samples were presented in triads (three samples at one time). In each triad, a prototype was compared to 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 preliminary sensory evaluations, a measurable difference from the Control was observed. The triads were served in trays that held a total of nine 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. In order 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 five 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 & 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 sec between samples. A rest period of 60 sec was enforced between samples. The evaluations had a total duration of approximately 45-50 min.

Statistical Analysis

A one-way analysis of variance (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, USA). 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*, etc.) referred 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 four categories: appearance, taste, flavour, texture. Liking data were submitted to a two-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 two-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 vers 13.1 software (Systat Software Inc, San José, USA). 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).

RESULTS AND DISCUSSION

Proximate Analyses and Syneresis Evaluation

Table 1 shows the proximate composition of the yogurt samples. Fortified omega-3 yogurts compared to 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 to 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). 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).

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), the p-Anisidine value (AnV) and acidity were then measured in the pure vegetable oils used for fortification (Table 3 a) and in all fortified yogurts at time 0 and at 21 d (Table 3 b). The peroxide value in the Control yogurt after the fermentation (T0) was 7.98 $mEqO_2$/kg. At the same time, the values of the fortified yogurts made with RAS (9.24 $mEqO_2$/kg), FS (11.90 $mEqO_2$/kg), CAM (4.68 $mEqO_2$/kg), EC (5.81 $mEqO_2$/kg) and BC oils (11.40 $mEqO_2$/kg) were significantly higher compared to the Control (P <0.05). After 21 d of storage, the PV values similarly increased in all of the samples with no significant differences (P >0.05). The results obtained in the pure vegetable oils are within acceptable limits according to Codex STAN 210-1999 reporting values up to 15 $mEqO_2$/kg and values up to 10 $mEqO_2$/kg oil for cold pressed and virgin oils and refined oils, respectively. Besides there are not specific limits of PV values for the dairy products, 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 3a). At time 0, there are similarities between the Control (0.65) and RAS (1.25) yogurts and between the FS (1.05) and CAM (0.30) yogurts while 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 a). During the 21 d of storage, the data show 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).

Omega-3 Quantification

The omega-3 PUFA content of yogurts fortified with vegetable oils and stored for 21 d at 4°C are shown in Table 4. The omega-3 PUFA concentration significantly increased ($p<0.05$) in all of the fortified yogurts compared to the Control yogurt at time 0 (8.52 mg/100 g). In particular, 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/100g) and BC (from 423.73 to 488.464 mg/100g).

It is well known that omega-3 PUFAs are highly susceptible to lipid oxidation (Let et al., 2005; Jacobsen, 2010), therefore a possible explanation for the observed decrease in omega-3 PUFA content could be attributed to 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 (Barrett et al., 2011; Salobir et al., 2010). Others identified the omega-3 PUFAs as ETE, EPA and DPA, but this did not significantly change during the storage of yogurts.

Despite the moderate decrease in the total amount of omega-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 behaviour 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 while 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, 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 omega-3 PUFAs 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 to 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, 53. In total, for FS, CAM and RAS were respectively discarded: 20, 29 and 18 comments. This number was composed by: the number of discarded comments because of a wrong answer in the triangle test (14, 24, 14) and the number of discarded comments, excluded because they were hardly understandable (6, 5, 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 four sensory modalities are reported in Figure 1. The omega-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 to the Control sample may be associated to their significant higher fat content, as fat content has been proved to increase creaminess in dairy products (Frost et al., 2001). More 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 Gustavo, 2011). When considering taste, the sourness 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 sourness 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. FS and CAM tended to be described as sweeter while for RAS sample, there was lower agreement among consumers whether to consider it sweeter than the Control. In general, fortified samples tended to be perceived as less sour and sweeter than 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 sourness 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 flavour deterioration (Sharareh, and McMahon, 1997). Comments on flavour (ortho- e retronasal sensations) suggested a discrimination of fortified samples from Control. FS was the sample described with the highest number of flavour descriptors, among which were the following types of flavors were cited: cereal, nuts, vegetable, fruity and metallic. Vegetable and nutty flavors were elicited also for the CAM sample, while 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 to those typically contributed by animal omega-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. While the type of oil does not influence the acceptability of the appearance, in particular for color (Bermúdez-Aguirre and

Barbosa-Cánovas, 2011), the type of omega-3 significantly affects flavor. In particular, unacceptable fish oil off-flavors are frequently found from the fish fortification (Jacobsen, 1999; Iafelice et al., 2008) while a higher acceptability from consumers were given to products fortified with omega-3 from vegetable flaxseed, canola or soybean oil. Similarly, samples prepared with fish oil showed 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 (yoghurts, creams) were suitable for fortification with fish oil but at very limited levels from 1 up to 5 g/kg (Kolanowski and Weiβbrodt. 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 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 FS sample.

The mixed Anova model applied to hedonic ratings allowed a deeper investigation of the 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 of 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. FS reached the acceptability score (considered as the central point of the scale 5.0 = neither dislike nor like) but it showed a significant lower liking compared to RAS and CAM. The EC sample obtained the lowest score.

Consumer segmentation based on liking data provided two 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 two clusters were superimposed on the internal preference map (Figure 2). Along with PC2, Cl1 clearly tended to prefer the Control while 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 clusters' data revealed a significant effect of product on liking both for Cl1 ($F=29.00$, $P<0.01$) and Cl2 ($F=16.86$, $P<0.01$). Cl1 significantly preferred the Control sample, which was considered highly likeable by this segment (Table 5). 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). 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 omega-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 (Cluster 2), is a very positive result. In general, our study confirms that vegetable omega-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

Omega-3 PUFA fortified yogurt was successfully produced, obtaining a product that was enhanced in ALA and microbially, physically and oxidatively stable within 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 to produce yogurts significantly higher amounts of ALA, providing to the consumer with a natural fortified product.

ACKNOWLEDGMENTS

This study was funded by the PSR - F.E.A.S.R. 2007/2013 - Misura 124 - Azione 1 from the Piedmont Region (Italy).

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Table 1. Proximate composition (mean±standard deviation) of non-fortified (Control) and fortified omega-3 yogurts and the results of the analysis of variance.

	Lactose (% w/w)	Protein (% w/w)	Fat (% w/w)	Moisture (% w/w)	Ash (% w/w)	Energetic value (kcal/100 g)
Control	3.12±0.01	3.57±0.01	1.65±0.02[a]	88.01±0.02	0.76±0.01	55.00±0.01[a]
Raspberry (RAS)	3.10±0.04	3.30±0.01	2.01±0.01[b]	87.38±0.01	0.74±0.01	59.00±0.03[a]
Flaxseed (FS)	2.72±0.05	3.43±0.01	3.18±0.01[b]	87.68±0.01	0.73±0.03	64.00±0.01[a]
Camelina sativa (CS)	2.99±0.03	3.40±0.03	2.00±0.02[b]	87.42±0.01	0.76±0.02	59.00±0.02[a]
Echium plantagineum (EC)	2.65±0.01	3.45±0.01	2.54±0.01[b]	87.31±0.01	0.73±0.01	62.00±0.01[a]
Blackcurrant (BC)	2.97±0.02	3.32±0.01	4.92±0.03[c]	85.11±0.02	0.75±0.01	84.00±0.01[b]
Statistical significance	ns	ns	**	ns	ns	*

Different letters in the same column indicate significant differences (Duncan Test, $P < 0.05$)

ns = not significant, * $P < 0.05$, ** $P < 0.01$

Table 2. Syneresis value (%; mean±standard deviation) of non-fortified (Control) and fortified yogurts made with vegetable oils and the results of the analysis of variance.

	Days			
	0	7	14	21
Control	25.60±0.05	17.90±0.15^{b}	12.70±0.50^{b}	5.10±0.01^{a}
Raspberry (RAS)	24.18±0.02	14.93±0.02^{a}	9.39±0.01^{a}	3.48±0.02^{a}
Flaxseed (FS)	25.12±0.01	18.27±0.01^{b}	9.88±0.30^{a}	4.25±0.05^{a}
Camelina sativa (CS)	24.17±0.01	16.70±0.20^{a}	9.00±0.14^{a}	4.88±0.01^{a}
Echium plantagineum (EC)	25.37±0.05	17.78±0.01^{b}	10.57±0.02^{a}	3.46±0.03^{a}
Blackcurrant (BC)	26.28±0.03	14.66±0.05^{a}	9.93±0.20^{a}	6.73±0.02^{b}
Statistical significance	ns	*	**	**

Different letters in the same column indicate significant differences (Duncan Test, $P<0.05$)
ns = not significant, * $P < 0.05$, ** $P < 0.01$

Table 3. Oxidation values (mean±standard deviation) for vegetable oils (a) and yogurts at time 0 and after 21 days of storage (b) and the results of the analysis of variance.

(a)

	Oil				
	Raspberry	Flaxseed	*Camelina sativa*	*Echium plantagineum*	Blackcurrant
Peroxide ($mEqO_2$/Kg)	1.99±0.07	3.47±0.01	1.19±0.01	12.00±0.59	1.91±0.07
p-Anisidine Value (AnV)	5.00±0.14	5.25±0.64	5.15±0.49	9.65±0.07	6.90±0.57
Acidity (% oleic acid)	0.22±0.01	0.14±0.01	0.01±0.00	0.08±0.00	0.13±0.00

(b)

	Control	Raspberry (RAS)	Flaxseed (FS)	*Camelina sativa* (CAM)	*Echium plantagineum* (EC)	Blackcurrant (BC)	Statistical significance
day 0							
Peroxide ($mEqO_2$/Kg)	$7.98±0.16^{abc}$	$9.24±0.89^{bcd}$	$11.90±1.34^{d}$	$4.68±0.73^{a}$	$5.81±0.54^{ab}$	$11.40±0.98^{cd}$	***
p-Anisidine Value (AnV)	$0.65±0.07^{a}$	$1.25±0.78^{a}$	$1.05±0.78^{a}$	$0.30±0.01^{a}$	$2.60±1.14^{b}$	$6.60±0.28^{c}$	***
Acidity (% oleic acid)	$0.17±0.04^{a}$	$0.33±0.18^{a}$	$0.26±0.09^{a}$	$0.59±0.01^{b}$	$0.26±0.01^{a}$	$0.22±0.03^{a}$	**
day 21							
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
p-Anisidine Value (AnV)	$1.15±0.07^{a}$	$1.65±0.07^{b}$	$1.65±0.07^{b}$	$3.05±0.07^{c}$	$3.35±0.35^{c}$	$7.00±0.01^{d}$	***
Acidity (% oleic acid)	$0.28±0.04^{a}$	$0.23±0.01^{a}$	$0.24±0.01^{a}$	$0.30±0.05^{a}$	$0.53±0.09^{b}$	$0.25±0.01^{a}$	**

Different letters in the same row indicate significant differences (Duncan Test, $P<0.05$).

ns = not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Table 4. Omega-3 content (mg/100 g yogurt; mean±standard deviation) of non-fortified (Control) and fortified yogurts made with vegetable oils and the results of the analysis of variance.

	Days	Control	Raspberry (RAS)	Flaxseed (FS)	*Camelina sativa* (CAM)	*Echium plantagineum* (EC)	Blackcurrant (BC)	Statistical significance
α-Linolenic C18:3n3 (α-ALA)								
	0	6.64±0.13^{A}	206.01±41.81^{B}	732.23±7.08^{cE}	188.31±7.14^{B}	560.00±47.33^{bD}	423.73±29.31^{aC}	***
	7	6.28±0.34^{A}	133.14±51.55^{B}	423.22±18.77^{bC}	161.11±45.37^{B}	390.19±2.05^{aB}	384.88±21.29^{aC}	***
	14	6.04±0.12^{A}	101.80±10.44^{B}	301.05±15.08^{aC}	132.78±12.64^{B}	185.47±25.67^{aB}	477.00±19.26^{bD}	***
	21	6.20±0.04^{A}	110.39±43.84^{B}	302.44±33.59^{aC}	182.11±63.89^{AB}	168.33±60.16^{aB}	488.46±14.83^{bD}	***
	Statistical significance	ns	ns	***	ns	**	*	
Eicosatrienoic C20:3n3 (ETE)								
	0	0.05±0.08^{abA}	0.51±0.10^{AB}	1.04±0.05^{bB}	7.92±0.29^{C}	0.59±0.05^{bAB}	0.44±0.63^{AB}	***
	7	0.00±0.00^{aA}	0.54±0.22^{A}	0.61±0.01^{aA}	7.37±0.81^{B}	0.41±0.04^{aA}	0.80±0.07^{A}	***
	14	0.10±0.00^{abA}	0.22±0.04^{A}	0.49±0.07^{aA}	5.58±0.54^{C}	0.17±0.10^{cB}	0.00±0.00^{A}	***
	21	0.11±0.00^{bA}	0.61±0.74^{A}	0.57±0.02^{aA}	7.51±2.57^{B}	0.61±0.05^{aA}	0.45±0.00^{A}	**
	Statistical significance	ns	ns	***	ns	***	ns	

Eicosapentaenoic C20:5n3 (EPA)								
	0	0.62±0.05^{A}	0.39±0.07^{A}	1.54±0.04^{bB}	0.61±0.27^{A}	1.37±0.12^{bB}	0.44±0.00^{aA}	***
	7	0.52±0.04	0.50±0.03	0.83±0.10^{a}	0.44±0.22	1.01±0.07^{a}	0.48±0.03^{a}	***
	14	0.58±0.02^{A}	0.66±0.06^{A}	0.86±0.20^{aB}	0.55±0.06^{A}	0.50±0.21^{aA}	0.60±0.05^{aA}	**
	21	0.59±0.04^{A}	0.49±0.17^{A}	0.92±0.03^{aB}	0.52±0.09^{A}	0.53±0.11^{aA}	0.89±0.23^{bB}	***
	Statistical significance	ns	ns	**	ns	**	*	
Docosapentaenoic C22:5n3 (DPA)								
	0	1.21±0.01^{aAB}	0.81±0.05^{A}	1.48±0.32^{B}	0.93±0.21^{A}	2.86±0.16^{C}	0.84±0.07^{aA}	***
	7	0.88±0.07^{bA}	0.93±0.01^{B}	1.53±0.01^{B}	0.77±0.35^{AB}	2.00±0.13^{B}	0.00±0.00^{bA}	***
	14	1.10±0.01^{aAB}	1.16±0.06^{AB}	2.65±1.19^{B}	1.10±0.00^{AB}	0.93±1.31^{AB}	0.65±0.19^{aA}	ns
	21	1.10±0.03^{aA}	1.29±0.92^{AB}	1.75±0.04^{AB}	0.94±0.05^{A}	2.11±0.28^{B}	1.09±0.38^{aA}	**
	Statistical significance	**	ns	ns	ns	ns	*	
Sum of Omega-3								
	0	8.52±0.05	207.72±8.40	736.29±1.50	197.77±1.58	564.82±9.53	425.46±0.60	
	7	7.68±0.09	135.12±10.36	426.18±3.78	169.69±9.35	393.61±0.46	386.15±4.28	
	14	7.83±0.03	103.84±2.12	305.05±3.31	140.01±2.65	187.08±5.46	478.24±3.90	
	21	8.00±0.02	112.78±9.13	305.67±6.74	191.08±13.32	171.58±12.12	490.89±3.09	
Sum of Omega-6								
	0	31.80±0.34	352.31±17.04	145.16±6.86	104.95±2.08	448.80±9.54	1676.96±27.02	
	7	25.50±0.32	135.69±13.60	104.75±1.34	164.01±2.33	313.90±1.19	1782.54±25.61	
	14	29.15±0.09	183.22±5.31	87.25±3.13	85.94±1.51	148.66±3.66	2051.19±10.17	

21	29.76±0.19	191.11±17.37	92.25±1.04	105.82±7.20	141.41±9.12	1941.77±15.15

Means followed by different lowercase letters in same row were significantly different at $P < 0.05$; means followed by different capital letters in same column were significantly different at $P < 0.05$.

ns = not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

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 non-fortified (Control) and 4 fortified yogurts (Raspberry RAS, Flaxseed FS, *Camelina sativa* CAM, *Echium plantagineum* EC) made with omega-3 vegetable oils. Mean values, standard errors of mean and Fisher's Least Significance Difference (LSD; $P < 0.05$) are reported. The yogurt enriched with blackcurrant (BC) oil was not examined in the liking test due to its evident objectionable odor.

Subjects	Control	Raspberry (RAS)	Flaxseed (FS)	*Camelina sativa* (CAM)	*Echium*
Mean (n=72)	6.46 ± 0.22^{A}	6.60 ± 0.22^{A}	5.83 ± 0.24^{B}	6.60 ± 0.21^{A}	
Cl 1 (n=18)	7.44 ± 0.49^{A}	6.22 ± 0.39^{B}	4.97 ± 0.43^{C}	5.81 ± 0.45^{B}	
Cl 2 (n=54)	5.47 ± 0.24^{B}	6.97 ± 0.27^{A}	6.69 ± 0.28^{A}	7.39 ± 0.24^{A}	

Different letters in the same row indicate significant differences Fisher's Least Significance Difference (LSD; $P < 0.05$).

Scale from 1 (extremely dislike) to 9 (extremely like) (Peryam & Pilgrim, 1957).

*** $P < 0.001$

Cluster segmentation was performed by conducting a Hierarchical Cluster Analysis on the overall liking scores given by 72 subjects.

Figure 1. Frequency of the sensory attribute elicitation (% on accepted comments) obtained to describe the three enriched yogurt (Flaxseed FS, *Camelina sativa* CAM, Raspberry RAS) after each triangle test: Flaxseed vs Control, *Camelina sativa* vs Control and Raspberry vs Control. Just comments from assessors who correctly identified the odd sample in the correspondent triangle test were considered. The sensory attributes were organized in four sensory modalities depicted (appearance, taste, flavour, texture). The yogurt enriched with blackcurrant (BC) oil was not examined in sensory test because of to its evident objectionable odor. The sample *Echium plantagineum* (EC) 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 Cl1 (n=18; males=9) and Cl2 (n=54; males=22) for 5 samples: the non-fortified (Control) and 4 fortified yogurts (Raspberry RAS, Flaxseed FS, *Camelina sativa* CAM, *Echium plantagineum* EC) made with Omega-3 vegetable oils. The yogurt enriched with blackcurrant (BC) oil was not examined in the liking test due to its evident objectionable odor.

Footnotes: 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 & Pilgrim, 1957). Cl1 and Cl2 represent respectively the mean liking scores of the two clusters. Cluster segmentation was performed by conducting a Hierarchical Cluster Analysis on the overall liking scores given by 72 subjects.

Figure

1.

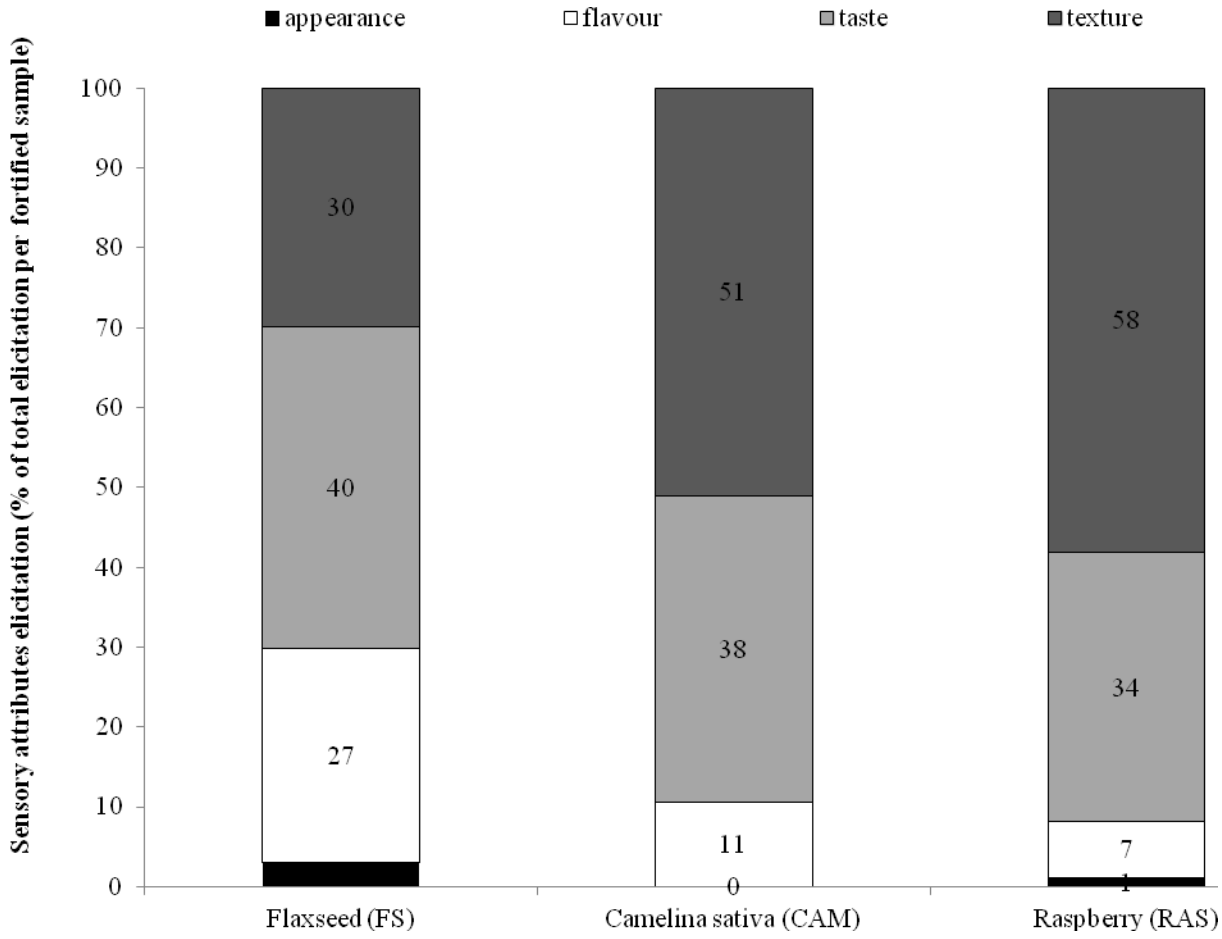
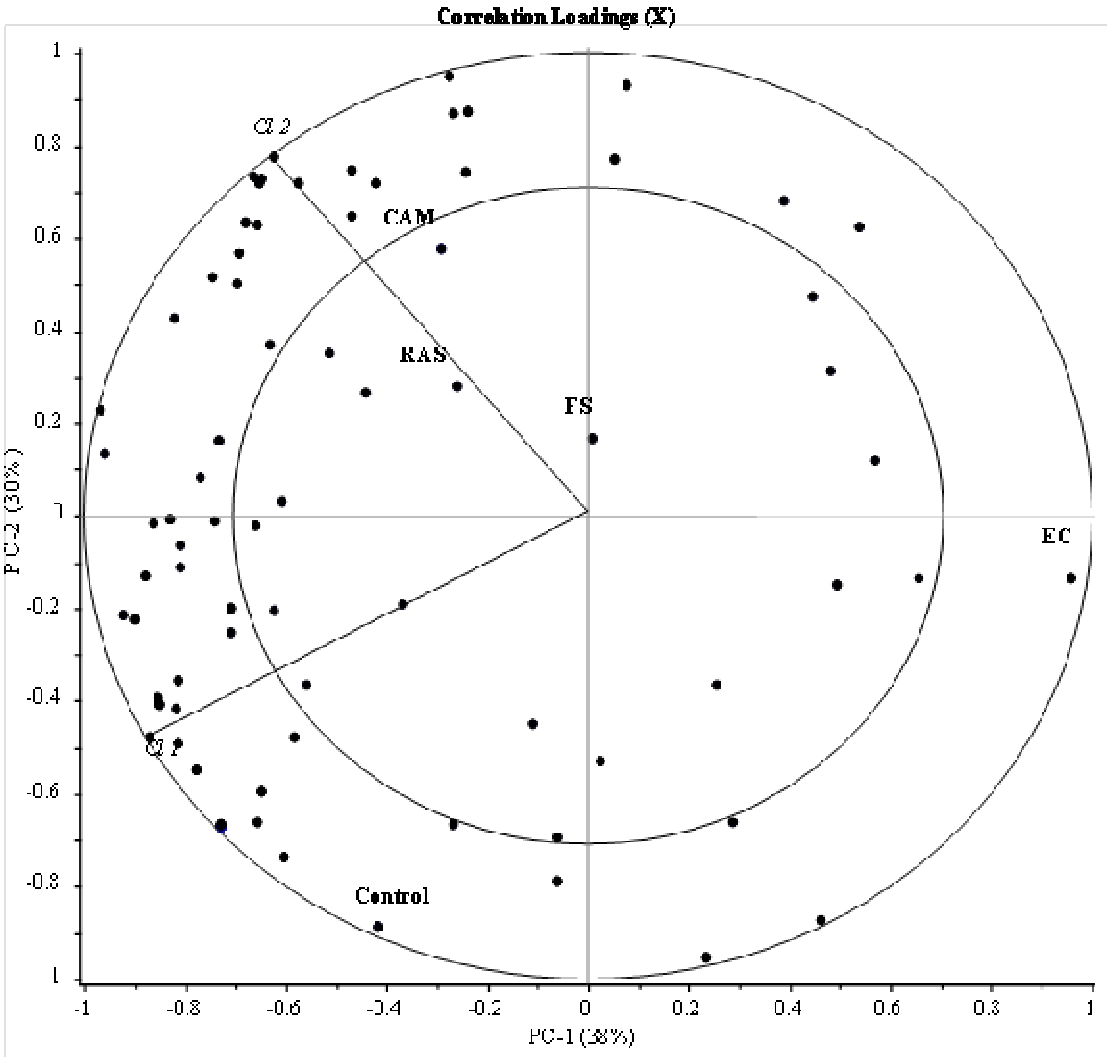

appearance
flavour
taste
texture
Sensory attributes elicitation (% of total elicitation per fortified sample)
100
90
80
70
60
50
40
30
20
10
0
30
40
27
51
38
11
0
58
34
7
1
Flaxseed (FS)
Camelina sativa (CAM)
Raspberry (RAS)

Figure 2.


Correlation Loadings (X)
PC-2 (30%)
PC-1 (38%)
Q 2
Q 1
CAM
RAS
FS
EC
Control
1
0.8
0.6
0.4
0.2
-0.2
-0.4
-0.6
-0.8
-1
-0.8
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
0.8
1