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Physicochemical traits and sensory quality of commercial butter produced in the Azores

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A B S T R A C T

In the volcanic islands of the Azores archipelago, pasture is available the entire year and affects the composition of bovine milk fat. The composition of cows' milk butter manufactured in this region was evaluated. All brands showed excellent microbiological and oxidative quality. The fatty acid profile range presented a low n-6/n-3 ratio (1.81–3.38), low atherogenic (2.86–3.11) and thrombogenicity (3.27–3.60) indices and reduced cholesterol content (136–143 mg 100 g^{-1}). In addition, Azorean butters have a high content of conjugated linoleic acid (0.44–0.64 mg 100 g^{-1}), β-carotene (0.12–0.17 mg 100 g^{-1}) and α-tocopherol (1.40–2.20 mg 100 g^{-1}). Sensory analysis revealed high scores for appearance, consistency and flavour of all brands. These results indicate that Azorean butters produced from cows' milk based on grass-feeding have a potentially healthier fat content and a desirable flavour that, associated with its "natural image", may be promoted for product differentiation.

1. Introduction

Milk fat is the most variable component of bovine milk and is characterised by an enormous complexity, owing to the large number of fatty acids with a variety of structures (Palmquist, 2003). Regarding the fatty acid profile of milk, about 65–75% are saturated fatty acids (SFAs), approximately 18–24% are mono-unsaturated fatty acids (MUFAs) and only 3% are polyunsaturated fatty acids (PUFAs) (MacGibbon & Taylor, 2003). Many fatty acids are specific to ruminant species, such as short-chain (C4:0 to C8:0), odd-chain (C15:0, C17:0, and C17:1) and branched chain, with origins in ruminal microbial lipids, as well as several *cis*-and *trans*-octadecenoic isomers and conjugated linoleic acid (CLA) isomers, resulting from rumen biohydrogenation of several MUFAs and PUFAs (Rego et al., 2009).

Consumption of butter and other high-fat dairy products has been declining in recent years due to a negative nutritional image of milk fat. This negative image is mostly related to the belief that cholesterol and saturated fatty acids elevate plasma cholesterol levels and increase risk of developing coronary heart disease (Huth & Park, 2012; Lovegrove & Givens, 2016; Parodi, 2003). Saturated medium- and long-chain FAs, notably lauric (C12:0), myristic (C14:0) and palmitic (C16:0) acids, are considered to have atherogenic action through increasing plasma cholesterol and low-density lipoprotein (LDL) levels (Parodi, 2003). In contrast, n-3 PUFAs, such as linolenic acid (C18:3 n-3), are effective in reducing plasma lipid levels, including cholesterol levels (Chang, Seo, Matsuzaki, Worgall, & Deckelbaum, 2009). About 20–25% of SFAs in milk fat (C4:0 to C10:0 and stearic acid-C18:0) are neutral with regard to influencing the metabolism of cholesterol. In addition, some minor components of milk fat, especially CLA, sphingomyelin, butyric acid, and polar lipids may have beneficial effects on health (Lordan & Zabetakis, 2017). CLA is a mixture of positional and geometric isomers of linoleic acid (C18:2 *cis*-9, *cis*-12) with a conjugated double bond system. These isomers are receiving increased attention due to their associated human health benefits, such as anti-carcinogenic, anti-obesity, anti-diabetic, anti-hypertensive and anti-atherogenic effects. Dairy fat is among the main dietary sources of CLA, in particular, the *cis*-9, *trans*-11 CLA isomer, also known as rumenic acid (RA). Vaccenic acid (VA, C18:1 *trans*-11) is the precursor of RA that has been shown to have anti-tumour effects by inhibiting proliferation of human malignant melanoma, colorectal, breast and lung cancer cell lines (Parodi, 2005). Butyric acid has also been shown to suppress colorectal cancer cell proliferation and induce apoptotic cell death (Zhang et al., 2016).

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Butter has been perceived as "unhealthy" due to the presence of saturated fatty acids and cholesterol, as they are associated with a higher risk of cardiovascular diseases, obesity or type 2 diabetes (Krause, Lopetcharat, & Drake, 2007), thus, there have been nutritional recommendations to avoid or reduce full-fat dairy intake. However, recent studies contradict these ideas, showing that some milk fat components can carry hypo-cholesterolaemic, anti-carcinogenic and anti-diabetogenic effects (Parodi, 2016; Pearce, 1996; Rodríguez-Alcalá, Castro-Gómez, Pimentel, & Fontecha, 2017; de Almeida et al., 2014). Moreover, full-fat dairy food intake was shown to be inversely associated with metabolic syndrome (Drehmer et al., 2015). Supported by these studies, dairy fat is being re-evaluated and there has been an increase in the interest and consumption of butter regarded as a "natural product" (Krause et al., 2007).

Much research has been focused on changing milk FA composition to create a FA profile that is considered more desirable for human health. Numerous studies indicated that milk from grazing-based production systems has less SFAs and a lower ratio of n-6/n-3 PUFAs, which are considered beneficial for human health (Elgersma, 2015). Grazing-based dairy cows have a more favourable FA profile with a lower n-6/n-3 ratio compared with more intensive production systems (Rego et al., 2016). Rego et al. (2004) compared the profiles of FAs in milk fat of pasture-fed cows with those of cows fed a mixture of maize silage and concentrate, observing that milk produced by pasture-feeding was substantially lower in medium and short chain saturated FAs and richer in long chain unsaturated FAs, CLA and n-3 PUFAs. In addition, a grazing-based cow diet has milk fat containing higher levels of α-tocopherol and β-carotene (Havemose, Weisbjerg, Bredie, & Nielsen, 2004). The level of antioxidants plays an important role in the oxidative stability of milk fat, since lipid oxidation is highly influenced by long chain unsaturated FAs, which are particularly susceptible to oxidation and can give rise to off-flavours.

The Azorean archipelago has excellent climate conditions for the growth of pasture allowing milk production to be heavily based on year-round grass grazing systems. Azorean butter is traditionally produced from the cream of cows' milk, without addition of starter culture. Generally, the grazing diet improves the dietary value of milk fat, increasing the concentration of components that exert beneficial effects. These aspects may distinguish the defining attributes and may contribute to the European certification of Azorean butter with a geographical indication such as protected designation of origin (PDO) or protected geographical indication (PGI). These EU certifications could help to strengthen consumer confidence, increase competitiveness and facilitate access to new markets. Thus, the objective of the present study was to characterise Azorean butter in terms of physicochemical and microbiological characteristics, fatty acid profile, cholesterol, α-tocopherol and β-carotene content, oxidative stability and sensory quality.

2. Materials and methods

2.1. Butter samples

Butters were sampled from local dairy production factories or retailers on three different islands of the Azores (Faial, Terceira and S. Miguel). In total, 16 samples of salted full-fat butters from two different batches from each of eight commercial brands were analysed. All sampled butters were kept in their original packages until they were opened for analysis. Sampling was carried out in accordance with the ISO 707 IDF 50 (EC, 2008). For sensory analysis, samples comprised the eight Azorean brands and a traditional salted full-fat butter produced on mainland Portugal, linked to the best-selling brand in Portugal.

2.2. Determination of proximate composition

Moisture, milk solids-non-fat (MSNF, excluding salt) and fat contents were evaluated using IDF recommended standards ISO 3727-1:2001 (IDF, 2001) and ISO 17189:2003 (IDF, 2003), respectively. Salt content and titratable acidity were measured following ISO 1738:2004 and ISO 1740:2004, respectively (IDF, 2004a,b). Ash, specific gravity, iodine and saponification values were evaluated by reference methods (Kirk & Sawyer, 1991).

2.3. Lipid and lipid-soluble antioxidant vitamins analysis

Fatty acid analysis was carried out according to Rego et al. (2016). Briefly, 200 mg of butter was weighed into culture tubes, and lipids were extracted according to the Folch, Lees, and Sloane Stanley (1957) procedure. Solvents were then evaporated under nitrogen, and FA methyl esters were prepared by transesterification with sodium methoxide (Christie, 2004). Extractions and analyses were performed in duplicate for each sample. Fatty acid methyl esters were analysed using a Shimadzu 2010 Plus gas chromatograph (Shimadzu, Kyoto, Japan), equipped with a flame ionisation detector and a fused silica capillary column SP-2560, 100 m × 0.2 mm × 0.20 μm (Supelco Inc., Bellefonte, PA, USA). Helium was used as carrier gas and the split ratio was 1:50. Injector and detector temperatures were set at 250 °C and 280 °C, respectively. The initial oven temperature of 50 °C was held for 1 min, then increased at 50 °C min^{-1} to 150 °C, where it was held for 20 min, then increased at 1 °C min^{-1} to 220 °C and held for 30 min.

Total cholesterol, tocopherols and β-carotene were analysed as described by Prates, Quaresma, Bessa, Fontes, and Alfaia (2006). Chromatographic analysis was performed by HPLC using a normal-phase silica column (Zorbax RX-Sil with corresponding 12.5 mm analytical guard column, 4.6 mm ID × 250 mm, 5 μm particle size: Agilent Technologies Inc., Palo Alto, CA, USA), with fluorescence detection for tocopherols (excitation wavelength of 295 nm and emission wavelength of 325 nm) and UV–Vis photodiode array detection for cholesterol (202 nm) and β-carotene (450 nm) in series. The solvent (1%, v/v, isopropanol in *n*-hexane) flow rate was 1 mL min^{-1}, the run lasted for 17 min and the temperature of the column oven was adjusted at 20 °C. Samples (two different batches from each brand) were analysed in duplicate.

2.4. Determination of lipid oxidation

Peroxide value of each butter was measured following ISO 3976:2006 (IDF, 2006). To assess the extent of lipid oxidation, the content of thiobarbituric acid reactive substances (TBARS) was determined using the method described by Botsoglou, Fletouris, Papageorgiou, Vassilopoulos, Mantis & Trakatellis (1994). Briefly, 2 g of butter was mixed with 8 mL of TCA (5%) and 5 mL of butylated hydroxytoluene (BHT, 0.8% in hexane, Sigma–Aldrich, St. Louis, MO, USA). The tube was vortexed and centrifuged for 3 min at 3000 × *g*. The bottom aqueous layer was completed to 10 mL with TCA (5%), and a 2.5 mL aliquot was mixed to 1.5 mL of 2-thiobarbituric acid (TBA, 0.8% aqueous, Merck, Darmstadt, Germany). Following incubation for 30 min at 70 °C, the reaction mixture was cooled and submitted to third-derivative spectrophotometry at 521.5 nm. Concentrations were calculated using 1,1,3,3-tetraethoxypropane (Sigma Aldrich) as a standard within the range from 0 to 0.24 μg mL^{-1}. Results were expressed as μg of malonaldehyde (MDA) kg^{-1} of butter.

2.5. Microbiological analysis

For the enumeration of microbial contamination levels of each batch, a representative sample of 25 g was aseptically taken and a preliminary decimal dilution was prepared by adding 225 mL of sterile 0.1% (w/v) buffered peptone water (Biokar, Beauvais, France). Samples were homogenised for 2 min at 230 rpm with a Stomacher (400 Circulator, Seward, UK). Further dilutions were made and quantification of aerobic mesophilic bacteria was carried out in duplicate on Plate Count Agar (Biokar) incubated at 30 °C for 72 h. Detection of coliforms in butter samples was performed in accordance with the reference method described in Annex X (Article 7) of Commission Regulation (EC) No 273/2008 (EC, 2008).

2.6. Sensory analysis

Sensory evaluation of butter was carried out according to Annex IV (Article 4) of Commission Regulation (EC) No 273/2008 (EC, 2008). Panellists were non-trained but were familiar with butter products and were competent to carry out sensory grading. The panel comprised 32 participants (10 male and 22 female), with ages ranging from 20 to 66 years old. Attributes judged were appearance, consistency and flavour/aroma. Scoring was conducted on a 1 to 5 scale, in which 1 stands for very poor (strong defects), 2 stands for poor (evident defects), 3 stands for fair (slight defects), 4 stands for good (no evident defects) and 5 stands for very good (ideal type, highest quality). Prior to assessment, each sample was divided into various portions, equilibrated at room temperature (15 °C ± 1 °C) and served with a glass of water and a slice of bread.

2.7. Statistical analysis

Two different samples from each batch and brand were analysed. Analysis of variance (ANOVA) was used to determine statistical differences among fatty acid compositions of different brands. The level of significance of each analysis is included in the tables. When a significant F was observed ($P < 0.05$), differences among means were evaluated by Post hoc Tukey's test. Principal component analysis (PCA) with Varimax rotation was employed to discriminate brands by major fatty acids, cholesterol, β-carotene and tocopherol contents. Sensory evaluations were analysed using Kruskal–Wallis test for independent samples. Post hoc multiple comparisons were determined by Dunn's pairwise tests. Differences were considered statistically significant at $P < 0.05$. Statistical analysis was performed using SPSS v24.0 (IBM Corporation, New York, NY, USA).

3. Results and discussion

3.1. Physicochemical characterisation

The average physicochemical characterisation and variability of the eight Azorean butter brands are shown in Table 1. The coefficient of variation (CV) was low (<10%) for moisture, fat content, specific gravity, iodine and saponification values. The highest variation in butter samples was observed in milk solids-non-fat (MSNF, excluding salt), ash, salt content, titratable acidity, peroxide value and thiobarbituric acid reactive substances (TBARS). Nevertheless, all the values with the exception of salt content were within legal compositional requirements (EC, 2008). Salt content of one brand (brand B) was slightly higher (2.16%) than the limit of 2% (EC, 2008). Level of fat oxidation, represented by peroxide values and TBARS, showed the highest variation among samples due to variation in storage time. Nevertheless, the maximum value of peroxide detected was below the regulatory limit of 0.3 mEq kg^{-1} (EC, 2008). The low level of peroxides in Azorean butter was confirmed by the relatively small extent of lipid oxidation, as measured by TBARS analysis. Both values of peroxide and TBARS were far below levels detected by other authors in commercial butter (Ozturk & Cakmakci, 2006; Simsek, 2011). Although milk fat from pasture feeding generally has a higher content of unsaturated fatty acids and is more susceptible to oxidation, increased levels of natural antioxidants such as tocopherol and carotenes have been related to high pasture-grass intake (Poulsen et al., 2012; Stergiadis et al., 2013). Some studies have demonstrated that oxidative stability of milk fat is positively correlated with β-carotene and α-tocopherol contents (Rafałowski, Żegarska, Kuncewicz, & Borejszo, 2014), and thus, higher level of antioxidants in Azorean butter, resulting from pasture feeding, can protect the milk fat against oxidation.

3.2. Microbiological analysis

All the samples tested had levels of bacterial contamination below the limit of detection (data not shown). These negative results included mesophilic bacteria and coliform counts, thus indicating excellent microbiological quality of all brands. The absence of pathogenic bacteria demonstrates efficiency of pasteurisation and good industrial practices. In contrast, other authors reported high counts of aerobic bacteria in traditional butters (Idoui, Benhamada, & Leghouchi, 2010; Samet-Bali, Ayadi, & Attia, 2009).

3.3. Fatty acid composition

Comparison of major fatty acid (FA) contents in the 8 different butter brands are reported in Table 2. A total of 39 FAs were quantified (g 100 g^{-1} fat) from each butter sample. Significant differences ($P < 0.05$) among the eight butter brands were observed for SFAs (except C4:0 and C16:0) and some unsaturated fatty acid isomers. Most of these fatty acids were minor components of butter (<2 g 100 g^{-1} fat), while no significant differences ($P > 0.05$) were found in major FAs such as palmitic (C16:0) and oleic (C18:1 c9) acids. However, nutritionally important n-3 and n-6 FAs did differ significantly ($P < 0.05$) among brands. This result was reinforced by the n-6/n-3 FA ratio, as it was significantly ($P < 0.05$) lower in two brands (A and B) as compared with all other brands (C, D, E. F, G and H). Interestingly, brands A and B were produced on the two smaller islands (Terceira and Faial, representing 26% and 3% of milk production in the Azores, respectively) while all the other brands were produced on the main island (S. Miguel, 62% of milk production in the Azores). As reported by Chassaing et al. (2016), a higher proportion of n-3 FAs is associated with the highest proportion of grass in the diet. Therefore, the n-6/n-3 ratio is an indicator of the feeding system, being lower in grass-based diets and higher in silage and/or concentrate supplementation (Capuano, Gravink, Boerrigter-Eenling, & van Ruth, 2015; Rego et al., 2016). To increase milk production on more intensive farms located on the main island, although mainly based on pasture, do need some energy supplementation from maize silage and concentrates, thus resulting in a higher n-6/n-3 ratio. Nevertheless, the n-3 FA contents of all brands from our study (ranging from 0.56 to 0.80 g 100 g^{-1} fat) were comparable to average values observed with grass-based diets by other authors (Chassaing et al., 2016; Ledoux et al., 2005; Rego et al., 2016; Soják, 2010). In addition, no differences ($P > 0.05$) were found in the amount of the major CLA isomer (c9, t11), among butter brands. The average CLA content of Azorean butters (0.53 g 100 g^{-1}) was higher than average values for butter (0.27 g 100 g^{-1}) as reported by the US Department of Agriculture (EFSA, 2010). Milk fat content of this CLA isomer is largely linked to grass-based diets (Soják, 2010), demonstrating that Azorean butters are mostly produced through pasture feeding systems.

3.4. Principal component analysis

Results of selected health-affecting FAs, cholesterol, carotene and tocopherol contents are visualised in a PCA plot (Fig. 1). The first two axes accounted for 77% of the total variation and separated

Table 1
Physicochemical characterisation and variability of the 8 Azorean butter brands.[a]

Parameter	Mean	Coefficient of variation (%)	Minimum	Maximum
Moisture (%)	14.3	8.4	11.8	16.3
MSNF (%)	0.91	26.5	0.51	1.24
Fat content (%)	84.2	1.9	82.9	87.9
Salt content (%)	1.53	22.7	1.08	2.16
Ash (%)	0.027	28.5	0.010	0.036
Titratable acidity (mg g^{-1} fat)	1.64	18.6	1.22	2.25
Specific gravity	0.928	1.1	0.902	0.944
Iodine value	38	9.3	33	44
Saponification value	211	2.1	204	220
Peroxide value (mEq kg^{-1})	0.13	46.4	0.04	0.23
TBARS (μg MDA kg^{-1} butter)	30.7	43.8	6.3	50.7

[a] Abbreviations are: MSNF, milk solids-non-fat excluding salt; TBARS, thiobarbituric acid reactive substances. $n = 16$ samples.

the eight brands into three distinct groups (*G1* to *G3*, Fig. 1a). Butters from brands A and B were clustered on the right side of the plot (*G1*), while all the other brands (C to H) were located on the left side (*G2* and *G3*). The butter samples from brands A and B (produced on smaller islands) were found to be associated with a more favourable FA profile linked to grass feeding, such as PUFAs, *trans*-FAs, CLA, VA, n3-FAs and also tocopherol and carotene (Fig. 1a). In contrast, butter samples from brands C to H (produced

Table 2
Fatty acid composition of different Azorean brands (labelled A–H) of butter.[a]

Fatty acid	A	B	C	D	E	F	G	H	*P*-value
C4:0	2.72	2.95	2.84	2.84	2.81	2.76	2.84	2.85	0.084
C6:0	1.87^{a}	2.02^{b}	1.90^{a}	1.84^{a}	1.87^{a}	1.86^{a}	1.84^{a}	1.86^{a}	0.002
C8:0	1.14^{ab}	1.23^{b}	1.13^{ab}	1.07^{a}	1.11^{a}	1.13^{ab}	1.08^{a}	1.10^{a}	0.006
C10:0	2.56^{ab}	2.80^{b}	2.57^{ab}	2.39^{a}	2.50^{ab}	2.60^{ab}	2.40^{a}	2.46^{ab}	0.023
C12:0	3.57^{a}	3.54^{a}	3.87^{ab}	3.92^{b}	3.99^{b}	3.83^{ab}	3.80^{ab}	3.77^{ab}	0.007
C14:0	11.72^{abc}	11.88^{c}	11.75^{abc}	11.58^{ab}	11.81^{bc}	11.74^{abc}	11.56^{a}	11.55^{a}	0.005
C14:1c9	1.04	0.89	0.94	1.02	1.08	1.00	0.97	0.94	0.086
C15:0	0.97^{ab}	1.00^{b}	0.95^{ab}	0.93^{ab}	0.92^{a}	0.93^{ab}	0.94^{ab}	0.93^{ab}	0.048
C16:0	32.15	30.78	31.74	32.76	33.51	32.26	32.27	31.63	0.264
C16:1c9	1.49^{ab}	1.21^{a}	1.37^{ab}	1.52^{ab}	1.58^{b}	1.46^{ab}	1.45^{ab}	1.39^{ab}	0.042
C17:0	0.46^{a}	0.53^{b}	0.47^{ab}	0.45^{a}	0.44^{a}	0.45^{a}	0.46^{a}	0.46^{ab}	0.011
C17:1c9	0.21^{b}	0.20^{ab}	0.20^{a}	0.21^{ab}	0.20^{ab}	0.20^{ab}	0.20^{ab}	0.20^{ab}	0.034
C18:0	10.56^{ab}	12.25^{b}	11.19^{ab}	10.53^{ab}	9.88^{a}	10.54^{ab}	10.95^{ab}	11.29^{ab}	0.029
C18:1t6/t7/t8	0.28^{ab}	0.25^{a}	0.28^{ab}	0.28^{ab}	0.28^{ab}	0.30^{b}	0.28^{ab}	0.29^{b}	0.045
C18:1t9	0.20^{ab}	0.18^{a}	0.21^{ab}	0.20^{ab}	0.20^{ab}	0.22^{b}	0.21^{ab}	0.21^{b}	0.023
C18:1t10	0.42^{ab}	0.29^{a}	0.45^{b}	0.44^{b}	0.47^{b}	0.53^{b}	0.44^{b}	0.47^{b}	0.004
C18:1t11	1.50^{ab}	1.78^{b}	1.41^{ab}	1.24^{a}	1.16^{a}	1.38^{ab}	1.35^{ab}	1.42^{ab}	0.035
C18:1t12	0.37^{b}	0.31^{a}	0.37^{b}	0.37^{b}	0.39^{b}	0.40^{b}	0.37^{b}	0.37^{b}	0.006
C18:1c9	21.75	21.19	21.58	21.83	21.19	21.44	21.87	22.02	0.072
C18:1t15	0.30	0.25	0.26	0.24	0.24	0.27	0.25	0.25	0.086
C18:1c11	0.44	0.41	0.43	0.43	0.44	0.46	0.43	0.44	0.527
C18:1c12	0.19^{bc}	0.14^{a}	0.19^{b}	0.19^{bc}	0.20^{bc}	0.21^{c}	0.19^{b}	0.19^{b}	0.000
C18:1c13	0.08^{b}	0.06^{a}	0.08^{b}	0.08^{b}	0.08^{b}	0.08^{b}	0.08^{b}	0.08^{b}	0.000
C18:1c14/t16	0.44^{b}	0.38^{ab}	0.37^{ab}	0.35^{a}	0.34^{a}	0.39^{ab}	0.36^{ab}	0.37^{ab}	0.044
C18:1c15	0.19	0.15	0.16	0.15	0.15	0.17	0.17	0.17	0.141
C18:2n6	1.43^{ab}	1.29^{a}	1.54^{ab}	1.48^{ab}	1.55^{ab}	1.64^{b}	1.50^{ab}	1.54^{ab}	0.031
C20:0	0.12^{a}	0.14^{b}	0.13^{ab}	0.13^{ab}	0.12^{a}	0.12^{a}	0.13^{ab}	0.13^{ab}	0.010
C18:3n-6	0.019	0.019	0.023	0.022	0.023	0.022	0.022	0.023	0.039
C18:3n-3	0.61^{bc}	0.65^{c}	0.50^{ab}	0.46^{a}	0.45^{a}	0.49^{ab}	0.50^{ab}	0.50^{ab}	0.003
C20:1	0.043	0.040	0.047	0.047	0.044	0.046	0.047	0.048	0.077
CLA-c9t11	0.78	0.78	0.67	0.65	0.62	0.69	0.67	0.68	0.073
C21:0	0.025^{ab}	0.030^{b}	0.026^{ab}	0.023^{a}	0.021^{a}	0.023^{a}	0.024^{a}	0.027^{ab}	0.005
C20:2n-6	0.015	0.016	0.018	0.017	0.017	0.018	0.017	0.017	0.096
C22:0	0.048^{a}	0.063^{b}	0.050^{a}	0.047^{a}	0.043^{a}	0.044^{a}	0.051^{a}	0.049^{a}	0.000
C20:3n-6	0.061^{a}	0.058^{a}	0.070^{b}	0.067^{b}	0.070^{b}	0.070^{b}	0.067^{b}	0.068^{b}	0.000
C20:4n-6	0.090^{ab}	0.078^{a}	0.101^{b}	0.101^{b}	0.104^{b}	0.101^{b}	0.097^{b}	0.097^{b}	0.002
C20:5n-3	0.050^{bc}	0.059^{c}	0.046^{ab}	0.040^{ab}	0.037^{a}	0.042^{ab}	0.043^{ab}	0.044^{ab}	0.001
C22:5n-3	0.085^{b}	0.085^{b}	0.073^{ab}	0.071^{ab}	0.068^{a}	0.072^{ab}	0.072^{ab}	0.072^{ab}	0.012
C22:6n-3	0.005	0.006	0.006	0.005	0.005	0.005	0.005	0.005	0.229
SFAs	67.92	69.22	68.61	68.50	69.02	68.31	68.35	68.11	0.280
MUFAs	25.87^{b}	24.67^{a}	25.37^{ab}	25.82^{b}	25.30^{ab}	25.45^{ab}	25.76^{b}	25.83^{b}	0.011
PUFAs	2.36	2.26	2.38	2.26	2.33	2.46	2.32	2.36	0.640
trans-FAs	3.07	3.07	2.98	2.77	2.74	3.10	2.90	3.01	0.420
n-3 FAs	0.75^{bc}	0.80^{c}	0.63^{ab}	0.58^{a}	0.56^{a}	0.61^{ab}	0.62^{ab}	0.62^{ab}	0.003
n-6 FAs	1.62^{ab}	1.46^{a}	1.75^{ab}	1.69^{ab}	1.77^{b}	1.85^{b}	1.70^{ab}	1.74^{ab}	0.019
n-6/n-3	2.17^{a}	1.83^{a}	2.78^{b}	2.93^{b}	3.17^{b}	3.03^{b}	2.77^{b}	2.81^{b}	0.000

[a] Values (in g 100 g^{-1} fat) are means; within a row, mean values followed by different superscript letters are different ($P < 0.05$). *trans*-FA is the sum of t6/7/8-, t9-, t10-, t11-, t12-and t15-C18:1; n-3 FA is the sum of C18:3n-3, C20:5n-3, C22:5n-3, C22:6n-3; n-6 FA is the sum of C18:2n-6, C18:3n-6, C20:2n-6, C20:3n-6, C20:4n-6.

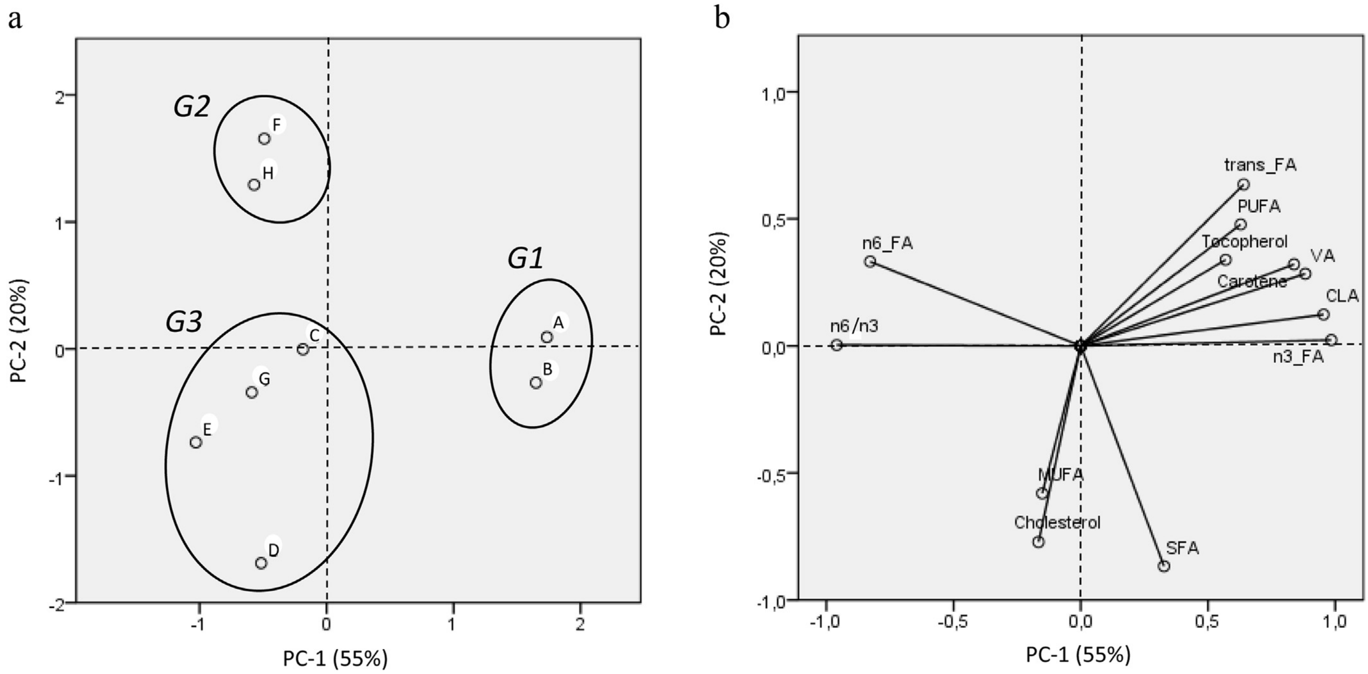

Fig. 1. Principal component analysis scores plot (a) and loadings plot (b) showing the clusters of the brands (A–H) of Azorean butter and categorising the butter brands into different groups, respectively, based on health-affecting fatty acids (SFAs, MUFAs, PUFAs, *trans*-FA, VA, CLA, n3-FAs, n6-FAs and n6/n3), cholesterol, carotene and tocopherol contents.

on the main island) were associated with a less favourable profile of n-6-FA and n-6/n-3 ratio, which is in agreement with the FA data shown in Table 2. The loading plot (Fig. 1b) showed that samples from brands F and H were also associated with lower content of SFAs, MUFAs and cholesterol. Although these differences of FA composition among brands were detectable, the variation of butters produced in the Azores was found to be rather small.

3.5. Selected health-affecting nutrients and nutritional indices

The coefficient of variation (CV) among different brands was low (<10%) for SFAs, MUFAs, PUFAs, butyric acid, CLA, atherogenic index, thrombogenicity index and cholesterol, and slightly higher (10–17%) for VA, n-3 PUFAs, n-6/n-3 ratio, carotene and tocopherol (Table 3). Average values for these health-affecting FAs were similar to butters produced from cows' milk when animal feeding was pasture-based (Chassaing et al., 2016; Larsen, Andersen, Kaufmann, & Wiking, 2014; Ledoux et al., 2005; Nantapo, Muchenje, & Hugo, 2014). Indeed, positive links have been established between grass-based diets and the resulting fatty acid profile of milk. CLA and VA levels of Azorean butters were slightly lower than values previously reported by other authors for pasture-based milk fat (Table 3). Several parameters of the cows' diets were reported to affect milk fat CLA levels such as stage of lactation, supplementation with silage or concentrate, fertiliser application and diverse flora species in the fields (Ledoux et al., 2005; Nantapo et al., 2014; Schwendel et al., 2017). Nevertheless, the average mean of SFAs and the n-6/n-3 ratio of Azorean butters was lower than the average of French butters produced from summer milk (52.6 g 100 g^{-1} versus 56.8 g 100 g^{-1} butter for SFAs and 2.69 versus 3.32 for n-6/n-3 ratio) with pasture based animal feeding (Ledoux et al., 2005). In contrast, MUFA and n-3 PUFA content of Azorean butters were slightly higher than the average of French butters produced in summer (19.6 versus 18.5 g 100 g^{-1} and 0.50 versus 0.47 g 100 g^{-1} for MUFAs and n-3 PUFAs, respectively).

Table 3
Average contents of selected health-affecting fatty acids, cholesterol, carotene and tocopherol in Azorean butter.[a]

Component	Mean	Coefficient of variation (%)	Minimum	Maximum
SFAs (g 100 g^{-1} butter)	52.6	2.3	50.8	54.8
MUFAs (g 100 g^{-1} butter)	19.6	2.8	18.4	20.5
PUFAs (g 100 g^{-1} butter)	2.33	4.9	2.16	2.57
Butyric acid (g 100 g^{-1} butter)	2.17	3.7	2.04	2.32
CLA (g 100 g^{-1} butter)	0.53	9.9	0.44	0.64
VA (g 100 g^{-1} butter)	1.08	14.6	0.82	1.42
n-3 PUFAs (g 100 g^{-1} butter)	0.50	13.8	0.41	0.63
n-6/n-3	2.69	16.8	1.81	3.38
Atherogenic index	2.97	2.54	2.86	3.11
Thrombogenicity index	3.41	2.71	3.27	3.60
Cholesterol (mg 100 g^{-1} butter)	140	1.4	136	143
Carotene (mg 100 g^{-1} butter)	0.14	11.3	0.12	0.17
Tocopherol (mg 100 g^{-1} butter)	1.87	12.9	1.40	2.20

[a] Abbreviations are: SFA, sum of saturated fatty acids; MUFA, sum of monounsaturated fatty acids; PUFA, sum of polyunsaturated fatty acids; CLA, conjugated linoleic acid (*cis*-9, *trans*-11); VA, (C18:1 *trans*-11). Atherogenic index was calculated using the equation proposed by Šimat, Bogdanović, Poljak & Petričević (2015); thrombogenicity index was calculated equation of O'Callaghan et al. (2017).

Table 4
Butter characteristics of different brands (labelled A–H) determined by sensory analysis.[a]

Sensory attribute	A	B	C	D	E	F	G	H	I	*P*-value
Appearance	4.7 ± 0.5	4.5 ± 0.7	4.6 ± 0.6	4.7 ± 0.5	4.8 ± 0.4	4.6 ± 0.7	4.7 ± 0.5	4.6 ± 0.6	4.4 ± 0.8	0.504
Consistency	4.7 ± 0.5	4.5 ± 0.7	4.6 ± 0.6	4.6 ± 0.6	4.6 ± 0.6	4.5 ± 0.6	4.6 ± 0.6	4.5 ± 0.6	4.4 ± 0.7	0.766
Flavour/aroma	4.2 ± 0.8^{ab}	3.9 ± 1.1^{ab}	3.9 ± 1.0^{ab}	4.1 ± 0.9^{ab}	4.3 ± 0.9^{ab}	4.4 ± 0.8^{a}	4.4 ± 0.5^{a}	3.9 ± 1.0^{ab}	3.5 ± 1.1^{b}	0.005

[a] Scores were given on a 1–5 scale. Values are the mean ± SD; mean values followed by different superscripts are significantly different ($P < 0.05$).

The WHO/FAO recommends an intake of PUFAs for 6–10% of energy intake (E%), of which 1–2 E% of n-3 PUFAs, to prevent diet-related chronic diseases (EFSA, 2010). The atherogenic and thrombogenicity indices are dietary risk indices for cardiovascular disease and express the detrimental effects of some fatty acids on human health (O'Callaghan et al., 2016). The atherogenic index (AI) indicates the relationship between the sum of the main saturated fatty acids being considered pro-atherogenic and those unsaturated FAs with anti-atherogenic properties (Ghaeni, Ghahfarokhi, & Zaheri, 2013). AI is calculated as [C12:0 + (4 × C14:0) + C16:0]/(MUFAs + PUFAs) according to Simat et al. (2015). Higher AI levels in milk fat are related to concentrate and grain feeding. The thrombogenicity index (TI) expresses the tendency to form clots in blood vessels and it is defined as the relationship between pro-thrombogenic (saturated) and the anti-thrombogenic FAs. TI is calculated using the equation proposed by O'Callaghan et al. (2017): TI = (C14:0 + C16:0 + C18:0)/[(0.5 × MUFAs) + (0.5 × n-6) + (3 × n-3) + n-6/n-3]. AI values obtained for Azorean butter (2.86–3.11) are lower than those reported for milk fat under a pasture-based dairy system by other studies: 4.08–5.13 and 3.40–3.56, as reported by Nantapo et al. (2014) and O'Callaghan et al. (2016), respectively. Similarly, Azorean butter TI (3.27–3.60) was lower than butters from cows that were maintained outdoors and ate fresh grass (4.06–4.26), described by O'Callaghan et al. (2016). In addition, the cholesterol content of Azorean butters was considerable lower (140 mg 100 g^{-1}) as compared with cholesterol levels ranging from 215 to 400 mg 100 g^{-1}, reported by other authors (Capuano et al., 2015; Pustjens, Boerrigter-Eenling, Koot, Rozijn, & van Ruth, 2017; Rodríguez-Alcalá et al., 2017; Seçkin, Gursoy, Kinik, & Akbulut, 2005). Several European countries such as Germany, Austria, and Switzerland and the WHO/FAO recommend a cholesterol intake lower than 300 mg per day (EFSA, 2010). The reduced levels of cholesterol, AI and TI are advantageous attributes of Azorean butter and are complemented by the greater content of carotene and tocopherol (Table 3). Due to the pasture-based dairy system, Azorean butters have an higher content of β-carotene and α-tocopherol compared with milk fat from mainland Portugal (1.75 versus 1.45 μg g^{-1} of fat and 23.4 versus 15.5 μg g^{-1} of fat for β-carotene and α-tocopherol, respectively), reported by (Ramalho, Santos, Casal, Alves, & Oliveira, 2012). Nevertheless, levels of β-carotene and α-tocopherol in milk fat described in literature vary greatly among studies (Marino et al., 2012). The average content of β-carotene of Azorean butter was within the range of those obtained from milk produced at grazing and organic farms (Agabriel, Cornu, Sibra, Grolier, & Martin, 2007; Bergamo, Fedele, Iannibelli, & Marzillo, 2003; Fauteux, Gervais, Rico, Lebeuf, & Chouinard, 2016), but lower than those observed in The Netherlands and Sicilian studies (Hulshof, van Roekel-Jansen, van de Bovenkamp, & West, 2006; Marino et al., 2014). In contrast, α-tocopherol content of Azorean butter (23.4 μg g^{-1} fat) was higher than those found in conventional and pasture milk (11–19 μg g^{-1} fat) reported by some authors (Agabriel et al., 2007; Bergamo et al., 2003; Fauteux et al., 2016; Gessner et al., 2015; Marino et al., 2012, 2014). Moreover, α-tocopherol content of Azorean butter was comparable with the high levels obtained from organic milk (19.0–26.2 mg g^{-1} fat) reported by Bergamo et al. (2003).

3.6. Sensory analysis

Panellist data scores (mean ± SD) for sensory analysis of Azorean butters brands (A–H) and one brand from mainland Portugal (brand I) are shown in Table 4. No significant differences ($P > 0.05$) among brands were observed for appearance and consistency attributes. Appearance was evaluated on colour, visible purity, absence of physical contamination, absence of mould growth and uniformity of water dispersion. For consistency, body, texture and firmness were assessed. Both attributes scored high (average ≥ 4.5) for Azorean butters indicating the highest quality of the butters analysed. Similar studies have revealed better texture and spreadability of pasture-based butters (O'Callaghan et al., 2016). For the flavour/aroma attribute, significant differences ($P = 0.005$) were recorded among butter brands. Butter from brand I scored significantly lower for flavour/aroma ($P < 0.05$) compared with butter from brands F and G. The average grading of brands A–H was ≥3.9, while brand I was attributed the lowest score (3.5) by the panellists. These results indicate that Azorean butters made of milk from cows grazed on pasture had a more favourable flavour/aroma. Likewise, O'Callaghan et al. (2016) reported that grass-derived butters display significantly higher scores for appearance and flavour over those from a mixed ration diet (grass silage, maize silage and concentrates). The distinctiveness of the fatty acid profile and antioxidant content (α-tocopherol and carotenes) of pasture-based milk can result in the enhancement of the sensory characteristics of butter.

4. Conclusion

In conclusion, no major differences were found among the compositions of all the commercial brands of Azorean butter. The results obtained in this work highlight the distinctive characteristics of Azorean butter that is linked to good quality pastures for grazing dairy cows during the entire year. Dairy production in the Azores results in healthier fat content and improved sensory attributes that distinguishes the Azorean butter from other sources. The Azorean butters showed a reduced n6/n3 fatty acid ratio and atherogenic and thrombogenicity indexes. In addition, they presented elevated contents of healthier components such as CLA, vaccenic acid, short-chain fatty acids and fat-soluble vitamins such as carotene and tocopherol. To our knowledge, this is the first description of an important reduction (approximately 50%) of cholesterol content in butter. In fact, the composition of butter produced in the Azores has a healthier nutritional profile that can be used as an advantage for promoting this high quality dairy product.

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