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Comparative study of pomegranate and jacaranda seeds as functional components for the conjugated linolenic acid enrichment of yogurt

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ABSTRACT

Pomegranate *(Punica granatum)* and jacaranda *(Jacaranda mimosifolia)* seeds represent high natural sources of conjugated linolenic acids (CLnAs), predominantly as punicic and jacaric acid, respectively. Both CLnAs are known as potent anticarcinogenic compounds; however, they are poorly used in food development. We characterized pomegranate and jacaranda seeds (PS and JS, respectively) and seed oil and employed seed powder for the bioactive lipid enrichment of yogurt. The fibre and lipid contents were quite similar in PS and JS. The seed oil fatty acid (FA) profile was dominated by unsaturated fatty acids (~87–89%), whereas the CLnA content varied from 31 to 69% (for JS and PS, respectively). A higher scavenging activity and phenolic content was determined for PS compared with JS oil. Compared to the control, yogurt enriched with 0.5% (w/v) PS or JS flour showed similar nutritional and pH values, but higher antioxidant activities, desirable FA and CLnA contents and lower atherogenicity indexes. Both bio-fortified yogurts showed high overall acceptability. For the first time, the feasibility of producing CLnA-enriched dairy foods using non-conventional plant seeds or waste seeds was performed, and these results encourage further studies on their functional effects for consumers.

1. Introduction

Consumers are continuously demanding foods with healthier or more functional properties that avoid the excessive intake of fat due to its association with obesity, cancer and diabetes. The lipid contents and the types of fatty acids (FAs) represent the most important compounds to be considered before food consumption. Thus, the production of dairy foods enriched in n-3 or n-6 FAs is continuously growing in the industry, and natural sources, such as uncommon oils, plant extracts or nuts, are usually incorporated for food fortification (Dal Bello, Torri, Piochi, & Zeppa, 2015; Oh et al., 2016; Ozturkoglu-Budak, Akal, & Yetisemiyen, 2016). Among functional dairy foods, and due to world-wide consumption, yogurt is often employed as a vehicle to add fibre, fruit or microorganisms as probiotics to improve its functionality (do Espírito Santo et al., 2012; Min et al., 2012). Moreover, algae oil emulsions and lipid extracts have been used for bioactive lipid yogurt-enrichment (Chee et al., 2005; Robertson et al., 2016).

Edible seeds are rich in fibre, protein, and oil contents and are considered as potential sources of bioactive compounds. In this context, lipids from vegetable oils are healthier than animal fats due to their high essential fatty acid contents that improve health and decrease the incidence of cardiovascular diseases. Overall, soybean, sunflower, olive and rapeseed oils represent the most common edible oils consumed by humans. In the last few years, it has been demonstrated that un-conventional oils represent an alternative to offer new healthy products to the food market (Ardabili, Farhoosh, & Khodaparast, 2011). Some seeds also contain substantial amounts of n-3, n-6 and n-9 FAs as well as other important bioactive compounds for human health care, such as conjugated linolenic acids (CLnAs) (Tsuzuki, Tokuyama, Igarashi, & Miyazawa, 2004; Gasmi & Thomas Sanderson, 2013). The term CLnA includes linolenic acid isomers with a conjugated double bond in the carbon length chain, which confer high bioactive potential through anti-carcinogenic and anti-inflammatory activities on cancer cells (Igarashi & Miyazawa, 2000). For example, punicic acid is found in pomegranate (*c9,t11,c13*-CLnA, 60%), α-eleostearic acid in tung (*c9,t11,t13*-CLnA, 60%) (Shinohara et al., 2012) and jacaric acid in jacaranda (*c8,t10,c12*-CLnA, 36%) seed oil (Gasmi & Thomas Sanderson, 2013).

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Pomegranate (*Punica granatum* L.), from the Punicaceae family, is a fruit that is widely grown and consumed in several countries either in the form of fresh fruit or juice, producing seeds and peels as waste by-products. Pomegranate seeds (PSs) can prevent DNA damage, inhibit cancer and alleviate the symptoms associated with menopause (Lansky & Newman, 2007), as well as being proposed as an emergent technology to improve food functionality. PSs represent 10–20% of total fruit weight and contain valuable pharmaceutical and nutritional compounds as conjugated fatty acids and phenolics. Recent studies to evaluate the inclusion of fruit-juice waste into the manufacture of novel and functional foods were conducted. Indeed, PSs or seed oil (PSO) have been included as natural ingredients for yogurt, bread and ice cream manufacturing (Bourekoua et al., 2018; Siano et al., 2016). Moreover, PSs were also used to supplement animal diets to enhance the CLnA contents of milk (Emami, Fathi Nasri, Ganjkhanlou, Rashidi, & Zali, 2016).

Jacaranda (*Jacaranda mimosifolia* D. Don), from the Bignoniaceae family, is a native tree from Central and South America and the Caribbean; its extracts are popularly consumed to avoid various diseases (Mostafa, Eldahshan, & Singab, 2014). Jacaranda seeds (JSs) also contain CLnAs and jacaranda seed oil (JSO) has demonstrated potent anticarcinogenic activity against tumour cell lines (Shinohara et al., 2012; Yamasaki et al., 2013; Gasmi & Thomas Sanderson, 2013), although there are not sufficient studies regarding its phytochemical composition or its inclusion for food manufacture. These studies are the baseline point for the biotechnological application of this important seed.

In the present work, a comparative study was performed focusing on the nutritional and fatty acid compositions of PS and JS, as well as the biotechnological potential for their use as additives for yogurt manufacture. The nutritional value, microbiological quality and fatty acid profile of fortified yogurts were evaluated over time.

2. Materials and methods

2.1. Chemicals

All solvents used were HPLC-grade (Avantor Performance Materials Poland S.A., Gliwice, Poland; Labscan brand and Sintorgan, Buenos Aires, Argentina). Folin-Ciocalteu's phenol reagent, gallic acid, DPPH (2,2-diphenyl-1-picryhydrazyl), fatty acid standards and other reagents and salts were from Sigma (99% pure, St. Louis, MO, USA). The M17 (Oxoid, London), MRS, potato dextrose and Mac Conkey (Britania, Buenos Aires, Argentina) agar broths were used for microorganism counting. The kit for measuring lactose was from Boehringer Mannheim (Mannheim, Germany). Other chemicals used were of analytical grade (Cicarelli, Reagents S.A., Santa Fe, Argentina).

2.2. Seed collection and analysis

Jacaranda fruits were manually picked from trees located in Tucumán (northwestern Argentina, 26° 51′ S Longitude 65° 12′ O) and the seeds were manually removed. Pomegranates were acquired from markets, and further processed to obtain juice and seeds. The seeds were crushed and sieved by 30 mesh screen to obtain a fine powder (< 0.5 mm diameter of sieved particles) called flour and then chemically analysed according to the official methods (AOAC, 1995). The moisture content was determined after seed drying at 105 ± 1 °C until a constant weight; the ash content was determined by incinerating the flour at 550 °C in a muffle furnace until attaining a constant weight; and protein content was determined by the Kjeldahl method (AOAC, 1995). The lipid content was estimated by Soxhlet using *n*-hexane, which was further evaporated in a rotary evaporator (Heidolph™ Hei-Vap™, Schwabach, Germany) at 45 °C. Total carbohydrate content was estimated by difference, subtracting the other parameters from 100. Total fibre was quantified using a gravimetric procedure (Prosky, Asp, Schweizer, DeVries, & Furda, 1985).

2.3. Phenolic contents and DPPH radical scavenging activity

Phenolic compounds were extracted from oil following the method described by Amri et al. (2017) with some modifications. Briefly, 1 g of oil was mixed with 1 mL of *n*-hexane and 2 mL of methanol/water (60:40, v/v), shaken and centrifuged to recover the hydroalcoholic phase. The hexane phase was extracted once again with 2 mL of methanol/water solution and the hydroalcoholic phases were mixed and stored at −20 °C. Total phenolic content (TPC) was measured by mixing 0.4 mL of the hydroalcoholic fraction with 10 mL of Folin-Ciocalteús reagent (1/10) and 8 mL of sodium carbonate solution (75 g/L). After 30 min of incubation in the dark, the absorbance was measured at 765 nm. Gallic acid was employed as a calibration standard and the results were expressed as gallic acid equivalents (mg GAE/kg of oil).

The DPPH radical scavenging activities of the oils and yogurts were determined by using a 1,1-diphenyl-2-picrylhydrazyl assay. All yogurt samples were firstly 10-fold diluted in methanol (1:10, v/v) and the oil was firstly dissolved in dimethyl sulfoxide (DMSO). Briefly, 0.1 mL of diluted sample was added to 0.4 mL of DPPH solution (0.1 mM) and 0.4 mL of 0.1 M buffer Tris HCl pH 7.6 and mixed; after 30 min, the absorbance was measured at 517 nm in a spectrophotometer (Varian Cary® 50 UV–Vis).

The DPPH scavenging activity was estimated according to following equation:

$$I\% = (A_{control} - A_{sample}) / A_{control} \times 100$$

where $A_{control}$ is the absorbance of the control reaction containing all reactivity without the tested sample and Asample is the absorbance of the tested sample.

2.4. Fatty acid analysis

Total lipids were extracted by the Folch method (Folch, Lees, & Stanley, 1957) by using a chloroform:methanol solution (2:1, v/v) and then further transmethylated and analysed as previously described (Taboada, Van Nieuwenhove, López Alzogaray, & Medina, 2015) with an Agilent Technologies (Palo Alto, CA, USA) gas chromatograph (model 6890N) equipped with a flame ionization detector and an automatic injector (model 7683) connected to a HP-88 capillary column (100 m × 0.25 mm x 0.20 μm, Agilent Technologies, USA). Fatty acids were identified by comparing their retention times with methylated standards (99% pure; Sigma, St. Louis, MO USA). The results were expressed as g/100 g of fatty acid methyl esters (FAME).

2.5. Yogurt manufacture

Yogurt was manufactured following standard procedures using a commercial starter culture (YC-380, Chr. Hansen, Denmark). Partially skimmed (fat: 1.3 g/100 g milk, lactose: 4.8/100 g milk, protein: 3.2/100 g milk) commercial cow milk (Ilolay, Santa Fe, Argentina) was enriched with JS or PS flour at 0.5% (w/v), mixed for 5 min, and then heated at 85 °C for 30 min for pasteurization. After the milk was cooled at 42 °C in an ice bath, a commercial culture composed of *Streptococcus thermpohilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* was incorporated according to the manufacturer's instructions. This mixture was agitated for 10 min and then incubated at 42 ± 1 °C in a water bath, where the pH was periodically monitored until the pH was approximately 4.6. At this point, the yogurts were fractioned into sterile plastic containers (100 g) and stored at 4 °C for 28 days. Yogurt manufactured without the addition of seed flour was used as a control.

2.6. Yogurt analysis

Yogurts were analysed weekly for pH, microbiological counts and DPPH scavenging activities. The approximate composition and the fatty acid profile were examined on day 1 and after 28 days of storage time. The pH was measured by using a Metrohm 962 pH meter (Herisau, Switzerland). The microbiological analyses of the yogurt samples were performed by plate counting on selective agar. Briefly, 10 mL of yogurt were added to 90 mL of 2% (w/v) sodium citrate solution, homogenized for 1 min in a Stomacher (Laboratory Blender Stomacher model 400, Seward Medical, London, U.K.), and then ten-fold diluted in peptone saline solution. For *Streptococcus thermophilus* enumeration, dilutions were plated on M17 agar (Oxoid, London) and incubated under aerobic conditions at 42 °C for 48 h. *Lactobacillus delbrueckii* subsp. *bulgaricus* was cultured and enumerated on acidified (pH 5.2) MRS agar (Britania, Buenos Aires, Argentina) under anaerobic conditions at 37 °C for 72 h. The counting of yeast and moulds was determined by plating on potato dextrose agar (Britania, Buenos Aires, Argentina) and incubating at 30 °C for 72–96 h. Coliforms were enumerated by using Mac Conkey agar (Britania, Buenos Aires, Argentina) cultured for 48 h at 30 °C. The results were expressed as the Log_{10} CFU (colony forming units)/mL.

The proteins, fat, and ash contents were evaluated according to AOAC International (1995). Lactose was determined by the UV lactose/D-galactose method (Boehringer Mannheim, Mannheim, Germany). The fatty acid profile was examined at day 1 and 28 by first performing lipid extraction according to Folch's procedure (Folch et al., 1957), followed by transmethylation per Taboada et al. (2015) before the FA was determined as described above. Desirable fatty acids (DFA) were determined as the sum of C18:0 plus unsaturated fatty acids (UFA), which were estimated as the sum of monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids. An atherogenicity index (AI) was estimated with the following formula described by Ulbricht and Southgate (1991):

$$AI = [C12:0 + (4 \times C14:0) + C16:0]/(MUFA + PUFA).$$

2.7. Sensory evaluation

After 7 days of refrigerated storage, yogurt samples were analysed by sensory evaluation by using the hedonic test (9-point scale). The panel consisted of 30 untrained panellists (20–41 years old, 18 females and 12 males) who were habitual consumers of yogurt. All samples were evaluated for appearance, taste, colour, texture, odour and overall acceptability, with categories ranging from “dislike extremely” (Amri et al., 2017) to “like extremely” (Dal Bello et al., 2015). Each yogurt sample was codified with three digits and served in 50 mL white plastic cups in a random order. Water was offered to panellists to cleanse their palates between samples.

2.8. Statistical analysis

All chemical determinations of seeds and yogurts were performed in triplicate and the results are expressed as the mean ± standard deviation (SD). All data were subjected to a two-way general linear model ANOVA with type III error and a level of significance of $\alpha = 0.05$, using the Tukey's HSD test for means comparison. Statistical analyses were performed using the STATISTICA version 10.0 software (StatSoft Inc, 2011).

3. Results and discussion

3.1. Proximate seed composition

Carbohydrates were the most abundant compounds in both seeds with values of 65.6 and 46.5 g/100 g for PS and JS, respectively (Table 1). Higher fibre, protein and ash contents were determined in JS compared with PS, whereas the lipid contents were quite similar (approximately 13 g/100 g seed). The general composition obtained for PS is within the range of values previously determined (Saeidi et al., 2018; Bourekoua et al., 2018). There is no available data about the phytochemical composition of JS, but the oil contents were similar to 12.9%, which was determined earlier for *Jacaranda semiserrata* (Chisholm & Hopkins, 1965).

Table 1
Proximate composition (g/100 g seed) of pomegranate and jacaranda seeds and total phenolic compounds (mg GAE/Kg oil) and DPPH (I%) activity of seed oil.

Parameter	PS	JS
Moisture	9.40 ± 0.40	6.41 ± 0.04
Carbohydrate	65.60 ± 0.34	46.51 ± 0.93
Total Fiber	22.57 ± 1.04	38.52 ± 0.91
Protein	10.10 ± 0.52	29.2 ± 0.81
Ash	1.98 ± 0.19	4.30 ± 0.09
Total lipid	12.91 ± 0.33	13.60 ± 0.59
*TPC (mg GAE/Kg oil)	88.45 ± 3.89	27.93 ± 1.10
DPPH (I%)	81.33 ± 3.51	48.30 ± 1.70

The results are expressed as the mean ± SD (n = 3). PS: pomegranate seeds. JS: jacaranda seeds. *TPC: total phenolic compounds. GAE: gallic acid equivalent. ND: not detected.

A higher TPC was observed in PSO compared with JSO (88.45 and 27.93 mg GAE/kg oil, respectively), which is consistent to a value previously determined for PSO (Amri et al., 2017).

The DPPH scavenging activity was higher in PSO than in JSO (81% vs. 48%, respectively). Siano et al. (2016) found a higher DPPH value (up to 98%) for PSO than in our study, but to our knowledge, there are no previous studies on JSO DPPH activities.

Saha, Patra, and Ghosh (2012) previously reported that some CLnA isomers have high antioxidant activities by using the DPPH method. The greater antioxidant activity of PSO compared to JSO could be attributed to the higher TPC content plus twice the CLnA contents compared with JSO.

3.2. Fatty acid profile of seed oil

The fatty acid profiles of PSO and JSO were characterized by a high UFA content (approximately 87–90 g/100 g FAME) and low saturated fatty acids (SFAs, ~10–13 g/100 g FAME), with palmitic (16:0) and stearic (18:0) acids being predominant in both oils (Table 2).

The MUFA contents were higher in JSO than PSO (12.38 vs. 8.86 g/100 g FAME, respectively). Among PUFAs, linoleic acid (18:2 n-6) was predominant in JSO (42 g/100 g FAME), followed by jacaric acid (32 g/100 g FAME), whereas punicic acid was the most abundant FA in PSO (near 60 g/100 g FAME). The total CLnA contents were 2-fold higher in PSO than JSO; jacaric acid (18:3 8c,10t,12c) was exclusively identified in JSO, whereas punicic (~58 g/100 g FAME), α-eleostearic (~8.5 g/100 g FAME) and catalpic (~2.6 g/100 g FAME) acids were detected in PSO. Similar SFA/UFA ratios and atherogenicity indexes were estimated for both seed oils. Our results are in agreement with previously reported results for JSO for SFAs with regard to their oleic and jacaric acid contents (Yamasaki et al., 2013). However, the proportion of SFAs, MUFAs and PUFAs appears to be different to those reported for *J. semiserrata* oil (Chisholm & Hopkins, 1965).

The FA composition of PSO varied according to the cultivar. Therefore, the SFA composition ranged from 4.8 to 9.7%, MUFA from 5.7 to 8.15% and PUFA from 69 to 85% (Verardo et al., 2014; Siano et al., 2016), with our results falling within these ranges. With regard to the CLnA contents, some authors exclusively found up to 75–84% punicic acid in PSO (Verardo et al., 2014), while Siano et al. (2016) reported the presence of punicic (55%), α-eleostearic (2.5%) and catalpic (1.6%) acids, which was consistent with our isomer CLnA profile.

Although conjugated linoleic acid (CLA) has been associated with many biological functions (Bhattacharya et al., 2006), CLnA appears to

Table 2
Fatty acid profile of pomegranate and jacaranda seed oil.

Fatty acid	PSO	JSO
16:0	5.35 ± 0.35	6.30 ± 0.12
18:0	3.26 ± 0.22	6.45 ± 0.13
18:1 n-9c	7.83 ± 0.52	10.70 ± 0.35
18:1 n-7	0.58 ± 0.07	0.42 ± 0.01
18:2 n-6	10.80 ± 0.70	41.81 ± 0.18
18:3 n-3	0.64 ± 0.04	0.52 ± 0.12
20:0	0.67 ± 0.06	1.26 ± 0.08
20:1 n-9	0.44 ± 0.01	0.60 ± 0.06
24:0	1.67 ± 0.08	ND
18:3 (9c,11t,13c; punicic acid)	57.65 ± 0.92	ND
18:3 (9c,11t,13t; α-eleostearic acid)	8.50 ± 0.56	ND
18:3 (9t,11t,13t; catalpic acid)	2.65 ± 0.78	ND
18:3 (8c,10t,12c; jacaric acid)	ND	31.2 ± 0.42
Others	0.47 ± 0.01	0.74 ± 0.04
Saturated Fatty acid (SFA)	10.55 ± 0.42	12.75 ± 0.25
Unsaturated Fatty acid (UFA)	89.52 ± 0.47	87.25 ± 0.30
Monounsaturated (MUFA)	8.86 ± 0.60	12.38 ± 0.26
Polyunsaturated (PUFA)	80.66 ± 0.13	74.87 ± 0.04
Total CLnAs	69.22 ± 0.78	31.20 ± 0.42
SFA/UFA	0.12 ± 0.01	0.15 ± 0.01
AI	0.06 ± 0.00	0.07 ± 0.00
Total CLnAs	69.23 ± 0.79	31.20 ± 0.42

The results correspond to the mean ± SD (n = 3). PSO: pomegranate seed oil. JSO: jacaranda seed oil. CLnAs: conjugated linolenic acids. AI: atherogenic index. ND: not detected.

have stronger *anti*-tumoural effects than CLA. Indeed, *in vitro* and *in vivo* studies showed the strongest *anti*-tumoural effects by α-eleostearic acid (Tsuzuki et al., 2004), although punicic acid enhanced the function of B cells involved in the humoural immune response pathway (Yamasaki et al., 2006) and inhibited proliferation of colon cancer cells (Shinohara et al., 2012). Moreover, Yamasaki et al. (2013) reported that JA has greater apoptotic effects on HL-60 cells through the induction of oxidative stress processes than either the t10,c12 or c9,t11 CLA isomers. Therefore, both PSO and JSO represent promising alternative source nutraceutical or food ingredients for humans that produce CLnAs, which are not naturally found in animal fats. According to the fatty acid profiles of the seeds, we estimated that 0.5% (w/v) was the minimum concentration of seed flour necessary to reach greater than 2 g/100 g FAME of CLnAs in yogurt.

3.3. pH, microbiological quality and scavenging activity of yogurt

The pH of the yogurts following production over 28 days of storage are shown in Table 3. The addition of PS or JS powder did not alter the pH compared to the control yogurt. At day 1, all samples reached a pH of 4.5, and this remained near a constant pH value of 4.4 by day 28.

The effect of the addition of seed powder on the viability of starter cultures was determined weekly (Table 3). The viable *St. thermophilus* cell count was not significantly different between the yogurts at any of the time points, remaining constant approximately 9 Log_{10} CFU/mL during the 28 days of refrigerated storage, indicating the good stability of the lactic acid bacteria strain. However, the viable cell count of *L. delbrueckii* subsp. *bulgaricus* was significantly affected by the addition of 0.5% PS and by the storage time. Therefore, PS 0.5% showed a lower initial count on day 1 (6 Log_{10} CFU/mL) compared to 6.5 Log_{10} CFU/mL for the control and JS 0.5% yogurts. This fact could be attributed, at least in part, to the presence of higher phenolic compounds in PS than JS, which could affect microorganism growth. The L. *delbrueckii* subsp. *bulgaricus* counts were also significantly affected by storage time and declined after 28 days of storage to 4.6 Log_{10} CFU/mL in 0.5% PS and 5.4 Log_{10} CFU/mL for the control and JS 0.5% yogurts, respectively. The starter viability at day 1 in our study (6–6.5 and over 9 Log_{10} CFU/mL for *L. delbrueckii* subsp. *bulgaricus* and *St. thermophilus*, respectively) was consistent with values reported by Robertson et al. (2016) using the same commercial starter (YC-380 Chr. Hansen) for yogurt manufacture. In addition to the changes determined to the number of *L. delbrueckii* subsp. *bulgaricus*, the viability of starter culture was still within the range of 8–9 Log_{10} CFU/mL for streptococci and 2–7 Log_{10} CFU/mL for lactobacilli as determined for Argentinean dairy products (Vinderola & Reinheimer, 2000).

Coliform, yeast and mould counts (Log_{10} CFU/ml) were quite similar in the control and fortified yogurts over time (Table 3). In all samples, the coliform counts during the 28 days were lower than 2 Log_{10} CFU/mL, which is the maximum allowed in most international standards and within the range established for commercial yogurt by the Argentinean legislation (ANMAT, 2014). The yeast and mould counts were not significantly affected by the addition of seed flour over the storage time, with counts less than 2 Log_{10} CFU/mL in all samples.

Yogurt enriched with 0.5% seed powder exhibited higher DPPH scavenging activities (41–61%) than plain yogurt (29–30%), and the differences were maintained over time. It was previously demonstrated that the antioxidant activity of foods could be improved by adding vegetable extract. For example, the addition of red ginseng extract increased the DPPH activity of yogurt up to 90% over 31 days of storage time (Jung et al., 2016). It was recently reported that the enrichment of yogurt with moringa extracts (0–0.2%) produced near 2-fold higher TPC and 7-fold higher DPPH activities than control yogurt (Zhang et al., 2019). Pomegranate is often used for the development of foods due to its functional activities. In a previous study, a yogurt rich in pomegranate juice showed high radical scavenging activity mainly associated with its anthocyanin contents (Trigueros, Wojdyło, & Sendra, 2014). Moreover, it was recently reported that bread manufactured with the addition of PS showed significantly higher TPC and antioxidant activity in comparison with unfortified bread (Bourekoua et al., 2018).

3.4. Yogurt proximate composition and fatty acid profile

The proximate compositions of control and bio-fortified yogurts are shown in Table 4. The addition of seed flour did not significantly affect the proximate composition over time. The protein content was approximately 3.4–3.5 g/100 g product in all yogurts over time. At day 1, lactose reached a value of 3.1 g/100 g product and slightly decreased over 28 days of storage time (3.0 g/100 g product). The ash contents were similar and constant in all the yogurts over time, with a value of 0.7 g/100 g product. The fat contents were close to 1.4 g/100 g product in the control and slightly increased by seed addition up to 1.5 g/100 g product. Even though PS and JS showed lipid contents near 13 g/100 g seed, fortified yogurts are still within the range of “low-fat yogurt” (less than 3 g/100 g of product), similar to the control. Our results are in agreement with the nutritional value of yogurt manufactured with skimmed milk and fortified with lipid algae extract (Robertson et al., 2016) or those enriched with n-3 sources (Dal Bello et al., 2015).

The GC analysis of yogurt fat showed significantly changes in the FA profile due to the addition of seed powder (Table 4). Because PS and JS showed high CLnA contents, we hypothesized that the addition of seed powder would improve the CLnA contents of the yogurts. This fact was confirmed by the significant increase in the UFA contents, especially for the observed PUFA levels, and the subsequent decrease in SFAs in both fortified yogurts with respect to the control ($P < 0.05$). SFAs in the control reached 68.5 g/100 g FAME on day 1 and 28, whereas an average value of 62–65 g/100 g FAME was determined for both fortified yogurts. Robertson et al. (2016) reported that the addition of different oils to yogurt significantly decreased the SFA and increased the UFA contents, which was consistent with our results. In fact, among UFAs, the most significant impact of seed powder addition was determined by the 1.5–2-fold higher linoleic acid content and the presence of high CLnA levels. Jacaric acid was exclusively found in JS yogurt, with an average value of 2.5 g/100 g FAME, whereas different CLnA isomers where found in PS yogurt, with punicic acid as the predominant isomer, followed by α-eleostearic and catalpic acid.

Table 3
pH, microbiological counts (log_{10} CFU/mL) and DPPH activity of yogurts at 1 and 28 days of refrigerated storage.

	Storage time					
	Samples	Day 1	Day 7	Day 14	Day 21	Day 28
pH	Control	4.51 ± 0.02^{a}	4.48 ± 0.02^{a}	4.48 ± 0.03^{a}	4.44 ± 0.02^{a}	4.42 ± 0.03^{a}
	PS 0.5%	4.50 ± 0.02^{a}	4.46 ± 0.02^{a}	4.45 ± 0.02^{a}	4.40 ± 0.03^{a}	4.39 ± 0.02^{a}
	JS 0.5%	4.51 ± 0.03^{a}	4.47 ± 0.01^{a}	4.46 ± 0.02^{a}	4.43 ± 0.02^{a}	4.41 ± 0.02^{a}
St. thermophilus	Control	9.13 ± 0.10^{a}	9.09 ± 0.09^{a}	8.91 ± 0.08^{a}	9.05 ± 0.11^{a}	8.89 ± 0.08^{a}
	PS 0.5%	9.17 ± 0.11^{a}	9.01 ± 0.11^{a}	8.82 ± 0.07^{a}	8.97 ± 0.09^{a}	8.86 ± 0.09^{a}
	JS 0.5%	9.08 ± 0.08^{a}	9.10 ± 0.08^{a}	8.90 ± 0.08^{a}	8.95 ± 0.09^{a}	8.94 ± 0.10^{a}
L. delbrueckii subsp. *bulgaricus*	Control	6.52 ± 0.07^{a}	6.13 ± 0.09^{a}	5.75 ± 0.09^{a}	5.51 ± 0.06^{a}	5.39 ± 0.05^{a}
	PS 0.5%	6.01 ± 0.06^{b}	5.70 ± 0.06^{b}	5.21 ± 0.06^{b}	5.03 ± 0.06^{b}	4.61 ± 0.06^{b}
	JS 0.5%	6.48 ± 0.08^{a}	6.08 ± 0.07^{a}	5.80 ± 0.08^{a}	5.48 ± 0.04^{a}	5.42 ± 0.07^{a}
Coliforms	Control	1.55 ± 0.07^{a}	1.53 ± 0.05^{a}	1.54 ± 0.06^{a}	1.52 ± 0.07^{a}	1.51 ± 0.11^{a}
	PS 0.5%	1.54 ± 0.08^{a}	1.52 ± 0.06^{a}	1.51 ± 0.07^{a}	1.52 ± 0.05^{a}	1.49 ± 0.07^{a}
	JS 0.5%	1.61 ± 0.10^{a}	1.55 ± 0.06^{a}	1.52 ± 0.08^{a}	1.50 ± 0.06^{a}	1.48 ± 0.09^{a}
Yeast and moulds	Control	1.36 ± 0.09^{a}	1.31 ± 0.07^{a}	1.26 ± 0.08^{a}	1.24 ± 0.08^{a}	1.25 ± 0.11^{a}
	PS 0.5%	1.41 ± 0.09^{a}	1.33 ± 0.05^{a}	1.25 ± 0.06^{a}	1.27 ± 0.06^{a}	1.28 ± 0.07^{a}
	JS 0.5%	1.31 ± 0.18^{a}	1.29 ± 0.05^{a}	1.26 ± 0.08^{a}	1.28 ± 0.07^{a}	1.27 ± 0.10^{a}
DPPH (I %)	Control	30.10 ± 0.85^{c}	30.05 ± 0.67^{c}	29.87 ± 1.29^{c}	29.33 ± 1.42^{c}	29.25 ± 1.20^{c}
	PS 0.5%	61.55 ± 2.05^{a}	60.23 ± 1.38^{a}	55.39 ± 1.56^{a}	55.71 ± 1.49^{a}	55.60 ± 2.19^{a}
	JS 0.5%	44.05 ± 1.77^{b}	44.16 ± 1.42^{b}	42.18 ± 1.71^{b}	41.56 ± 1.39^{b}	41.65 ± 1.63^{b}

Values are expressed as means ± SD (n = 3). PS: pomegranate seed. JS: jacaranda seed. $^{a,b,c:}$ different superscript letters indicate significant differences among the yogurts at the same time ($P < 0.05$).

Some microorganisms, including lactic acid bacteria and ruminal bacteria, are able to produce CLnA isomers (Terán et al., 2015), but CLnA contents in dairy and fermented dairy products are scarce (Van Nieuwenhove, Terán, & González, 2012). Through the incorporation of 2.5% PSO to goat diets, Emami et al. (2016) reported a 2.5-fold increment in CLA contents in milk fat and 1.32 g/100 g FAME of punicic acid. To our knowledge, this is the first study in which CLnA-yogurt enrichment was performed by the incorporation of PS or JS powder. The enhancement of the CLnA contents herein is twice those found in goat milk fed PSO (Emami et al., 2016). Moreover, the CLnA levels are comparable with other fermented products directly enriched with the addition of CLA, another health-beneficial conjugated fatty acid (Özer & Kiliç, 2015).

The DFA contents were also significantly improved by seed flour incorporation. Therefore, the control yogurt reached a value ~43–44 g/100 g FAME, while 46–50 g/100 g FAME was determined for both fortified yogurts over time, with the highest value determined for JS 0.5%.

The atherogenicity index (AI) infers the atherogenicity power of foods and is calculated with the following formula: AI = [C16:0 + (4*C14:0) +C12:0]/UFA. Our results showed that AI was significantly lower in both fortified yogurts (~2.1–2.5) than the control (~3) over time ($P < 0.05$).

To determine the fatty acid contents offered by each product to consumers, the total and particular conjugated fatty acids and n-3 and n-6 fatty acids were estimated as mg/100 g of product (Table 5). Seed addition significantly improved the n-3 and n-6 contents offered by the yogurts. Therefore, both fortified yogurts offered higher n-3 contents compared with the control (11–13 vs. 9–10 mg/100 g of product, respectively). The n-6 contents in fortified yogurt were also higher compared to the control (54–79 vs. 42 mg/100 g product, respectively). Regarding the CFA, the CLA contents offered by each product were similar, within the range previously determined for yogurt (Shanta el al., 1995) and in agreement with other fermented milks (Florence et al., 2012). However, it is remarkable that the fortification of yogurt with JS produced a jacaric acid-enriched food, offering nearly 38 mg/100 g of product. In contrast, the addition of PS produced a 1.8-fold increase in CLnAs compared with JS yogurt; there were a variety of CLnA isomers, which was in accordance to that determined in PSO. The PS 0.5% yogurt consisted of a total CLnA content of 62–63 mg/100 g of product. There are few natural sources of CLnAs for humans, with PSO being the most widely studied potential functional ingredient to enrich foods. Daily recommended doses of CLnAs are not yet well-established. Since there are fewer CLnAs in dairy products than CLAs, the possibility of using seed wastes as CLnA sources for animal diet or as a food ingredient is a promising alternative for functional food development.

3.5. Sensory properties

The results of the sensory analysis performed with yogurt after 7 days of refrigerated storage are reported in Table 6. In general, the panellists similarly qualified the appearance of all the samples, with mean scores greater than 7. There were significant differences among the samples for colour, with the lowest value for JS (6.2) and the highest value for PS (7.8) yogurt ($P < 0.05$). Panellists also distinguished between the tastes of both fortified yogurts; yogurt with 0.5% of JS had a mean score of 6.3 and with 0.5% of PS had a mean score of 7.3 ($P < 0.05$). It was previously shown that using pomegranate juice or seed for the production of fortified foods gave an attractive colour and acceptable taste, and consumers frequently prefer to consume new products with its addition (Bourekoua et al., 2018). There were no differences among the texture and odour, with scores ranging from 6.8 to 7.4 and 7.4 to 7.9, respectively. Based on the hedonic tests, all the yogurts were found to be acceptable, with an overall acceptability ranging from 7.1 to 7.5.

4. Conclusion

Even though different properties have been attributed to PSO and JSO over last few years, the proximate composition of jacaranda seeds and oil inclusion for food manufacture remains unexplored. To our knowledge, this is the first study where the general composition of JS was examined, showing its nutritional value and the presence of functional compounds of interest, including its fatty acid composition.

Table 4
Proximate composition (g/100 g of yogurt) and fatty acid profile of yogurt at 1 and 28 days storage at 4°C.

Parameter	Day 1			Day 28		
	Control	PS 0.5%	JS 0.5%	Control	PS 0.5%	JS 0.5%
Protein	3.46 ± 0.11^{a}	3.49 ± 0.12^{a}	3.53 ± 0.09^{a}	3.50 ± 0.10^{a}	3.47 ± 0.18^{a}	3.55 ± 0.08^{a}
Lactose	3.11 ± 0.05^{a}	3.10 ± 0.04^{a}	3.06 ± 0.06^{a}	3.00 ± 0.05^{a}	3.01 ± 0.07^{a}	2.95 ± 0.07^{a}
Ash	0.74 ± 0.04^{a}	0.77 ± 0.03^{a}	0.76 ± 0.03^{a}	0.73 ± 0.03^{a}	0.76 ± 0.03^{a}	0.75 ± 0.02^{a}
Fat	1.38 ± 0.06^{a}	1.51 ± 0.11^{a}	1.49 ± 0.13^{a}	1.36 ± 0.09^{a}	1.45 ± 0.11^{a}	1.48 ± 0.08^{a}
Fatty acid*						
4:0	0.50 ± 0.04^{a}	0.52 ± 0.03^{a}	0.46 ± 0.04^{a}	0.45 ± 0.05^{a}	0.51 ± 0.04^{a}	0.51 ± 0.02^{a}
6:0	0.95 ± 0.02^{a}	0.90 ± 0.04^{ab}	0.79 ± 0.08^{b}	0.81 ± 0.08^{a}	0.81 ± 0.02^{a}	0.82 ± 0.012^{a}
8:0	0.90 ± 0.00^{a}	0.88 ± 0.03^{ab}	0.78 ± 0.05^{b}	0.85 ± 0.05^{a}	0.85 ± 0.04^{a}	0.73 ± 0.03^{b}
10:0	1.39 ± 0.26^{b}	1.65 ± 0.13^{a}	1.70 ± 0.14^{a}	1.44 ± 0.10^{c}	1.85 ± 0.06^{a}	1.61 ± 0.06^{b}
10:1 9c	0.29 ± 0.01^{a}	0.27 ± 0.01^{a}	0.16 ± 0.01^{b}	0.17 ± 0.02^{a}	0.10 ± 0.02^{b}	0.16 ± 0.03^{a}
12:0	4.98 ± 0.12^{a}	3.81 ± 0.09^{b}	3.53 ± 0.32^{b}	3.81 ± 0.41^{a}	3.69 ± 0.27^{a}	3.38 ± 0.20^{a}
12:1 9c	0.11 ± 0.01^{a}	0.08 ± 0.00^{b}	0.07 ± 0.01^{b}	0.07 ± 0.01^{a}	0.07 ± 0.01^{a}	0.08 ± 0.00^{a}
13:0	0.05 ± 0.01^{b}	0.13 ± 0.01^{a}	0.13 ± 0.00^{a}	0.29 ± 0.00^{a}	0.13 ± 0.01^{b}	0.13 ± 0.01^{b}
14:0	14.44 ± 0.12^{a}	12.65 ± 0.14^{b}	12.36 ± 0.27^{b}	14.71 ± 0.06^{a}	12.55 ± 0.49^{b}	12.08 ± 0.13^{b}
14:1 9c	1.34 ± 0.21^{a}	1.10 ± 0.16^{a}	0.96 ± 0.12^{a}	1.05 ± 0.06^{a}	1.03 ± 0.03^{a}	0.98 ± 0.05^{a}
15:0	1.28 ± 0.01^{a}	1.40 ± 0.21^{a}	1.28 ± 0.04^{a}	1.22 ± 0.09^{a}	1.40 ± 0.08^{a}	1.23 ± 0.11^{a}
16:0	31.55 ± 0.64^{a}	31.18 ± 0.45^{a}	28.63 ± 0.46^{b}	31.8 ± 0.28^{a}	31.15 ± 0.78^{a}	28.15 ± 0.21^{b}
16:1 9t	0.28 ± 0.01^{a}	0.26 ± 0.02^{a}	0.28 ± 0.02^{a}	0.26 ± 0.02^{a}	0.26 ± 0.01^{a}	0.29 ± 0.02^{a}
16:1 9c	1.64 ± 0.12^{a}	1.56 ± 0.09 ^{a}	1.28 ± 0.07^{b}	1.64 ± 0.10^{a}	1.59 ± 0.04^{a}	1.52 ± 0.05^{a}
17:0	0.57 ± 0.02^{a}	0.56 ± 0.05^{a}	0.57 ± 0.02^{a}	0.52 ± 0.02^{a}	0.57 ± 0.03^{a}	0.56 ± 0.04^{a}
17:1 10c	0.13 ± 0.01^{a}	0.13 ± 0.01^{a}	0.11 ± 0.01^{a}	0.15 ± 0.02^{a}	0.13 ± 0.02^{a}	0.11 ± 0.02^{a}
18:0	11.77 ± 0.19^{a}	11.82 ± 0.08^{a}	12.18 ± 0.20^{a}	12.51 ± 0.43^{a}	11.64 ± 0.08^{b}	12.73 ± 0.18^{a}
18:1 11t	0.03 ± 0.00^{a}	0.02 ± 0.01^{a}	0.04 ± 0.00^{a}	0.01 ± 0.00^{b}	0.01 ± 0.00^{b}	0.05 ± 0.00^{a}
18:1 9c	22.15 ± 0.35^{b}	20.22 ± 0.46^{c}	23.56 ± 0.42^{a}	22.51 ± 0.43^{b}	20.30 ± 0.28^{c}	23.32 ± 0.47^{a}
18:1 11c	0.61 ± 0.01^{c}	0.90 ± 0.05^{b}	1.06 ± 0.06^{a}	0.59 ± 0.01^{b}	0.93 ± 0.02^{a}	1.06 ± 0.09^{a}
18:1 13c	0.21 ± 0.00^{c}	0.46 ± 0.04^{b}	0.59 ± 0.02^{a}	0.21 ± 0.01^{b}	0.51 ± 0.04^{a}	0.58 ± 0.03^{a}
18:2 n-6t	ND	0.10 ± 0.00^{a}	0.10 ± 0.01^{a}	ND	0.10 ± 0.00^{a}	0.11 ± 0.00^{a}
18:2 n-6c	2.82 ± 0.16^{c}	3.56 ± 0.16^{b}	4.77 ± 0.17^{a}	2.87 ± 0.04^{c}	3.37 ± 0.23^{b}	4.94 ± 0.06^{a}
18:3 n-6	0.09 ± 0.00^{a}	0.06 ± 0.01^{a}	0.08 ± 0.00^{a}	0.10 ± 0.00^{a}	0.11 ± 0.01^{a}	0.09 ± 0.00^{a}
18:3 n-3	0.58 ± 0.02^{b}	0.66 ± 0.07^{ab}	0.75 ± 0.01^{a}	0.62 ± 0.03^{b}	0.73 ± 0.03^{a}	0.78 ± 0.04^{a}
18:2 c9t11	0.80 ± 0.02^{a}	0.73 ± 0.04^{a}	0.79 ± 0.03^{a}	0.83 ± 0.03^{a}	0.88 ± 0.05^{a}	0.85 ± 0.06^{a}
20:0	0.15 ± 0.01^{a}	0.18 ± 0.03^{a}	0.13 ± 0.02^{a}	0.15 ± 0.02^{b}	0.23 ± 0.00^{a}	0.13 ± 0.01^{b}
20:1 n-9	0.13 ± 0.01^{a}	0.13 ± 0.01^{a}	0.14 ± 0.00^{a}	0.10 ± 0.01^{a}	0.12 ± 0.01^{a}	0.14 ± 0.01^{a}
20:3 n-6	0.11 ± 0.01^{a}	0.12 ± 0.01^{a}	0.10 ± 0.00^{a}	0.11 ± 0.01^{a}	0.15 ± 0.02^{a}	0.10 ± 0.01^{a}
20:4 n-6	0.13 ± 0.01^{a}	0.07 ± 0.00^{b}	0.13 ± 0.00^{a}	0.14 ± 0.01^{a}	0.11 ± 0.01^{a}	0.14 ± 0.01^{a}
18:3 (PA)	ND	3.29 ± 0.24	ND	ND	3.43 ± 0.41	ND
18:3 (EA)	ND	0.59 ± 0.06	ND	ND	0.53 ± 0.06	ND
18:3 (CA)	ND	0.10 ± 0.01	ND	ND	0.11 ± 0.01	ND
18:3 (JA)	ND	ND	2.53 ± 0.11	ND	ND	2.59 ± 0.03
SFA	68.55 ± 0.65^{a}	65.60 ± 0.68^{b}	62.45 ± 0.31^{c}	68.56 ± 0.26^{a}	65.34 ± 0.91^{b}	62.06 ± 0.34^{c}
UFA	31.45 ± 0.82^{c}	34.40 ± 0.62^{b}	37.33 ± 0.09^{a}	31.45 ± 0.36^{c}	34.66 ± 0.83^{b}	37.69 ± 0.41^{a}
MUFA	26.91 ± 0.69^{b}	25.01 ± 0.60^{c}	28.19 ± 0.23^{a}	26.75 ± 0.43^{b}	25.02 ± 0.29^{c}	28.21 ± 0.33^{a}
PUFA	4.54 ± 0.12^{b}	9.39 ± 0.10^{a}	9.25 ± 0.32^{a}	4.69 ± 0.06^{b}	9.64 ± 0.50^{a}	9.60 ± 0.08^{a}
DFA	43.22 ± 1.01^{c}	46.20 ± 0.47^{b}	49.51 ± 0.09^{a}	43.97 ± 0.85^{c}	46.23 ± 0.92^{b}	50.42 ± 0.24^{a}
AI	3.05 ± 0.14^{a}	2.49 ± 0.08^{b}	2.19 ± 0.03^{c}	3.00 ± 0.05^{a}	2.45 ± 0.03^{b}	2.12 ± 0.03^{c}

The results correspond to the mean ± SD (n = 3). PS: pomegranate seed. JS: jacaranda seed. *Fatty acid composition expressed as g/100 g of FAME. PA: punicic acid. EA: α-eleostearic acid. CA: calendic acid. JA: jacaric acid. UFA: unsaturated fatty acids. MUFA: monounsaturated fatty acids. PUFA: polyunsaturated fatty acids. DFA: desirable fatty acids. AI: atherogenicity index. $^{a,b,c:}$ different superscript letters indicate significant differences among the yogurts at the same storage time ($P < 0.05$).

Therefore, PS and JS could be potential sources of CLnAs and could be considered as ingredient for foods. In fact, the manufacture of yogurt with 0.5% seed addition improved the fatty acid profile and DPPH activities of the fermented products and maintained good nutritional, microbiological and sensorial characteristics. Jacaranda seed is a unique naturally available source of jacaric acid for human consumption, while pomegranate seed offers punicic acid as its predominant CLnA. The healthy fatty acid profile of fortified yogurts herein plus the great

Table 5
Comparison of n-3, n-6 and conjugated fatty acids offered by different yogurts.

Fatty acid (mg/100 g yogurt)	Day 1			Day 28		
	Control	PS 0.5%	JS 0.5%	Control	PS 0.5%	JS 0.5%
n-3	9.5 ± 0.6^{c}	11.1 ± 1.0^{b}	12.6 ± 0.6^{a}	10.1 ± 0.5^{b}	12.2 ± 0.6^{a}	13.1 ± 0.9^{a}
n-6	42.3 ± 1.1^{c}	56.9 ± 1.6^{b}	75.7 ± 2.1^{a}	42.4 ± 1.0^{c}	54.7 ± 3.0^{b}	78.3 ± 0.3^{a}
CLA (c9,t11)	11.0 ± 0.6^{a}	10.8 ± 0.7^{a}	11.8 ± 0.8^{a}	11.3 ± 0.8^{a}	12.8 ± 0.9^{a}	12.6 ± 0.9^{a}
CLnA	–	61.8 ± 2.9^{a}	37.7 ± 1.3^{b}	–	67.8 ± 2.3^{a}	38.5 ± 0.3^{b}
Total CFAs	11.0 ± 0.1^{c}	73.6 ± 3.5^{a}	49.5 ± 1.6^{b}	11.3 ± 0.4^{c}	80.6 ± 1.5^{a}	51.1 ± 0.6^{b}

The results correspond to the mean ± SD (n = 3). CLA: conjugated linoleic acid. CLnA: conjugated linolenic acid. CFAs: conjugated fatty acids (CLA + CLnA). $^{a, b, c}$: Different superscript letters indicate significant differences among the samples at the same time ($P < 0.05$).

Table 6
Sensory evaluation of yogurts.

Attribute	Control	PS 0.5%	JS 0.5%
Appearance	7.7 ± 0.8[a]	7.7 ± 0.8[a]	7.1 ± 1.3[a]
Colour	6.7 ± 0.9[ab]	7.8 ± 0.9[a]	6.2 ± 1.1[b]
Taste	6.8 ± 0.8[ab]	7.3 ± 1.0[a]	6.3 ± 0.9[b]
Texture	7.4 ± 0.9[a]	7.1 ± 1.1[a]	6.8 ± 1.0[a]
Odour	7.4 ± 1.2[a]	7.7 ± 0.8[a]	7.9 ± 1.0[a]
Overall acceptability	7.3 ± 0.7[a]	7.5 ± 0.7[a]	7.1 ± 0.9[a]

The results are the mean score ± SD (n = 30). [a, b]: Different superscript letters indicate significant differences among the samples ($P < 0.05$).

CLnA contents offered by these products is a technological alternative with remarkable health benefits. To our knowledge, this is the first study focused on the development of CLnA-enriched yogurt by the addition of seed flour and must be taken as a starting point for the production of new functional fermented products. Thus, pomegranate and jacaranda seed and seed oil might have wide biotechnological applications as nutraceuticals or food ingredients. As with other medicinal plants, several studies must be carried out to confirm that their consumption and daily dosing promote beneficial health effects.

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