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Kinetics of Formation of Butyric and Pyroglutamic Acid during the Shelf Life of Probiotic, Prebiotic and Synbiotic Yoghurt

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Abstract: Butyric acid (C4) and pyroglutamic acid (pGlu) exert significant beneficial effects on human health. In this study, the influence of probiotics (*Lactobacillus acidophilus* and *Bifidobacteria*) and/or prebiotics (1 and 3% inulin and fructo-oligosaccharides) on the content of C4 and pGlu in yoghurt during the shelf-life period was evaluated. The contents of C4 and pGlu were determined in probiotic, prebiotic and synbiotic yoghurts during 30 days of storage at 4 °C by solid-phase microextraction coupled with gas chromatography/mass spectrometry and HPLC analysis. Traditional yoghurt and uninoculated milk were used as control. Prebiotic yoghurt contained more C4 (2.2–2.4 mg/kg) than the uninoculated milk, and no increase was detected with respect to traditional yoghurt. However, probiotic yoghurt showed 10% more C4 than traditional yoghurt. Adding fibre to probiotics (synbiotic yoghurt) the C4 content increased by 30%. Regarding pGlu, probiotic yoghurt presented the highest content of approximately 130 mg/100 g. Fibre did not affect pGlu content. Finally, C4 and pGlu contents generally increased up to 20 days of storage and then decreased up to 30 days of storage. The results might be useful for the preparation of other functional foods rich in C4 and pGlu using lactic acid bacteria.

Keywords: inulin; fructo-oligosaccharides; *Bifidobacteria*; *Lactobacillus acidophilus*; pidolic acid; butanoic acid; fermented milk

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1. Introduction

Consumers show a growing interest in the consumption of foods that can directly contribute to health, particularly after the COVID-19 pandemic disease [1,2]. A survey found that in Italy during the 2020 lockdown, eating habits changed, and approximately 10% of the population had increased their consumption of milk and yoghurt [3]. Yoghurt, in fact, has a positive image among consumers because of its diverse nutritional and therapeutic properties. It can be considered a functional food because of its role as a vector for bioactive compounds that can carry out positive actions on human health, especially on the immune system [4]. Yoghurt is a dairy product fermented by *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophiles*. By adding probiotic microorganisms such as *Lactobacillus acidophilus* and *Bifidobacterium bifidum* to yoghurt starter cultures, the nutritional value of the yoghurt is increased because they synthesise folic acid, niacin, thiamine, riboflavin, pyridoxine and vitamin K; they increase the bioavailability of mineral salts and the digestibility of proteins [5]. Regular consumption of yoghurt (400–500 g/week) containing 1.0×10^6 CFU/g *Bifidobacterium* spp. and *L. acidophilus*, which can survive in the upper regions of the gastrointestinal tract, is essential to obtain therapeutic benefits [6,7]. The vitality and activity of the bacteria are important prerequisites since, to be effective, they need to combat the low pH values of products such as yoghurt, the antagonistic action of other fermenting bacteria, the hostile gastrointestinal environment and the competition of the gastrointestinal microbiota. Several studies have shown that the buffering capacity of yoghurt protects the microorganisms of the gastrointestinal tract. Most strains of *L. acidophilus* and *B. bifidum* have the ability to establish themselves among gastrointestinal microbiota as natural inhabitants of the human intestine [8,9], unlike *L. bulgaricus* and *S. thermophilus*, which is still not clear whether or not they are able to resist the hostile environment of the gastrointestinal tract [10,11]. *L. acidophilus* is more tolerant to the acidic pH generated by *Bifidobacterium bifidum*, and the growth of the latter is significantly retarded at pH values below 5. Nonetheless, the tolerance of *Bifidobacterium* to the acidic conditions of the stomach has been reported to be strain-specific [8].

The health properties of yoghurt are also improved by the addition of prebiotics such as inulin and fructo-oligosaccharides (FOS) [12,13].

Recent studies have suggested that the healthful properties of yoghurt are also due to butyric acid (C4) and pyroglutamic acid (pGlu) [14,15].

C4 is one of the most studied short-chain fatty acids and is known for its extra- and intra-intestinal effects, linked above all to its anti-inflammatory and anticancer activities [16]. Naturally, the majority of C4 is generated in the colon of mammals by anaerobic microorganisms belonging to the genera *Clostridium*, *Butyrvibrio*, *Butyribacterium*, *Eubacterium*, *Fusobacterium*, *Megasphera*, and *Sarcina*. These bacteria produce C4 by fermenting dietary fibre, undigested starch, and proteins [17–19]. Instead, lactic acid bacteria, such as *Lactobacillus plantarum*, produce C4 releasing it from the triglycerides of milk thanks to the activity of strain-specific lipase [20]. The C4 healthful properties are present not only when butyrate is produced by the gastrointestinal microbiota, but also when butyrate is taken up orally by consuming products such as yoghurt [20]. Exogenous intake of butyrate could prevent obesity and related metabolic diseases, and may be effective in the treatment of paediatric obesity [21,22].

pGlu, instead, is the cyclic lactam of glutamic acid that has been less studied compared to C4, although it has antimicrobial, antitumoral, mitogenic, anxiolytic and antidiabetic activity [23]. Specifically, the administration of a dose of 3 g/day of pGlu in the form of arginine salt to people aged between 60 and 80 has been shown to improve age-associated memory impairment [24]. As for the formation, pGlu has obtained from: the spontaneous cyclisation of glutamate due to high temperatures; the degradation of glutathione; the incomplete reactions following glutamate activation; the degradation of proteins containing pyroglutamic acid at the N-terminus by the action of glutaminyl cyclase and pyroglutamyl peptidase [23]. The presence of pGlu has been reported in yoghurts from sheep and goat milk [25] and in probiotic and synbiotic yoghurt [26]. It has recently been reported that pGlu content does not increase during the fermentation phase of the yoghurt production process but, rather, increases during storage due to the release of bacterial cyclases by traditional starter cultures [15].

Both compounds (C4 and pGlu) have an effect not only on the nutritional value but also on the flavour of foods. In fact, C4 is associated with a rancid taste at a concentration of about 46 mg/kg, as reported by Scanlan et al. [27], while pyroglutamic acid is correlated to a bitter taste when it exceeds 1 g/L in aqueous solution [28]. Therefore, their concentration should not exceed these limits by much to not negatively affect the acceptance of the yoghurt.

To the best of our knowledge, there is a lack of studies that correlate the presence of probiotics and prebiotics in yoghurt with C4 and pGlu, which have significant effects on health. Therefore, this work is focused on the production of C4 and pGlu in different types of yoghurt (probiotics, prebiotics, symbiotic and traditional yoghurt) and how it can vary during the shelf life at 4 °C. Probiotic, prebiotic and synbiotic yoghurts were produced using *L. acidophilus* and *B. bifidum*, and/or inulin/FOS in addition to traditional microbial starter cultures.

2. Materials and Methods

2.1. Yogurt Production

Yoghurt preparations were manufactured with a dairy pilot plant facility of 100 L milk capacity at YMA s.r.l. (Pignataro Maggiore, Caserta, Italy). Raw milk was homogenised at 200 bars, heated at 85 °C for 15 min, and finally concentrated by removing 15% (*w*/*w*) water using centrifugal vacuum concentrators (Farck, Cremona, Italy). Then, the concentrated milk was cooled at 40 °C and inoculated with a traditional yoghurt starter made of *L. delbrueckii* subs. *bulgaricus* (5%) and *S. thermophilus* culture (95%) (TY) or with probiotic *L. acidophilus* + *Bifidobacteria* spp. culture (1.0×10^6 CFU/mL for each strain) to the traditional starter to prepare probiotic yoghurt (yoghurt ProY). Finally, TY and ProY were fortified with a prebiotic formulation based on the inulin and fructo-oligosaccharides (FOS) (Probiotic S.P.A, Novara, Italy) at a concentration of 1% (*w*/*w*) (PreY1 and SY1) or 3% (*w*/*w*) (PreY3 and SY3) according to the reported Table 1.

Table 1. pH values and lactic acid content in control milk and yoghurt samples during storage at 4 °C for 30 days. The results are expressed as the mean ± ds.

Samples	Composition Specifications	Storage (Days)	pH	Lactic Acid (mg/100 g)
Concentrated milk (control milk)	-	0	6.54 ± 0.02^{c}	nd
		10	6.60 ± 0.04^{c}	nd
		20	$6.59 \pm 0.03^{b,c}$	nd
		30	$6.63 \pm 0.04^{a,b}$	nd
Traditional yoghurt used as control (TY)	*L. delbrueckii* subs. *bulgaricus*	0	4.32 ± 0.00^{d}	878.0 ± 1.90^{l}
	S. thermophilus	10	4.29 ± 0.01^{d}	890.1 ± 8.98^{l}
		20	4.17 ± 0.02^{e}	909.0 ± 2.33^{k}
		30	$4.12 \pm 0.01^{e,f}$	$923.5 \pm 3.26^{j,k}$
Probiotic yoghurt (ProY)	*L. delbrueckii* subs. *bulgaricus*	0	4.06 ± 0.01^{g}	$1161.5 \pm 4.94^{a,b,c}$
	S. thermophilus	10	4.05 ± 0.00^{g}	1174.0 ± 7.17^{a}
	L. acidophilus	20	4.05 ± 0.01^{g}	1172.0 ± 4.14^{a}
	Bifidobacteria spp.	30	4.05 ± 0.01^{g}	1176.1 ± 0.24^{a}
Prebiotic yoghurt (PreY1)	*L. delbrueckii* subs. *bulgaricus*	0	$4.14 \pm 0.01^{e,f}$	$929.2 \pm 2.91^{i,j}$
	S. thermophilus	10	$4.13 \pm 0.00^{e,f}$	$951.1 \pm 7.12^{g,h}$
	1% (50% inulin + 50% FOS)	20	4.15 ± 0.01^{e}	$942.8 \pm 1.34^{g,h,i}$
		30	$4.14 \pm 0.01^{e,f}$	$950.4 \pm 1.77^{g,h}$
Prebiotic yoghurt (PreY3)	*L. delbrueckii* subs. *bulgaricus*	0	$4.13 \pm 0.01^{e,f}$	$927.2 \pm 2.85^{i,j}$
	S. thermophilus	10	$4.12 \pm 0.00^{e,f}$	$935.3 \pm 1.64^{h,i,j}$
	3% (50% inulin + 50% FOS)	20	$4.14 \pm 0.01^{e,f}$	$940.5 \pm 1.93^{g,h,i,j}$
		30	$4.13 \pm 0.01^{e,f}$	953.8 ± 0.86^{g}
Synbiotic yoghurt (SY1)	*L. delbrueckii* subs. *bulgaricus*	0	4.15 ± 0.01^{e}	1108.3 ± 2.87^{f}
	S. thermophilus	10	$4.09 \pm 0.00^{f,g}$	$1135.5 \pm 4.07^{d,e}$
	L. acidophilus	20	4.06 ± 0.01^{g}	$1144.9 \pm 6.29^{c,d,e}$
	Bifidobacteria spp. 1% (50% inulin + 50% FOS)	30	$4.13 \pm 0.01^{e,f}$	$1163.1 \pm 3.24^{a,b}$
Synbiotic yoghurt (SY3)	*L. delbrueckii* subs. *bulgaricus*	0	$4.04 \pm 0.01^{e,f}$	1128.0 ± 1.84^{e}
	S. thermophilus	10	$4.06 \pm 0.00^{e,f}$	$1137.0 \pm 1.33^{d,e}$
	L. acidophilus	20	$4.15 \pm 0.01^{e,f}$	$1150.0 \pm 8.63^{b,c,d}$
	Bifidobacteria spp. 3% (50% inulin + 50% FOS)	30	$4.04 \pm 0.01^{e,f}$	1172.9 ± 3.26^{a}

$^{a–l}$ Different letters in the same column indicate statistically significant differences ($p < 0.05$). n.d.: not detectable.

After incubation at 40 °C for 16 h, fermentation continued up to the final pH of 4.3. At the end of incubation in a tank, the coagulum was broken prior to cooling and packing. An aliquot of concentrated milk (milk control) was incubated at the same temperature-time couple used for the yoghurt preparations, without incorporating any bacterial culture; this sample and yoghurt TY were used as control and were referred to as “control milk” and “control TY”, respectively. All samples were cooled and stored at 4 °C for 30 days.

2.2. Fatty Acid Determination

The fatty acid profile was determined by gas chromatographic analysis of the methyl esters of the fatty acids (FAMEs) obtained by transesterifying the fat extracted from each sample. The extraction was performed according to the method described in D.M. 1986 [29], which is based on the Schimith–Bondzynski–Ratzla traditional method, with some modifications. Specifically, approximately 5 g of yoghurt was placed in a 50 mL centrifuge tube and added with 7 mL of ethanol and 10 mL of the ethyl ether/n-heptane (2:1) mixture. After centrifuging at 8000 rpm for 10 min, the supernatant was collected in a flask. The extraction protocol was repeated three times. The flask content was dried in a rotary evaporator at 4 °C. The fat was recovered with hexane in a 15 mL tube and added with 2–3 mL of sodium chloride (saturated solution). After vortexing and centrifugation under the same conditions described above, the supernatant was transferred into a 15 mL glass test tube after filtration on anhydrous sodium sulphate. Finally, the samples were dried in a stream of nitrogen.

For the gas chromatographic analysis, a solution of the extracted fat in 1% hexane was prepared, and 300 µL of a 2 M KOH solution was added to transesterify. Then, 1 µL of this solution was injected into an Agilent Technologies 6890N gas chromatograph equipped with a programmed temperature vaporiser (PTV) and flame ionisation detector (FID). A capillary column (100 m × 0.25 mm internal diameter, 0.20 µm film thickness) with a 90% biscyanopropyl/10% cyanopropylphenyl siloxane stationary phase (Supelco, Bellofonte, OH, USA) was employed. The operating conditions of the oven, the PTV, and the carrier gas were the same as reported by Manzo et al. [30].

The identification and the quantification of separated peaks were performed using the Supelco 37 Component FAME MIX (Supelco Bellofonte, PA, USA) and Conjugated Linoleic Acid (CLA) FAME Isomers (Sigma-Aldrich, Milano, Italy) as external standards. The fatty acids were expressed as a percentage of total fatty acids.

2.3. Free Butyric Acid Determination

The extraction and analysis of free C4 were performed by solid phase microextraction (SPME) coupled with gas chromatography analysis and mass spectrometry, following the method described by Manzo et al. [30] with modifications. Briefly, 2 g of yoghurt/milk was weighed in a 10-mL vial, and 1 g of sodium chloride and 15 µL of 2-methyl-3-heptanone (10 mg/L) were added as an internal standard. The samples were placed on a heating magnetic stirrer at 50 °C for 10 min. Then, an SPME fibre (coated with 50/30 µm thick divinylbenzene/carboxy/polydimethylsiloxane) of 2 cm length was hermetically inserted into the vial containing the samples and left for 1 h at 50 °C. Next, the fibre was introduced directly into the inlet of a 6890 N GC equipped with a 5973-mass detector, and the thermal desorption of the analytes was performed at 250 °C for 10 min. Splitless injection was used, and the analytes were separated on a 30 m × 0.250 mm capillary column coated with a 0.25 µm film of 95% phenyl and 5% dimethylpolysiloxane. The column oven temperature was held at 40 °C for 2 min and increased from 40 °C to 160 °C at 6 °C/min and from 160 °C to 210 °C at 10 °C/min and then maintained at 210 °C for 10 min. The injection and ion source temperatures were 250 °C and 230 °C, respectively. Helium was used as the carrier gas at a flow rate of 1 mL/min. The energy of the ionising electrons was 70 eV, and the mass range scanned in full scan acquisition mode was 40–450 amu. Compounds were identified using the NIST Atomic Spectra Database version 1.6 and verified by retention rates. For quantification, a C4 calibration curve was constructed using standard solutions of C4 with the internal standard.

2.4. Pyroglutamic and Lactic Acid Determination

Organic acid extraction was carried out according to the method described by Bevilacqua and Califano [31], with some modifications. Then, pGlu and lactic acid were determined by HPLC according to the method reported by Marconi et al. [32], with the same modifications reported by Aiello et al. [15].

2.5. Statistical Analysis

All experiments and determinations were performed in triplicate, and the reported results are the average values (±standard deviation) of the three repetitions. The data were tested by one-way analysis of variance (ANOVA) and Tukey's multiple range test ($p \leq 0.05$) using XLSTAT software version 2022.3.2 (Addinsoft, New York, NY, USA).

3. Results and Discussion

3.1. pH Determination

In general, the pH of fermented foods is naturally low due to the transformation of fermentable sugars into organic acids by the starter microorganisms; therefore, the greater the concentration of the acids in the substrate, the lower the pH will be due to their dissociation in an aqueous environment, from which the release of H+ ions occurs [33].

Table 1 shows the pH values at 0, 10, 20 and 30 days of storage, both for the control milk and the different types of yoghurt. The pH values are expressed as the average of two determinations for each sample.

Samples of the different types of yoghurts (TY, ProY, PreY1, PreY3, SY1 and SY3) showed average pH values over the shelf-life period of 4.22 ± 0.02, 4.05 ± 0.01, 4.14 ± 0.01, 4.13 ± 0.01, 4.10 ± 0.04 and 4.07 ± 0.05, respectively, in line with the range of 4.00–4.60 reported in the literature [34–36]. The control milk sample showed a pH value of 6.60 ± 0.06, also in line with the data reported in the literature. In fact, due to the presence of casein (in which acid groups prevail) and anions of phosphoric and citric acids, bovine milk is weakly acidic, between pH 6.6 and 6.8 [37]. On the other hand, milk, due to the presence of proteins that have groups with positive and negative charges of variable numbers according to the pH of the medium, is a buffered solution. For this reason, even relatively small deviations from the indicated values are considered abnormality indices [38].

The pH values of ProY were significantly lower than those of TY; the decrease is attributable to a more efficient conversion of lactose into lactic acid by Lactobacillus acidophilus [39]. Several studies have also reported that the production of yoghurt using a starter culture associated with probiotics allows it to rapidly reach optimal pH values, reducing the fermentation time [40]. Conversely, inulin and FOS do not significantly affect the pH of yoghurts [26]. For TY, there is a progressive further reduction of pH as the duration of storage increases; several studies [41,42] confirm residual acidification by the starter culture during storage.

3.2. Lactic Acid Content

Lactic acid is the main fermentation product in yoghurt, where it is present in concentrations between 0.8% and 1.3%; its importance concerns not only its influence on the flavour (acidic and refreshing) of yoghurt but also its contribution to the prolongation of shelf life (preventing the development of putrefactive bacteria), the digestibility of caseins, the absorption of mineral salts and the pH and bowel regularity [43,44].

The control milk sample showed negligible values of lactic acid, which was expected because lactic acid is a fermentation product and is almost absent in fresh milk [45]. Furthermore, heat treatment at high temperatures, such as UHT, reduces the microbial load but does not alter the concentration of lactic acid, which therefore becomes an indicator of the freshness of the product. The samples of the different types of yoghurt showed lactic acid values during the shelf-life period of 878.0–923.5 (TY), 1161.5–1176.1 (ProY), 929.2–951.1 (PreY1), 927.2–953.8 (PreY3), 1108.3–1163.1 (SY1), and 1128.0–1172.9 (SY3), expressed as mg/100 g fresh weight (Table 1). The values between 800 mg/100 g fresh weight and

1300 mg/100 g fresh weight were in line with those reported by various sources in the literature [43,44]. The values of lactic acid in ProY were significantly higher than those in TY; the increase is attributable to a more efficient conversion of lactose into lactic acid by *Lactobacillus acidophilus* [39]. The same increase was also found in SY1 and SY3, which had concentrations in the same range as those in ProY. Conversely, inulin and FOS do not significantly affect the variation in lactic acid in prebiotic yoghurts [26]. Considering the variation in concentration during the entire shelf-life period analysed, the kinetics of lactic acid production show an increasing trend for all types of yoghurt. Several studies [41,42] have reported residual acidification activity by starter cultures during storage. The highest concentration of lactic acid (1176.1 ± 0.24 mg/100 g) was found on the thirtieth day of storage in ProY; studies [46] evaluating the sensory acceptability of the latter by consumers revealed a positive consensus.

3.3. Fatty Acid Profile

The fatty acid profiles of the samples of the different types of yoghurts at time 0 are shown in Table 2. The results obtained were in agreement with Serafeimidou et al. [47] concerning the fatty acid composition of yoghurts produced from cow's milk, in which palmitic acid (C16:0) was the most abundant (31.62–33.09%) among saturated fatty acids (SFA), oleic acid (C18:1) among monounsaturated fatty acids (MUFA) (10.64–11.53%) and linoleic acid (C18:2n9c,12c) among polyunsaturated fatty acids (PUFA) (2.73–3.61%).

Table 2. Fatty acid profile (%) of control milk and yoghurt samples at time 0 of the shelf-life.

Fatty Acids	Control Milk	Control TY	ProY	PreY1	PreY3	SY1	SY3
C4:0	2.78 ± 0.014 ^{a}	3.67 ± 0.011 ^{b}	3.18 ± 0.011 ^{b}	3.66 ± 0.09 ^{b}	3.66 ± 0.09 ^{b}	4.13 ± 0.05 ^{a}	3.42 ± 0.06 ^{b}
C6:0	1.84 ± 0.25 ^{a}	2.41 ± 0.06 ^{a}	1.95 ± 0.17 ^{a}	2.42 ± 0.10 ^{a}	2.40 ± 0.07 ^{a}	2.50 ± 0.30 ^{a}	2.24 ± 0.10 ^{a}
C8:0	1.02 ± 0.15 ^{a}	1.29 ± 0.10 ^{a}	1.05 ± 0.14 ^{a}	1.29 ± 0.06 ^{a}	1.28 ± 0.10 ^{a}	1.44 ± 0.07 ^{a}	1.27 ± 0.06 ^{a}
C10:0	2.67 ± 0.09 ^{a}	2.89 ± 0.17 ^{a}	2.61 ± 0.04 ^{a}	3.00 ± 0.17 ^{a}	2.89 ± 0.17 ^{a}	3.18 ± 0.11 ^{a}	2.99 ± 0.03 ^{a}
C11:0	0.32 ± 0.01 ^{b}	0.36 ± 0.02 ^{a}	0.31 ± 0.01 ^{b}	0.35 ± 0.02 ^{a}	0.35 ± 0.02 ^{a}	0.36 ± 0.02 ^{b}	0.35 ± 0.02 ^{a}
C12:0	3.47 ± 0.03 ^{a}	3.49 ± 0.11 ^{a}	3.47 ± 0.13 ^{a}	3.49 ± 0.11 ^{a}	3.49 ± 0.11 ^{a}	3.65 ± 0.21 ^{a}	3.58 ± 0.01 ^{a}
C13:0	0.16 ± 0.00 ^{a}	0.15 ± 0.04 ^{a}	0.15 ± 0.00 ^{a}	0.15 ± 0.04 ^{a}	0.15 ± 0.04 ^{a}	0.15 ± 0.00 $^{a, b}$	0.17 ± 0.03 ^{a}
C14:0	11.48 ± 0.02 ^{a}	11.25 ± 0.11 ^{a}	11.50 ± 0.22 ^{a}	11.26 ± 0.02 ^{a}	11.22 ± 0.07 ^{a}	11.60 ± 0.07 ^{a}	11.69 ± 0.08 ^{a}
C14:1n9	1.03 ± 0.01 ^{a}	0.98 ± 0.02 ^{a}	1.01 ± 0.03 ^{a}	1.00 ± 0.05 ^{a}	0.98 ± 0.02 ^{a}	1.01 ± 0.07 ^{a}	1.01 ± 0.00 ^{a}
C15:0	1.23 ± 0.05 ^{a}	1.19 ± 0.08 ^{a}	1.22 ± 0.00 ^{a}	1.14 ± 0.00 ^{b,c}	1.19 ± 0.08 ^{a}	1.24 ± 0.03 ^{a}	1.21 ± 0.01 ^{a}
C16:0	33.20 ± 0.12 ^{a}	31.62 ± 0.04 ^{c}	32.96 ± 0.23 ^{a}	31.63 ± 0.05 ^{c}	31.63 ± 0.05 ^{c}	31.72 ± 0.05 ^{c}	32.82 ± 0.04 ^{a}
C16:1n7	1.97 ± 0.05 ^{a}	1.85 ± 0.06 ^{a}	2.01 ± 0.00 ^{a}	1.89 ± 0.09 ^{a}	1.86 ± 0.05 ^{a}	2.05 ± 0.05 ^{a}	2.13 ± 0.01 ^{a}
C17:0	0.56 ± 0.02 ^{b}	0.55 ± 0.02 ^{a}	0.57 ± 0.03 ^{a}	0.56 ± 0.02 ^{a}	0.56 ± 0.01 ^{a}	0.60 ± 0.04 ^{a}	0.61 ± 0.03 ^{a}
C17:1n7	0.21 ± 0.02 ^{a}	0.20 ± 0.01 ^{a}	0.25 ± 0.02 ^{a}	0.24 ± 0.03 ^{a}	0.20 ± 0.01 ^{a}	0.27 ± 0.03 ^{a}	0.30 ± 0.02 ^{a}
C18:0	11.3 ± 0.18 ^{a}	11.37 ± 0.19 ^{a,b}	11.09 ± 0.06 ^{a}	11.45 ± 0.08 ^{a}	11.39 ± 0.16 ^{a}	10.15 ± 0.25 ^{b}	11.36 ± 0.13 ^{a}
C18:1n11t	0.09 ± 0.01 ^{a}	0.09 ± 0.00 ^{a}	0.10 ± 0.02 ^{b}	0.09 ± 0.00 ^{a}	0.09 ± 0.00 ^{a}	0.09 ± 0.00 ^{a}	0.12 ± 0.01 ^{a}
C18:1n9t	0.99 ± 0.02 ^{a}	0.93 ± 0.01 ^{a,b}	1.05 ± 0.03 ^{a}	0.94 ± 0.00 ^{a}	0.94 ± 0.00 ^{a}	0.90 ± 0.02 ^{a}	0.87 ± 0.02 ^{a}
C18:1n9c	20.74 ± 0.05 ^{a}	20.03 ± 0.19 ^{a}	20.87 ± 0.08 ^{a}	20.06 ± 0.16 ^{a}	20.03 ± 0.18 ^{a}	20.45 ± 0.25 ^{b,c}	20.34 ± 0.11 ^{a}
C18:2n9t,12t	0.40 ± 0.03 ^{a}	0.37 ± 0.06 ^{a}	0.35 ± 0.00 ^{a}	0.40 ± 0.02 ^{a}	0.37 ± 0.06 ^{a}	0.34 ± 0.03 ^{a}	0.34 ± 0.02 ^{a}
C18:2n9t,12c	0.07 ± 0.01 ^{a}	0.09 ± 0.01 ^{a}	0.06 ± 0.01 ^{a}	0.09 ± 0.01 ^{a}	0.09 ± 0.01 ^{a}	0.07 ± 0.01 ^{a}	0.08 ± 0.01 ^{a}
C18:2n9c,12t	0.08 ± 0.01 ^{a}	0.10 ± 0.01 ^{a}	0.08 ± 0.02 ^{a}	0.10 ± 0.01 ^{a}	0.10 ± 0.01 ^{a}	0.10 ± 0.01 ^{b}	0.11 ± 0.01 ^{a}
C18:2n9c,12c	2.85 ± 0.11 ^{a}	3.61 ± 0.01 ^{a}	2.77 ± 0.01 ^{a}	3.60 ± 0.02 ^{a}	2.79 ± 0.01 ^{a}	2.39 ± 0.11 ^{a}	2.44 ± 0.00 ^{a}
C20:0	0.18 ± 0.01 ^{a}	0.19 ± 0.00 ^{a}	0.13 ± 0.03 ^{b}	0.19 ± 0.01 ^{a}	0.19 ± 0.02 ^{a}	0.19 ± 0.01 ^{a}	0.19 ± 0.01 ^{a}
C18:3n3	0.43 ± 0.09 ^{a}	0.42 ± 0.09 ^{a}	0.39 ± 0.00 ^{a}	0.42 ± 0.08 ^{a}	0.39 ± 0.02 ^{a}	0.36 ± 0.09 ^{a}	0.36 ± 0.02 ^{a}
CLA	0.42 ± 0.02 ^{a}	0.39 ± 0.02 ^{a}	0.43 ± 0.01 ^{a}	0.38 ± 0.02 ^{a}	0.41 ± 0.00 ^{a}	0.45 ± 0.02 ^{a,b}	0.45 ± 0.02 ^{a}
C22:0	0.14 ± 0.00 ^{a}	0.11 ± 0.01 ^{a}	0.11 ± 0.01 ^{a}	0.11 ± 0.00 ^{a}	0.13 ± 0.01 ^{a}	0.14 ± 0.04 ^{a}	0.12 ± 0.00 ^{a}
C20:3n6	0.06 ± 0.01 ^{a}	0.08 ± 0.01 ^{a}	0.08 ± 0.00 ^{a}	0.08 ± 0.01 ^{a}	0.06 ± 0.02 ^{a}	0.11 ± 0.02 ^{a}	0.12 ± 0.01 ^{a}
C22:1n9	0.16 ± 0.03 ^{b}	0.17 ± 0.02 ^{a}	0.16 ± 0.00 ^{b}	0.17 ± 0.02 ^{a}	0.24 ± 0.08 ^{a}	0.20 ± 0.01 ^{a}	0.22 ± 0.02 ^{a}
C20:3n3	0.02 ± 0.00 ^{b}	0.02 ± 0.00 ^{a}	0.01 ± 0.01 ^{a}	0.01 ± 0.01 ^{a}	0.02 ± 0.00 ^{a}	0.02 ± 0.00 ^{b}	0.03 ± 0.00 ^{a,b}
C22:2n6	0.05 ± 0.01 ^{a}	0.07 ± 0.00 ^{a}	0.03 ± 0.03 ^{a}	0.07 ± 0.00 ^{a}	0.06 ± 0.00 ^{a}	0.09 ± 0.00 ^{a,b}	0.01 ± 0.00 ^{a}
C24:1n9	0.06 ± 0.01 ^{a}	0.06 ± 0.00 ^{a,b}	0.05 ± 0.00 ^{b}	0.05 ± 0.01 ^{a}	0.07 ± 0.00 ^{a}	0.08 ± 0.02 ^{a}	0.07 ± 0.00 ^{a}

$^{a–c}$ Different letters in the same column indicate statistically significant differences ($p < 0.05$).

Considering the storage time, the profile differed only for some fatty acids (Tables 3–9). Among these, there was an increase in C4 (C4:0) between days 10 and 20, in line with Güler and Gürsoy-Balcı [48]. It may be related to the increased activity of *L. delbrueckii* subsp. *bulgaricus* during storage [49] on longer-chain fatty acids. During the shelf-life, a loss of viability, especially for lactobacilli, has been demonstrated [50]. This could lead to the release of their intracellular esterases and, although the pH and temperature conditions are not optimal for these enzymes, to the partial hydrolysis of milk fat [51]. The hydrolysis of fatty acids would make them more subject to oxidation phenomena, already observed during yoghurt storage [52].

Table 3. Fatty acids (%) of control milk with statistically significant differences during shelf life.

	Days of Storage			
Fatty Acids	**0**	**10**	**20**	**30**
C11:0	0.32 ± 0.01 [b]	0.35 ± 0.00 [a]	0.37 ± 0.01 [a]	0.37 ± 0.00 [a]
C16:1	1.97 ± 0.05 [a]	1.86 ± 0.03 [a,b]	1.77 ± 0.00 [b]	1.77 ± 0.01 [b]
C17:0	0.56 ± 0.02 [b]	0.65 ± 0.00 [a]	0.70 ± 0.00 [a]	0.71 ± 0.02 [a]
C18:1n9t	0.99 ± 0.02 [a]	0.93 ± 0.00 [b]	0.93 ± 0.00 [b]	0.93 ± 0.00 [b]
C22:1n9	0.16 ± 0.03 [b]	0.30 ± 0.00 [a]	0.30 ± 0.01 [a]	0.28 ± 0.04 [a]
C20:3n3	0.02 ± 0.00 [b]	0.023 ± 0.00 [a,b]	0.024 ± 0.00 [a]	0.024 ± 0.00 [a,b]

[a,b] Different letters in the same column indicate statistically significant differences ($p < 0.05$).

Table 4. Fatty acids (%) of traditional yoghurt used as control (control TY) with statistically significant differences during shelf life.

	Days of Storage			
Fatty Acids	**0**	**10**	**20**	**30**
C4:0	3.67 ± 0.011 [b]	4.14 ± 0.15 [a]	3.72 ± 0.02 [a,b]	3.36 ± 0.10 [b]
C16:0	31.62 ± 0.04 [c]	32.66 ± 0.20 [a,b]	33.09 ± 0.17 [a]	32.30 ± 0.14 [b]
C18:0	11.37 ± 0.19 [a,b]	10.64 ± 0.08 [c]	10.78 ± 0.04 [b,c]	11.53 ± 0.21 [a]
C18:1n9t	0.93 ± 0.01 [a,b]	0.85 ± 0.00 [b]	0.87 ± 0.01 [a,b]	1.00 ± 0.07 [a]
C18:2n9c,**12**c	3.61 ± 0.01 [a]	2.76 ± 0.15 [b]	2.73 ± 0.08 [b]	2.76 ± 0.04 [b]
C24:1n9	0.06 ± 0.00 [a,b]	0.05 ± 0.00 [b]	0.07 ± 0.00 [a]	0.07 ± 0.00 [a]

[a–c] Different letters in the same column indicate statistically significant differences ($p < 0.05$).

Table 5. Fatty acids (%) of probiotic yoghurt (ProY) with statistically significant differences during shelf life.

	Days of Storage			
Fatty Acids	**0**	**10**	**20**	**30**
C4:0	3.18 ± 0.011 [b]	3.29 ± 0.11 [b]	3.67 ± 0.00 [a]	3.21 ± 0.01 [b]
C11:0	0.31 ± 0.01 [b]	0.32 ± 0.01 [b]	0.37 ± 0.01 [a]	0.34 ± 0.01 [a,b]
C17:1n7	0.25 ± 0.02 [a]	0.33 ± 0.00 [b]	0.20 ± 0.02 [b]	0.24 ± 0.02 [b]
C18:0	11.09 ± 0.06 [a]	11.07 ± 0.08 [a]	10.82 ± 0.08 [a,b]	10.70 ± 0.12 [b]
C18:1n11t	0.10 ± 0.02 [b]	0.23 ± 0.01 [a]	0.09 ± 0.02 [b]	0.10 ± 0.01 [b]
C18:1n9t	1.05 ± 0.03 [a]	1.05 ± 0.03 [a]	0.86 ± 0.04 [b]	1.04 ± 0.01 [a]
C18:2n9c,**12**c	2.77 ± 0.01 [a]	2.72 ± 0.06 [b]	2.77 ± 0.07 [b]	2.75 ± 0.02 [b]
C20:0	0.13 ± 0.03 [b]	0.16 ± 0.00 [a,b]	0.17 ± 0.02 [a,b]	0.22 ± 0.01 [a]
C22:1n9	0.16 ± 0.00 [b]	0.16 ± 0.02 [b]	0.17 ± 0.01 [b]	0.24 ± 0.02 [a]
C24:1n9	0.05 ± 0.00 [b]	0.06 ± 0.01 [a,b]	0.07 ± 0.01 [a]	0.06 ± 0.00 [a,b]

[a,b] Different letters in the same column indicate statistically significant differences ($p < 0.05$).

Table 6. Fatty acids (%) of prebiotic yoghurt (PreY1) with statistically significant differences during shelf life.

	Days of Storage			
Fatty Acids	**0**	**10**	**20**	**30**
C4:0	3.66 ± 0.09 [b]	4.14 ± 0.15 [a]	3.73 ± 0.04 [a,b]	3.38 ± 0.13 [b]
C15:0	1.14 ± 0.00 [b,c]	1.12 ± 0.01 [a]	1.26 ± 0.00 [a]	1.15 ± 0.01 [b]
C16:0	31.63 ± 0.05 [c]	32.66 ± 0.20 [a,b]	33.04 ± 0.10 [a]	32.33 ± 0.12 [b]
C18:0	11.45 ± 0.08 [a]	10.69 ± 0.02 [b]	10.82 ± 0.10 [b]	11.54 ± 0.19 [a]
C18:n**9t,12t**	0.40 ± 0.02 [a]	0.35 ± 0.01 [b]	0.34 ± 0.03 [b]	0.37 ± 0.01 [a]
C18:2n**9**c**,12**c	3.60 ± 0.02 [a]	3.60 ± 0.02 [a]	2.77 ± 0.14 [a]	2.79 ± 0.01 [b]
C24:1n9	0.05 ± 0.01 [a]	0.06 ± 0.01 [a,b]	0.05 ± 0.00 [b]	0.07 ± 0.00 [a]

[a–c] Different letters in the same column indicate statistically significant differences ($p < 0.05$).

Table 7. Fatty acids (%) of prebiotic yoghurt (PreY3) with statistically significant differences during shelf life.

	Days of Storage			
Fatty Acids	**0**	**10**	**20**	**30**
C4:0	3.66 ± 0.09 [b]	4.14 ± 0.15 [a]	3.73 ± 0.04 [a,b]	3.36 ± 0.12 [b]
C12:0	3.49 ± 0.11 [a]	3.58 ± 0.26 [a]	3.58 ± 0.06 [a]	3.55 ± 0.07 [b]
C15:0	1.19 ± 0.08 [a]	1.12 ± 0.01 [c]	1.26 ± 0.00 [a]	1.15 ± 0.01 [a]
C16:0	31.63 ± 0.05 [c]	32.66 ± 0.20 [a,b]	33.04 ± 0.10 [a]	32.41 ± 0.12 [b]
C18:0	11.39 ± 0.16 [a]	10.69 ± 0.02 [b]	10.82 ± 0.10 [b]	11.54 ± 0.19 [a]
C18:2n**9**t**,12**t	0.37 ± 0.06 [a]	0.35 ± 0.01 [a]	0.34 ± 0.03 [a]	0.37 ± 0.01 [b]
C18:2n**9**c**,12**c	2.79 ± 0.01 [a]	2.77 ± 0.14 [b]	2.73 ± 0.08 [b]	2.78 ± 0.01 [a]

[a–c] Different letters in the same column indicate statistically significant differences ($p < 0.05$).

Table 8. Fatty acids (%) of synbiotic yoghurt (SY1) with statistically significant differences during shelf life.

	Days of Storage			
Fatty Acids	**0**	**10**	**20**	**30**
C4:0	4.13 ± 0.05 [a]	3.50 ± 0.01 [b]	3.68 ± 0.00 [a]	3.75 ± 0.06 [b]
C6:0	2.50 ± 0.30 [a]	2.28 ± 0.01 [a]	2.09 ± 0.00 [a]	2.37 ± 0.01 [a,b]
C10:0	3.18 ± 0.11 [a]	2.97 ± 0.01 [a]	2.89 ± 0.04 [a]	2.75 ± 0.04 [b]
C11:0	0.36 ± 0.02 [b]	0.34 ± 0.00 [a]	0.34 ± 0.00 [a]	0.32 ± 0.01 [a]
C13:0	0.15 ± 0.00 [a,b]	0.19 ± 0.00 [a]	0.20 ± 0.00 [a]	0.14 ± 0.00 [b]
C14:0	11.60 ± 0.07 [a]	11.75 ± 0.01 [a]	11.78 ± 0.01 [a]	11.34 ± 0.01 [b]
C15:0	1.24 ± 0.03 [a]	1.10 ± 0.01 [b]	1.11 ± 0.01 [b]	1.24 ± 0.03 [a]
C16:0	31.72 ± 0.05 [c]	32.79 ± 0.01 [a]	32.91 ± 0.01 [a]	32.34 ± 0.06 [a]
C16:1n7	2.05 ± 0.05 [a]	2.08 ± 0.00 [b]	2.07 ± 0.00 [b]	2.12 ± 0.01 [a]
C17:0	0.60 ± 0.04 [a]	0.60 ± 0.00 [a,b]	0.56 ± 0.00 [a,b]	0.66 ± 0.03 [a]
C18:0	10.15 ± 0.25 [b]	10.25 ± 0.01 [a]	10.34 ± 0.00 [a]	10.46 ± 0.09 [b]
C18:1n9t	0.90 ± 0.02 [a]	0.89 ± 0.00 [a]	0.89 ± 0.00 [a]	0.87 ± 0.03 [b]
C18:1n9c	20.45 ± 0.25 [b,c]	20.30 ± 0.01 [a]	20.34 ± 0.00 [a]	20.79 ± 0.08 [a,b]
C18:2n**9**c**,12**t	0.10 ± 0.01 [b]	0.13 ± 0.01 [a]	0.12 ± 0.01 [a]	0.21 ± 0.03 [a]
CLA	0.45 ± 0.02 [a,b]	0.44 ± 0.00 [a]	0.44 ± 0.00 [a]	0.45 ± 0.00 [a,b]
C20:3n3	0.02 ± 0.00 [b]	0.03 ± 0.00 [a]	0.03 ± 0.00 [a,b]	0.02 ± 0.00 [a,b]
C22:2n6	0.09 ± 0.00 [a,b]	0.01 ± 0.00 [a]	0.02 ± 0.00 [a]	0.01 ± 0.00 [c]

[a–c] Different letters in the same column indicate statistically significant differences ($p < 0.05$).

Table 9. Fatty acids (%) of synbiotic yoghurt (SY3) with statistically significant differences during shelf life.

	Days of Storage			
Fatty Acids	**0**	**10**	**20**	**30**
C4:0	3.42 ± 0.06 [b]	4.09 ± 0.02 [a]	3.50 ± 0.08 [c]	3.63 ± 0.12 [a]
C6:0	2.24 ± 0.10 [a]	2.82 ± 0.14 [a]	2.30 ± 0.02 [b]	2.12 ± 0.06 [a]
C10:0	2.99 ± 0.03 [a]	3.22 ± 0.02 [a]	2.65 ± 0.03 [b]	2.90 ± 0.06 [a]
C12:0	3.58 ± 0.01 [a]	3.69 ± 0.07 [a]	3.44 ± 0.04 [a]	3.51 ± 0.03 [b]
C13:0	0.17 ± 0.03 [a]	0.16 ± 0.01 [a]	0.15 ± 0.00 [a,b]	0.22 ± 0.03 [a]
C14:0	11.69 ± 0.08 [a]	11.61 ± 0.07 [a]	11.10 ± 0.01 [c]	11.81 ± 0.01 [a]
C15:0	1.21 ± 0.01 [a]	1.19 ± 0.00 [a]	1.15 ± 0.01 [a]	1.02 ± 0.00 [c]
C16:0	32.82 ± 0.04 [a]	31.76 ± 0.08 [c]	32.07 ± 0.03 [b]	32.92 ± 0.10 [a]
C16:1n7	2.13 ± 0.01 [a]	2.02 ± 0.03 [a]	2.07 ± 0.08 [a]	2.08 ± 0.01 [b]
C17:0	0.61 ± 0.03 [a]	0.60 ± 0.00 [a]	0.67 ± 0.02 [a]	0.53 ± 0.02 [b]
C18:0	11.36 ± 0.13 [a]	10.19 ± 0.10 [b]	11.09 ± 0.12 [a]	10.37 ± 0.10 [a]
C18:1n9t	0.87 ± 0.02 [a]	0.82 ± 0.02 [b]	0.91 ± 0.05 [b]	0.88 ± 0.04 [a]
C18:1n9c	20.34 ± 0.11 [a]	20.13 ± 0.07 [c]	21.29 ± 0.03 [a]	20.45 ± 0.00 [a]
C18:2n9c,**12**t	0.11 ± 0.01 [a]	0.12 ± 0.00 [b]	0.12 ± 0.00 [b]	0.11 ± 0.00 [a]
C20:0	0.19 ± 0.01 [a]	0.15 ± 0.00 [b]	0.17 ± 0.0 [a,b]	0.19 ± 0.02 [a]
CLA	0.45 ± 0.02 [a]	0.44 ± 0.00 [b]	0.51 ± 0.02 [a]	0.43 ± 0.00 [a]
C20:3n3	0.03 ± 0.00 [a,b]	0.02 ± 0.00 [a,b]	0.00 ± 0.00 [a]	0.02 ± 0.00 [b]
C22:2n6	0.01 ± 0.00 [a]	0.10 ± 0.01 [a]	0.08 ± 0.00 [b]	0.01 ± 0.00 [a]

[a–c] Different letters in the same column indicate statistically significant differences ($p < 0.05$).

As regards the CLA content during storage, it remained constant in all types of yoghurt, with the exception of the synbiotic preparations (Tables 8 and 9), in which a significant increase was observed around the twentieth day. The results confirm those of some studies according to which no change in the CLA content was observed in yoghurt or other dairy products, when stored at 4 °C for 6 weeks [53]. However, the influence of microbial and storage time on CLA content in dairy products is still a matter of discussion [54].

3.4. Butyric Acid Content

In general, TY exhibited higher free butanoic acid concentrations (2.13–2.36 mg/100 g) than control milk (1.77–1.81 mg/100 g), as shown in Figure 1. In fact, during fermentation and storage, small quantities of free fatty acids are released due to the activity of lipases and microbial esterases [20]. However, most of the free fatty acids are not derived from milk fat but from amino acids [49]. PreY1 and PreY3 showed concentrations of C4 that were not different from those of TY: 2.14–2.37 and 2.13–2.37 mg/100 g, respectively. Fibre may not affect the metabolic activity of starter cultures, confirming that, in contrast to other bacteria, such as Clostridia, these lactic acid bacteria are unable to ferment oligosaccharides to produce butyrate [55]. In contrast, ProY showed a higher concentration of C4 (2.03–2.76 mg/100 g) than control milk, but not significantly higher than TY, in line with what was reported by Chang et al. [56], in which a high content of SCFA was found in ProY containing *B. bifidum* and *L. acidophilus.* SY1 exhibited the highest concentrations of C4—2.97 mg/100 g at 30 days of shelf life, and the final concentration of C4 in the yoghurt with prebiotics of 1% indicates that C4 production is more efficient in that yoghurt. This increase could be due to the presence of oligosaccharides, which could ensure better survival of *L. acidophilus* and *B. bifidum* in yoghurt. The kinetics of butyric production indicated an increasing trend for all samples up to 20 days and then a decreasing trend. This reduction could be related to C4 being converted into other flavour compounds, such as methyl ketones, secondary alcohols, esters and lactones [57].

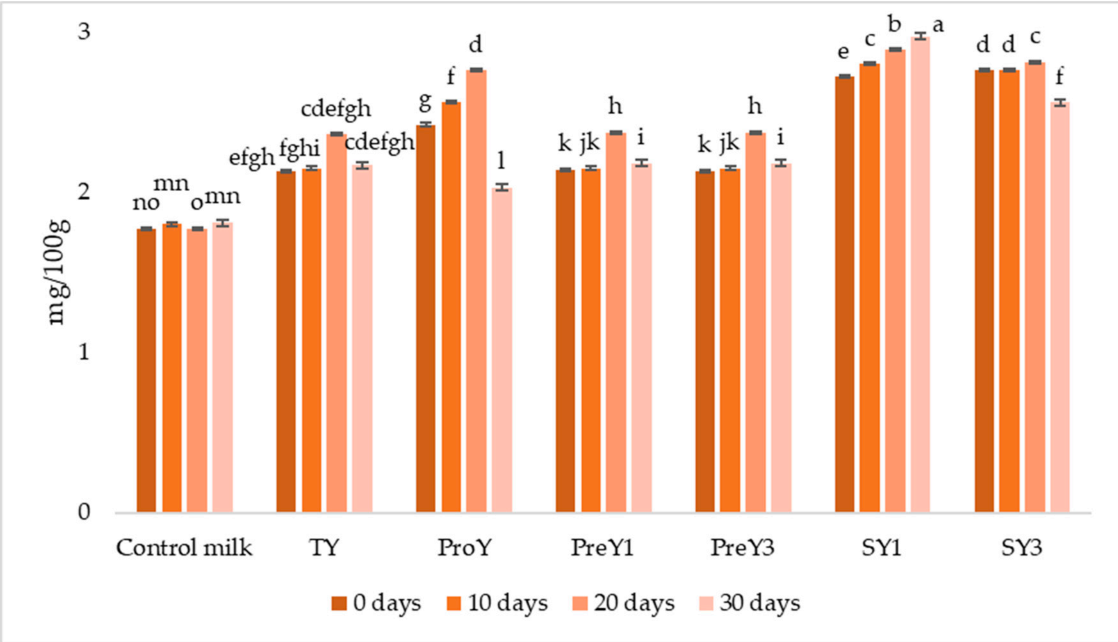

Figure 1. Butyric acid content (mg/100 g) in control milk and yoghurt samples. [a–o] Different letters on bars indicate statistically significant differences (*p*-value < 0.05).

3.5. Pyroglutamic Acid Content

In regard to pGlu content (Figure 2), ProY presented the highest content (136.6 mg/100 g), while milk presented the lowest content (113.5 mg/100 g) due to the absence of lactic ferments, which did not allow the synthesis of pidolic acid. At 10 days, an increase in pyroglutamic acid was visible in all samples due to the gradual conversion of glutamine into the respective lactam and the possible hydrolysis of the latter from the terminal ends of the proteins by the fermenting microorganisms present, as reported by Liu et al. [58]. However, except TY, at 30 days, the concentration of pyroglutamic acid was lower than that at time 0 for all yoghurt types, and was significantly different between all yoghurt types. The reduction in pGlu concentration could be influenced by its conversion to glutamic acid in an aqueous substrate, as reported in the literature. These two organic acids convert reversibly into each other, rapidly depending on the environmental conditions [59]. In probiotic yoghurt (ProY), made by adding the probiotic strains *L. acidophilus* and *Bifidobacteria bifidum* to the starter culture, a higher concentration of pyroglutamic acid was found each time considered for storage at 4 °C. This result is probably due to the addition of probiotic microorganisms inducing an intensification of proteolytic activity [26], contributing to the synthesis of pGlu by extracellular cyclases and its release from the N-terminal protein ends mediated by exopeptidases. However, it is also thought that the antagonistic relationship existing between probiotics and starter cultures indirectly contributed to the variation in pyroglutamic acid content. The starter cultures usually used for the production of yoghurt (*S. thermophilus* and *L. bulgaricus*) cause a lowering of the pH during fermentation, which results in the inhibition of probiotic bacteria [60]. Playne [61] reported that *L. acidophilus* does not grow well below a pH value of 4.0, while Shah and Lankaputhra [62] reported that the growth of *B. bifidum* is impeded at pH values below 5.0. Furthermore, the viability of the probiotic *L. acidophilus* is also compromised by the production of inhibitory substances produced by *L. bulgaricus*, such as H_2O_2 [63]. These factors induce an advancement of probiotic microorganisms towards the stationary phase and the release, following cell lysis, of intracellular exopeptidases and cyclases that contribute to the production of pyroglutamic acid. In particular, exopeptidases remove the molecule from the terminal end of proteins and peptides, while cyclases induce the cyclisation of glutamic acid. An intracellular exopeptidase, the α-aminoacyl-peptide hydrolase, responsible for the main

N-terminal proteolytic activities, was isolated from the microorganism *L. acidophilus* [64]. In the case of SY1 and SY3, the presence of oligosaccharides (inulin and FOS) favours the survival of *L. acidophilus* and *B. bifidum* in yoghurt [65], and in particular, the growth of *B. bifidum* is stimulated [66]. These conditions, attributed to the composition of the growth substrate, counteract the inhibitions induced by starter cultures, delaying the advancement of microorganisms towards the stationary phase and, consequently, cell lysis, which determines the release of the main enzymes responsible for the synthesis of pyroglutamic acid. TY, while having the lowest amount of pyroglutamic acid among all yoghurt types tested, was the only one that had a higher concentration of pyroglutamic acid at the end of storage (125.8 mg/100 g) than at time zero (121 mg/100 g), in agreement with Aiello et al. [15]. The microorganisms of the traditional starter cultures are characterised by an intense proteolytic activity consistent with what was reported by Shihata and Shah [67] and by the presence of specific cyclases that induce the conversion of glutamine into the respective lactam [68].

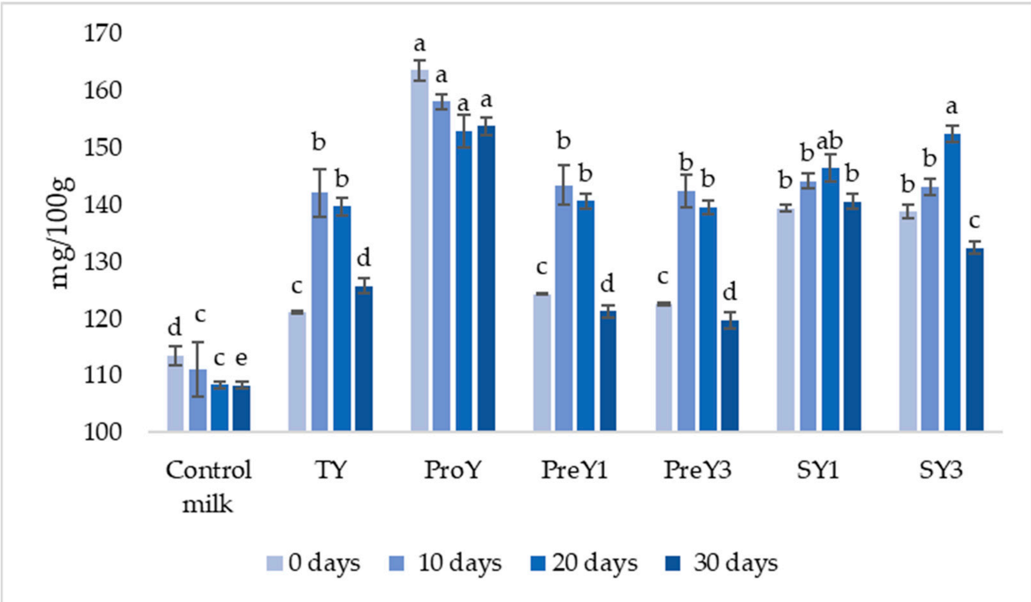

Figure 2. Pyroglutamic acid content (mg/100 g) in control milk and yoghurt samples. [a–e] Different letters on bars indicate statistically significant differences (p-value < 0.05).

4. Conclusions

The beneficial health properties of yoghurt can be associated with two naturally occurring bioactive molecules, pGlu and C4, of which the positive physiological effects on human health are known. The results of this study showed how adding probiotic strains to the traditional starter culture positively influenced the synthesis of pGlu and C4. In particular, probiotic yoghurt showed 10% more C4 content than traditional yoghurt, and this content was also intensified up to 30% by adding fibre to probiotics (synbiotic yoghurt), resulting in favourable growth conditions. Probiotic yoghurt also presented the highest content of pGlu (approximately 130 mg/100 g), while fibre did not affect pGlu content. C4 and pGlu contents generally increased up to 20 days of storage and then decreased up to 30 days of storage. It can be concluded that the production of pGlu and C4 is presumably influenced by the microbiological biodiversity of the samples analysed and by the relationships of symbiosis and antagonism that occur between the microorganisms of the starter cultures. This work can offer opportunities for process optimisation towards nutritional quality.

Further studies on the bioaccessibility and bioavailability of pGlu and C4 taken by these types of yoghurt are necessary to establish the daily intake capable of exerting beneficial health effects.

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