

Article

Seasonal Variations of the Protein Fractions and the Mineral Contents of the Cheese Whey in the Parmigiano Reggiano Cheese Manufacture

Piero Franceschi ¹, Francesca Martuzzi ², Paolo Formaggioni ¹, Massimo Malacarne ^{1,*}
and Andrea Summer ¹

¹ Department of Veterinary Science, University of Parma, Via del Taglio 10, 43126 Parma, Italy

² Department of Food and Drug, University of Parma, Parco Area delle Scienze, 27/A, 43124 Parma, Italy

* Correspondence: massimo.malacarne@unipr.it; Tel.: +39-0521032615

Abstract: The milk whey remaining at the end of the cheese-making process is the main by-product of the dairy industries and it is currently used as a source of high added-value compounds by the food and pharmaceutical industries. The aim of this research was to study the effects of the season on the residual whey characteristics in the Parmigiano Reggiano cheese-making process. Over two years, a total of 288 cheese-making trials of Parmigiano Reggiano PDO (Protected Designation of Origin) cheese were performed in three commercial cheese factories and, in each trial, a sample of the vat milk (V-milk) and of the residual whey (C-whey) were collected. The C-whey values of dry matter and non-fat matter were higher in winter and autumn than in spring and summer. Moreover, the C-whey fat and crude protein contents were also higher in autumn (0.52 and 0.89 g/100 g, respectively) and lower in spring (0.44 and 0.83 g/100 g, respectively) and summer (0.46 and 0.84 g/100 g, respectively). Furthermore, crude whey protein resulted to be the major fraction of crude protein (97.96%). Crude whey protein and true whey protein were higher in autumn and lower in spring and summer and their values mainly depended on milk whey protein. Finally, the C-whey average contents of phosphorus and magnesium were higher in autumn and winter than in summer.

Keywords: Parmigiano Reggiano cheese; cheese whey; cheese whey chemical characteristics; protein fraction; mineral contents; cheese whey seasonal variations; milk seasonal variations



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1. Introduction

Cheese whey is the residual whey remaining in the vat at the end of the cheese-making process. With a world production of about 183 million tons in the year 2020, cheese whey is the main waste of cheese factories [1].

At present, in many countries around the world, European Union included, spilling untreated cheese whey in water bodies or on soil is forbidden [2], since cheese whey promotes eutrophication and acidification in water and in soil [3]. Therefore, the cheese factories' efforts were led to reduce their cheese whey load and to reconsider cheese whey as a by-product.

For this reason, in recent years many efforts have been made to re-evaluate cheese whey and nowadays this is no longer considered as a waste product but as a resource and about 50% of it is currently used by food and pharmaceutical industries as a source of compounds with high added-value [4].

Cheese whey is used both as an animal feed [4–6] and as an important source of valuable raw materials [4,7,8], such as lactose [9], lactic acid [9,10], whey protein of high biological value, mainly lactalbumin and beta-lactoglobulin [11,12], and minerals [4]. In addition, at present, cheese whey is also used as an ingredient in many food preparations, both as it is [4,9], and as concentrate in powders [8,11]. In this second case, one of the

most widespread utilizations of residual whey is the recovery of protein and minerals, concentrated by the ultrafiltration process or reduced into protein powder [4,8,12].

Consequently, due to their economic relevance, the characteristics of cheese whey were significant object of investigation. As a matter of fact, since the economic value of cheese whey mainly depends on the quantitative content of its compounds [7], many studies investigated the chemical composition [9,11,13] and microbiota characteristics [8,9] of cheese whey, the factors affecting them [8,14,15], and their repercussions on the cheese whey quality [8,11,13–15].

In Italy, hard and cooked Protected Designation of Origin (PDO) cheeses such as Grana Padano and Parmigiano Reggiano cheese are produced in high quantities, and the greatest quantity of national milk is destined to their production. As a consequence of their production, the greatest quantity of residual whey was derived.

Parmigiano Reggiano cheese is an Italian hard cooked cheese included in the list of the PDO products. Parmigiano Reggiano cheese is produced starting from partially skimmed raw milk and the cheese-making process of the milk into cheese basically consists in the formation and rapid dehydration, by syneresis, of a rennet–acid curd. To facilitate the syneresis, the curd is broken into granules which are successively cooked at the temperature of about 55 °C [14]. For this reason, the residual whey of Parmigiano Reggiano cheese-making (approximately 1000 kg for each vat) is named cooked whey.

After the cheese-making process, approximately from 10.31% to 27.38% of the milk fat and approximately from 22.44% to 31.59% of the milk proteins remain in the cooked whey [15]; these latter consist mainly of the whey protein of milk [16].

One of the main features of this cheese-making process is the use of a natural whey starter, which is added to the vat milk at the start of the cheese-making process, immediately before its clotting. A natural whey starter is produced directly in the cheese factory by the natural fermentation overnight of 30 kg cooked whey of the previous day's cheese-making. It is rich in thermophilic lactic acid bacteria [17], which are very important, since these bacteria added into the vat milk play a pivotal role for the correct acidification of the cheese wheels during the cheese's first hours of life. This is the driver of the process, preventing the development of abnormal fermentations which could damage the cheese [17].

Moreover, the whey starter addition into the milk, resulting rich in lactic acid, affects the cheese whey properties [17]. As a consequence, the cooked whey at the end of the cheese-making process has a pH value of about 6.25 [9,17], and therefore it can be considered a sweet cheese whey [7].

Since the Parmigiano Reggiano is a rennet-coagulated cheese, the structure of curd consists of a paracasein network that entraps the fat globules [18]. In cheeses produced starting from raw milk, such as Parmigiano Reggiano, the casein content and the integrity of casein micelles are the most important factors conditioning the clotting process and, consequently, the recovery of milk protein and fat in the cheese [18–20], with repercussion on the quantities of fat and protein remaining in the cheese whey [15].

Among the factors influencing milk characteristics, season is one of the most important. Indeed, seasonal variations of milk characteristics were reported in several studies [21–23] and some of them directly investigated the changes in both chemical composition and microbiological characteristics of the milk destined to Parmigiano Reggiano cheese production [24,25].

Nevertheless, there are no studies regarding the season effect on the chemical characteristics of the cooked whey of Parmigiano Reggiano cheese, whereas the importance of these traits stands out considering that in the Parmigiano Reggiano district, cooked whey is commonly paid in accordance with its quality and in particular in accordance with its protein content.

Therefore, in full correspondence with the dairy sector efforts to increase sustainability, with the Union of Nations Sustainable Development Goals and with the European Union directives aiming to prevent food waste and promoting circular economy, the aim of this research was to study the seasonal variations of protein fractions and mineral contents

in the cheese whey derived from the cheese-making process in the Parmigiano Reggiano cheese manufacture.

2. Materials and Methods

2.1. Experimental Design and Sampling Procedure

Throughout two years, a total of 288 Parmigiano Reggiano cheese-makings processes were carried out. Cheese-making was performed in field conditions and in agreement with the Parmigiano Reggiano regulation [26].

Operating in three different cheese factories (CF1, CF2, and CF3) located in the Parma province (inside the Parmigiano Reggiano PDO area), 6, 3, and 3 cheese-makings were followed monthly in the cheese factory CF1, CF2, and CF3, respectively.

Each vat was filled with the milk of one single herd, which remained the same throughout the entire research. All involved herds reared Italian Friesian cows.

A sample of vat milk (V-milk) was taken from the vat, before the whey starter addition, at the start of each cheese-making process [27]. Furthermore, a sample of cooked whey (C-whey) was taken after the extraction of the cheese wheels directly from the vat, following the IDF standard [27].

2.2. Cheese-Making Process

The raw full cream milk obtained from the evening milking was collected directly from the cooling tanks of the farm at a temperature not lower than 18 °C. Afterward, it was delivered to the cheese factory, and it was stored into a creaming tank overnight. During the night, for about 10 h a natural creaming of milk took place. Thus, the morning after, the milk cream was at the top of the creaming tank and the partially skimmed milk was on the bottom of it. The partially skimmed milk was then extracted from the bottom of the creaming tank and placed into the cheese-making vat, while the cream was sold. Afterward, into the vat the raw full cream milk obtained from the morning milking was added. It was collected from the same farm as the one of the evening. From this commingling the “vat milk” was obtained, that is a partially skimmed milk with about 2.6% of fat and with a fat to casein ratio of about 1.1. Successively, the natural whey starter was added to the vat milk (about 3% of vat milk). The natural whey starter was obtained by natural fermentation of a part of the cooked whey that remained from the cheese-making of the previous day. Afterward, 2.5 g/100 kg of milk of calf rennet powder was added to the vat milk, its strength was 1:120000 containing about 98% of chymosin and about 2% of pepsin (Caglio Bellucci Srl, IT-41123 Modena, Italy). Milk clotting happened at approximately 12 min and, afterward, the curd was broken into small grains (around 5 mm of size). Then, the temperature was increased up to 55 °C in about 15 min. This is carried out to cook the curd and to favor a correct purging of the cheese whey from it. After the cooking phase, the cheese was left to settle down at the bottom of the vat and then, after one hour, the cheese mass was extracted from the bottom of the vat and divided into two twin cheese wheels that were placed in molds, where they remained for about two days. Finally, the cheese wheels were salted in brine and placed into the ripening room.

2.3. Analytical Methods

By means of the Kjeldahl method, total nitrogen (TN) [28] and non-casein nitrogen (NCN) [29] were assessed both on V-milk and on C-whey, whereas non-protein nitrogen (NPN) was determined after treatment with trichloroacetic acid (TCA 120 g/L) only on C-whey [30]. From these values, crude protein ($TN \times 6.38/1000$), crude whey protein ($NCN \times 6.38/1000$), casein ($(TN - NCN) \times 6.38/1000$), NPN $\times 6.38$ ($NPN \times 6.38/1000$), true protein ($(TN - NPN) \times 6.38/1000$), true whey protein ($(NCN - NPN) \times 6.38/1000$), casein number ($(TN - NCN) \times 100/TN$), and whey protein number ($NCN \times 100/TN$) were calculated, according to Malacarne et al. [31].

Fat content was assessed on V-milk and C-whey by Mid-IR spectroscopy, by FT-plus of Foss, (Foss Electric, DK-3400 Hillerød Denmark) [32], and by volumetric method of Gerber [33].

Dry matter was assessed on V-milk and on C-whey after drying at 102 °C [34] and ash after calcination in muffle at 530 °C [35].

Moreover, starting from hydrochloric ash solution of V-milk and C-whey, the contents of calcium and magnesium were determined with atomic absorption spectrometry [31] while the phosphorus content was assessed with the colorimetric method proposed by Allen [36].

The pH value was assessed in C-whey samples by potentiometer (Crison Instruments, Alella, Barcelona 08328, Spain), and the titratable acidity values were determined by titration of 50 mL of V-milk or C-whey with sodium hydroxide 0.25 Normal [37] using an electro-titrators Crison Compact Titrator D.

Furthermore, on each sample of V-milk, the rennet coagulation parameters were assessed using Formagraph (Foss Electric, DK-3400 Hillerød, Denmark) [38]. Briefly, into 10 mL of milk at 35 °C, 0.2 mL of rennet with a strength of 1:19000 was added (Chr. Hansen, I-20094 Corsico MI, Italy). The time of analysis was of 30 min and, by observing the graph produced by the device, milk rennet clotting time (RCT), curd firming time (k_{20}), and curd firmness (a_{30}) were assessed. Rennet clotting time is the time passing from the addition of rennet into the milk until the beginning of coagulation; curd firming time is the time taken by the device to draw a graph reaching a width of 20 mm; and curd firmness is obtained measuring the width of the graph expressed in millimeters 30 min after the addition of the rennet.

Finally, the somatic cells count was determined by the fluoro-opto-electronic method with Fossomatic FC (Foss Electric, DK-3400 Hillerød, Denmark) [39].

2.4. Statistical Analysis

Data collected were analyzed by SPSS general linear model procedure (SPSS Statics 27, Armonk, New York 10504-1722, NY, USA), using the following univariate model:

$$Y_{ijk} = \mu + A_i + B_j + \varepsilon_{ijk} \quad (1)$$

where Y_{ijk} is the dependent variable; μ is the average value; A_i is the effect of the season ($i = 1, \dots, 4$; winter, from January to March; spring, from April to June; summer, from July to September, Autumn, from October to December); B_j is the effect of cheese factory ($j = 1, \dots, 3$); ε_{ijk} = residual error.

Furthermore, the significance of differences among the seasons was tested by ANOVA with the Bonferroni post hoc test.

Moreover, the Pearson correlations were calculated among V-milk and C-whey parameters.

According to Malacarne et al. [31], the statistically significant correlations were considered weak with r values less than 0.3, moderate with r values between 0.3 and 0.7, and strong with r values over than 0.7.

3. Results

The ANOVA evidenced that the factors season and cheese factory influenced most of the parameters analyzed here. However, the seasonal trends of C-whey and vat milk were the same in the three cheese factories considered. The differences among cheese factories related to the contents of C-whey and vat milk characteristics due to differences among herds in milk quality and little differences in cheese-making operations among cheese factories, which are typical of an artisanal cheese such as Parmigiano Reggiano. These latter will not be discussed here.

In Table 1, the seasonal variations of chemical composition and of physico-chemical properties of the C-whey are shown.

Table 1. Seasonal variation of chemical characteristics and physico-chemical properties of the cooked whey (least square means values and standard errors).

Parameters		Winter <i>n</i> ¹ = 72		Spring <i>n</i> ¹ = 72		Summer <i>n</i> ¹ = 72		Autumn <i>n</i> ¹ = 72		Overall Mean <i>n</i> ¹ = 288	ES ²	<i>p</i> ³
Dry matter	g/100 g	7.97	c	7.76	b	7.58	a	7.98	c	7.85	0.04	***
Fat	g/100 g	0.45	a	0.44	a	0.46	a	0.52	b	0.45	0.01	***
Solids-not-fat	g/100 g	7.52	c	7.31	b	7.12	a	7.46	c	7.39	0.04	***
Crude protein	g/100 g	0.87	b	0.83	a	0.84	a	0.89	c	0.86	0.01	***
Crude whey protein	g/100 g	0.85	c	0.81	a	0.83	b	0.88	d	0.84	0.01	***
Casein	g/kg	0.20	b	0.22	b	0.15	a	0.16	a	0.02	0.01	**
NPNx6.38	g/100 g	0.24		0.24		0.23		0.23		0.24	0.01	NS
True protein	g/100 g	0.63	b	0.60	a	0.61	a	0.66	c	0.62	0.01	***
True whey protein	g/100 g	0.61	b	0.58	a	0.60	a	0.64	c	0.60	0.01	***
Whey protein number	%	97.68	a	97.42	a	98.12	b	98.28	b	97.96	0.18	**
Casein number	%	2.32	bc	2.58	c	1.88	ab	1.72	a	2.04	0.18	**
Ash	g/100 g	0.55	b	0.55	b	0.54	a	0.55	b	0.55	0.01	*
Ca	mg/100 g	43.62		43.36		43.96		43.69		43.58	0.32	NS
P	mg/100 g	45.80	c	44.71	b	44.28	a	45.55	c	45.05	0.02	***
Mg	mg/100 g	8.22	bc	8.04	b	8.00	a	8.39	c	8.14	0.06	***
Titratable acidity	°SH/50 mL	2.62		2.63		2.63		2.63		2.63	0.01	NS
pH	Value	6.31		6.31		6.31		6.31		6.31	0.01	NS

¹ Number of samples; ² Standard error; ³ Significance of differences. NS: $p > 0.05$; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; a, b, c, d different with $p \leq 0.05$.

Among the considered parameters, the average values of dry matter, fat, solids-not-fat, crude protein, crude whey protein, true whey protein, P, and Mg resulted different among the seasons all with $p \leq 0.001$. Moreover, the average values of casein, whey protein number, and casein number exhibited significant differences throughout the seasons all with $p \leq 0.01$. Finally, the mean values of ash were also different depending on the season with $p \leq 0.05$.

The average values of the C-whey contents of dry matter and solids-not-fat were higher in winter and autumn when compared to the spring and summer ones. In addition, the contents of fat and crude protein were also higher in autumn, when compared to the spring and summer contents. Moreover, the average contents of P and Mg were higher in autumn and winter and lower in summer.

In Table 2, the seasonal variations of chemical characteristics, of physico-chemical properties, and of somatic cell count of the vat milk are shown.

Table 2. Seasonal variations of chemical characteristics, physico-chemical properties, and somatic cell count of the vat milk (least square means values and standard errors).

Parameters		Winter <i>n</i> ¹ = 72		Spring <i>n</i> ¹ = 72		Summer <i>n</i> ¹ = 72		Autumn <i>n</i> ¹ = 72		Overall Mean <i>n</i> ¹ = 288	ES ²	<i>p</i> ³
Dry matter	g/100 g	11.83	c	11.63	b	11.52	a	11.95	d	11.73	0.03	***
Fat	g/100 g	2.74	b	2.61	a	2.57	a	2.82	c	2.68	0.02	***
Crude protein	g/100 g	3.22	b	3.10	a	3.13	a	3.27	c	3.18	0.01	***
Crude whey protein	g/100 g	0.73	c	0.69	a	0.71	b	0.75	d	0.72	0.01	***
Casein	g/100 g	2.50	b	2.40	a	2.42	a	2.52	c	2.46	0.01	***
Casein number	g/100 g	77.39	bc	77.60	c	77.21	ab	77.11	a	77.33	0.09	**
Fat-to-casein ratio	-	1.10	bc	1.09	ab	1.06	a	1.12	c	1.09	0.01	***
Ash	g/100 g	0.73	bc	0.73	ab	0.72	a	0.73	c	0.73	0.01	**
Ca	mg/100 g	118.54	ab	117.24	a	119.90	b	122.55	c	119.59	0.73	***
P	mg/100 g	90.08	b	87.33	a	86.98	a	90.10	b	88.62	0.32	***
Mg	mg/100 g	10.77	ab	10.53	a	10.48	a	11.03	b	10.67	0.12	***

Table 2. Cont.

Parameters		Winter n ¹ = 72		Spring n ¹ = 72		Summer n ¹ = 72		Autumn n ¹ = 72		Overall Mean n ¹ = 288	ES ²	p ³
Titratable acidity	°SH/50 m	3.32	b	3.28	a	3.26	a	3.29	ab	3.29	0.01	**
Clotting time (RCT)	minutes	18.17	ab	17.84	a	19.49	c	18.59	b	18.52	0.23	***
Curd firming time (k ₂₀)	minutes	6.32	a	6.10	a	8.80	b	6.80	a	7.01	0.30	***
Curd firmness (a ₃₀)	millimeters	28.00	bc	28.41	c	22.27	a	26.37	b	26.25	0.62	***
Somatic cell count	10 ³ Cells/mL	136	a	162	ab	186	b	176	b	165	4.42	***

¹ Number of samples; ² Standard error; ³ Significance of differences. ** p ≤ 0.01; *** p ≤ 0.001; a, b, c, d different with p ≤ 0.05.

The V-milk average values of dry matter, fat, crude protein, crude whey protein, casein, fat-to-casein ratio, Ca, P, and Mg were different among the seasons with p ≤ 0.001. The values of RCT, k₂₀, and a₃₀ as well as the milk somatic cell contents exhibited differences among the seasons, all with p ≤ 0.001. Moreover, the average values of casein number and titratable acidity and the average content of ash exhibited differences, also with p ≤ 0.01.

The V-milk produced during the autumn showed the highest average values of dry matter, fat, crude protein, casein, fat-to-casein ratio, ash, P, and Mg. On the other hand, the lowest values of the same parameters were recorded in summer.

Moreover, also during the summer season, V-milk exhibited the longest RCT and k₂₀ and the lowest values of a₃₀.

The lowest values of somatic cell count were recorded in winter, when the values of titratable acidity were higher than those shown in spring and summer.

Furthermore, V-milks collected in spring and summer were, also, characterized by lower average contents of ash, P, and Mg.

In Table 3, Pearson correlation coefficients between the V-milk and the C-whey chemical characteristics are reported.

Table 3. Pearson correlation coefficients (r) among the vat milk and cooked whey chemical characteristics. Only significant correlations (p ≤ 0.05) are reported.

Milk Parameters	Cooked Whey Parameters											
	Crude Protein		Whey Protein		Fat		Ca		P		Mg	
	r	p ¹	r	p ¹	r	p ¹	r	p ¹	r	p ¹	r	p ¹
Crude protein	0.691	***	0.711	***			0.282	***	0.334	***	0.342	***
Whey protein	0.674	***	0.688	***					0.172	***	0.306	***
Fat	0.420	***	0.406	***	0.281	***	0.277	***	0.101	**	0.298	***
Ca	0.280	***	0.282	***			0.210	*			0.308	***
P	0.475	***	0.491	***			0.192	***	0.471	***	0.351	***
Mg	0.221	**	0.229	**			0.150	*			0.460	***

¹ Significance of correlation: * p ≤ 0.05; ** p ≤ 0.01; *** p ≤ 0.001.

The C-whey content of crude protein resulted positively correlated with moderate r values in the V-milk contents of crude protein, of crude whey protein, of fat, and of P and with weak r values in the contents of Ca and Mg.

Furthermore, the C-whey contents of crude whey protein resulted positively correlated with strong r values in the V-milk contents of crude protein, with moderate r values in the V-milk contents of crude whey protein, of fat, and of P and with weak r values in the contents of Ca and Mg.

Moreover, the C-whey content of fat resulted positively correlated with weak r values in the contents of fat in V-milk.

Among the considered minerals, the C-whey content of Ca were positively correlated, with weak r values, with the V-milk contents of crude protein, fat, Ca, P, and Mg. The C-whey content of P resulted positively correlated with the V-milk contents of crude protein and P with moderated r values, and with the crude whey protein content with weak r values.

The C-whey content of Mg was positively correlated with moderated r values with the V-milk contents of crude protein, crude whey protein, Ca, P, and Mg. Moreover, the C-whey content of Mg correlated positively with weak r values in the content of fat in V-milk.

4. Discussion

The overall means of protein and fat contents of the C-whey are in agreement with those reported in the literature. For example, Prazeres et al. [40] in a review reported that, in general, sweet cheese whey contains from 0.6 to 1.1% crude protein and from 0.06 to 0.5% fat.

In particular, the average values of C-whey crude protein and fat contents are in agreement with those shown by Malacarne et al. [41] in a study conducted in ten different cheese factories. They reported average values of 0.82 g/100 g for crude protein and of 0.49 g/100 g for fat.

Crude whey protein was the major constituent among protein fractions, amounting to 97.96% of crude protein, whereas casein (2.04%) represents only a minor fraction of crude protein components.

Furthermore, the overall means of ash content are also in agreement with what is reported in the literature for sweet cheese whey. Recently, Osorio-González et al. [7] reported, for sweet cheese whey obtained by cow milk, an average value of 0.6% of ash content. In addition, among the main minerals of cheese whey, the overall content of Ca and P are also in agreement with those reported by Malacarne et al. [41], who found average values of Ca and P of 40.68 and of 43.83 mg/100 g, respectively, in the Parmigiano Reggiano cheese whey.

In general, C-whey in winter and autumn was characterized by higher values of dry matter and solids-not-fat when compared to those of spring and summer. Among the constituents of the dry matter, the contents of crude protein, crude whey protein, true whey protein, fat, P, and Mg were higher in autumn than in spring and in summer.

This is mainly due to the higher contents of these compounds in the V-milk of autumn with respect to the V-milk of spring and summer.

This is confirmed by the positive moderate correlation among the C-whey crude protein content and the V-milk crude protein and whey protein contents, by the strong positive correlation among the C-whey crude whey protein content and the V-milk crude protein and whey protein contents and, also, by the weak correlation between the C-whey and V-milk fat contents.

Furthermore, it should be considered that the C-whey fat content was strongly influenced by the value of V-milk fat-to-casein ratio [15]. This last parameter was higher in autumn and lower in summer.

However, it is noteworthy that both the autumn and summer average values of the V-milk fat-to-casein ratio should be considered within the normal range in the Parmigiano Reggiano production. For example, Franceschi et al. [16], in a study conducted on 48 cheese-making processes, reported average values of the V-milk fat-to-casein ratio that ranged from 1.05 to 1.16.

Moreover, the C-whey Ca, P, and Mg contents mainly depended on their contents in V-milk, but also, on the V-milk crude protein content.

The higher contents of V-milk crude protein, crude whey protein, and fat in autumn compared to the spring were likely mainly due to the effects of the lactation stage of cows.

Indeed, Summer et al. [25] reported that in the Parmigiano Reggiano area, during the spring and the autumn seasons, most cows are in the early and late lactation stages, respectively.

Moreover, many authors observe that the beginning of lactation of the cow is characterized by increased milk production and by low contents of fat and protein in the yielded milk and that afterward, during the lactation period, their contents progressively increase [25,42–44].

On the other hand, during the late lactation stage, cows are characterized by a decrease in milk production and their milk shows an increase in fat and protein contents [22,23,25,44].

The lower contents of V-milk crude protein, crude whey protein, and fat of summer V-milk, when compared to that collected in autumn, are due instead to the effect of the season.

Indeed, several studies carried out on milk samples collected in the Parmigiano Reggiano area confirm a decrease in protein and fat contents in milk and a worsening of its rennet coagulation parameters during the summer season [24,25,45].

The effect of the season can be explained because, in the Parmigiano Reggiano area, the summer is characterized by average values of temperature and humidity provoking heat stress in cows [24,45,46]. It is well recognized that heat-stressed cows produce less milk, with a reduction in fat and protein contents and with a worsening of its rennet coagulation properties [24,45,46].

For example, Bertocchi et al. [24], in a study that involved 508,613 herd samples of milk, showed an average value of milk fat content of 3.75 g/100 g in summer and 3.85 g/100 g and 3.97 g/100 g in spring and autumn, respectively. These observations were confirmed by Bernabucci et al. [45], that reported a decrease in milk fat content in summer (3.20 g/100 g) with respect to the winter and spring values (3.80 and 3.61 g/100 g, respectively).

Moreover, the same observation made about the milk fat content can be made for the milk protein compounds. For example, Cowley et al. [47] reported differences between casein content of milk produced by heat-stressed cows when compared to those raised in comfortable temperatures (26.8 vs. 28.1 g/L, respectively). This finding is in agreement with that reported by Bernabucci et al. [45], who reported higher milk casein content during winter and spring (2.75 and 2.48 g/100 g, respectively) than in summer (2.27 g/100 g). These decreases in milk fat, protein, and casein contents are due, as reported by Summer et al. [46] and by Cowley et al. [47], to the heat stress of the cows, causing a reduction in feed intake by the animals.

Furthermore, the milk produced in the summer season is characterized by a lower content of ash and P as well. This change in milk during summer is related to the increase in milk somatic cells content [48].

Both Summer et al. [25] and Bernabucci et al. [45], indeed, reported that in northern Italy, during the summer, there is an increase in the milk somatic cells count in comparison to the other seasons, and they also reported that this somatic cell count increase is the result of mammary gland inflammation processes.

In this case, the response to the gland inflammation processes is characterized by a diffusion of some components from the blood into the milk [49,50], as, for example, sodium chloride. Furthermore, the inflammation process is also characterized by a decrease in the contents of some milk components, such as casein and P [49,51], caused by a decrease in the secretory activity of the mammary gland and by the increasing activity of proteolytic enzymes [50,52].

Furthermore, since the V-milk contents of casein and P are highly correlated with the titratable acidity value [53], the decrease in these first two components causes the consequent decrease in the latter one.

In addition, since the milk P content and titratable acidity values are correlated with the milk rennet coagulation properties [38], the decrease in P content and titratable acidity value are therefore related to a worsening of the enzymatic milk coagulation properties.

With regard to C-whey content of Ca, P, and Mg, it is important to emphasize that these elements in milk are mainly divided into two different phases. About two-thirds of Ca, half the content of P, and one-quarter of Mg are in the colloidal phase as a constituent of the casein micelles, while the remaining part of them is in solution [14,38].

During the cheese-making process, there is a partial solubilization out of Ca, P, and Mg from the casein micelles into the C-whey, as a consequence of the acidification of V-milk induced by the addition of the natural whey starter [54].

However, the main part of Ca, P, and Mg remains in the casein micelles and participates in the constitution of the cheese [18,55].

Thus, the Ca, P, and Mg remaining in the C-whey are mainly constituted by the fractions of these elements that were in the V-milk solution phase and by those solubilized from the casein micelles after the addition of the natural whey starter [15].

5. Conclusions

In conclusion, the seasonal variations of the protein fraction and of the mineral contents of the cooked whey are significant, and their variability mainly depends on the variability of the milk chemical composition from which cheese whey derives.

Indeed, the seasonal variations in milk's chemical characteristics and in its physico-chemical properties strongly affect the milk rennet coagulation, which in turn influences the recovery of matter from the milk into the cheese. Therefore, the season affects the quantity of fat, proteins, and mineral salts in the cooked whey, and these constitute the main components of its dry matter.

Furthermore, the composition of milk, in turn, is strongly influenced by the physiological conditions of the cows that produce it, which in turn are influenced by the environmental conditions imposed by the seasons.

In other words, the cooked whey composition strictly depends on milk characteristics and, overall, since the season affects the milk chemical composition and its coagulation properties, the seasons exert influence on the cooked whey chemical composition as well.

Finally, since the cooked whey produced in autumn has about 18% of fat and 10% of true whey protein, more than the one produced in the spring, it could have higher economic value. Therefore, a payment system based on quality may also be introduced for cooked whey, similar to the one that already exists for the payment of the milk, to reward the cooked whey of higher quality.

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