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A study on the quality of bovine colostrum: physical, chemical and safety assessment

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Abstract

Colostrum, as a bioactive substance for humans, could be a functional food ingredient ; today colostrum products are the object of health claims, even on line, especially from some foreign Countries. The aim of the present paper is to assess the quality of bovine colostrum samples taken in some farms in Sicily and in Calabria, in view of lack of information on physical and chemical and safety criteria on this product for human consumption. Comparing the overall picture of parameters under study, a relationship between these criteria was not easy, due to several factors, as for example animals breed, diversified farms management and other intrinsic and extrinsic additional factors relating to colostrum sampling. The aspects observed in the present study would suggest a great accuracy by competent authority during the qualitative controls of raw colostrum.

Introduction

In the last decades, numerous changes have characterized the economic and social development and they have been followed by significant modification in consumer habits, in particular in the dynamics of food consumption. The market of some food, therefore, has a phase of fast growing: traditional and organic products, fair trade products and food with an high added value and service content. Together with these products, it is necessary to take into account a growing demand for more and more specific foods, such as the so-called "functional foods". A functional food may be: i) a food enriched with some components that produce a benefit (for example probiotics, selected bacterial flora with a positive effect on bowel function); ii) a food in which one or more components have been modified to induce a benefit to the health condition of consumer (e.g. protein hydrolysates added to infants formulas, to reduce the allergic risk); iii) a product in which the bioavailability of one or more of its components have been enhanced with the aim to increase the assimilation of a component with beneficial properties, etc.

Moreover, scientific research is taking attention on numerous biological activities, that are linked to milk derivatives (Mills *et al*., 2001). In recent years, bovine colostrum has become popular as a product for human consumption, because it is an excellent source of bioactive proteins. The latter would have the ability to prevent bacteria and viruses, and to improve the gastrointestinal and body condition (Houser *et al*., 2008). Really, the exploitation of the beneficial properties of colostrum is not a new concept. In fact, in India, where cows are sacred, colostrum is distributed to the homes with milk. The population benefits of colostrum therapeutic action to treat flu symptoms in the elderly. This custom began thousands of years ago with the activity of Ayurvedic physicians and with the so-called "holy healers", known as "Rishis". In the Scandinavian towns the colostrums was used to cook a pudding to celebrate a calf birth. At the end of XVIII century some researches proved the benefits of colostrum on the cattle life; this was the first step for its therapeutic use in humans (Keinsmith, 2011).

Nowadays it is possible to buy the pure colostrum or the products colostrum based, mixed with other components in lyophilized or in liquid form. It is also possible to buy on line some supplements based on bovine colostrum; some products based on goat colostrum also exist. Human colostrum cannot be put on the market; so the cattle colostrum is used as a food supplement for human consumption (Li and Aluko, 2006). The marketing of these product must take into account the quality control of raw material

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and the production chain, till product sale. The importance of the product control was highlighted in the Commission Regulation (EC) No 1662/2006; together with this rule, the Commission Regulation (EC) No 1663/2006 was issued (Official Journal of the European Union No L 320 18.11.2006). The aspects concerning colostrum production were integrated with the Regulation (EC) No. 1662/2006 where this fluid " is considered as a product of animal origin but is not covered by the definition of raw milk" as referred in the Annex I of Regulation (EC) No. 853/2004 (Official Journal of the European Union No. L226, 25.6.2004). Furthermore, colostrum "is produced in a similar way and can be considered as presenting a similar risk to human health as raw milk. It is therefore necessary to introduce specific hygiene rules for colostrum production". In particular it is useful to report some essential requirements of Regulations (EC) No. 1662/2006 and N. 1663/2006. Commission Regulation (EC) No. 1662/2006 shows the following definitions:

1. "Colostrum" means the fluid secreted by the mammary glands of milk-producing animals up to three to five days post parturition that is rich in antibodies and minerals, and precedes the production of raw milk.
2. "Colostrum-based products" means processed products resulting from the processing of colostrum or from the further processing of such processed products.

Criteria for raw milk and colostrum are also mentioned; particularly:
" National criteria for colostrum, as regards plate count, somatic cell count or antibiotic residues, apply pending the establishment of specific Community legislation". Requirements for heat treatment are also defined :" When raw milk, colostrum, dairy or colostrum-based products undergo heat treatment, food business operators must ensure that this satisfies the requirements laid down in Chapter XI of Annex II to Regulation (EC) No 852/2004. In particular, they shall ensure, when using the following processes, that they comply with the specifications mentioned: (a) pasteurization is achieved by a treatment involving: (i) a high temperature for a short time (at least 72 °C for 15 seconds);
(ii) a low temperature for a long time (at least 63 °C for 30 minutes); or (iii) any other combination of time-temperature conditions to obtain an equivalent effect such that the products show, where applicable, a negative reaction to an alkaline phosphatase test immediately after such treatment".

REGULATION (EC) No 1663/2006, on specific rules for the organization of official controls on products of animal origin intended for human consumption, specifies this concept: "If the food business operator has not corrected the situation within three months of first notifying the competent authority of non-compliance with the criteria with regard to plate count and/or somatic cell count, delivery of raw milk and colostrum from the production holding is to be suspended or — in accordance with a specific authorization of, or general instructions from, the competent authority — subjected to requirements concerning its treatment and use necessary to protect public health. This suspension or these requirements are to remain in place until the food business operator has proved that the raw milk and colostrum again complies with the criteria" .

Table 1. Composition of bovine colostrum

Hours after birth	Total nitrogen (%)	Fat (%)	Lactose (%)	Total solids (%)
0	17,57	5,10	2,19	26,99
6	10,00	6,85	2,71	20,46
12	6,05	3,80	3,71	14,53
24	4,52	3,40	3,98	12,77
36	3,98	3,55	3,97	12,22
48	3,74	2,80	3,97	11,46

(by Kleinsmith, 2011, modified)

From the above mentioned (EC) Regulations it is clear that the control of the cattle colostrum at primary production is one of the purposes of the competent authority in order to grant the quality of raw food and its derivatives. Despite the Regulation (EC) No. 1662 refers to " national criteria for colostrum, as regards plate count, somatic cell count or antibiotic residue", in Italy a lack of national legislation on physical, chemical, hygienic and sanitary requirements for the product for human consumption was underlined. As a consequence of this, the aim of this study is to make a screening on cow colostrum samples taken in some farms of Sicily and Calabria. Some brief notices on colostrum will be given, as follows. The cattle colostrum composition modifies gradually after birth because it is reabsorbed by cow; this happens soon after the birth (Table 1). The best colostrum has a high concentration of active biological substances (Kleinsmith, 2011). Immune factor, immune - regulator substances and growth factors are biological active components of colostrums. These factors can be useful for the organism against the external agents (virus, bacteria, mycetes, protozoa etc.). Among them we find: immuno globulins, polipeptides with high content of prolin, lactoferrin, cytokines; some enzymes, glycoproteins, trypsin inhibitors, lysozyme, limphokynes, oligo-polysaccharids and glycoconjugates. Immune-globulins are considered

Table 2 . Comparison between bacterial number in colostrum samples from farms in Pennsylvania, together with some favorable limits for bacteria in calves fed with milk

Microrganisms	Limits (CFU/ml)	Bacterial load in colostrums samples (CFU/ml)			
		Standard	*Mean*	*Minimum*	*Maximum*
Standard plate count	<20,000	997,539	15,300	140	9,070,000
Coliforms	<100	323,372	600	0	3,950,000
Staphylococcus aureus	0	306	0	0	12,000
Streptococcus agalactiae	0				
Salmonella[4]	0				
Environment Streptococci	<5,000	256,722	2,140	0	5,600,000
Environment Staphylolococci	<5,000	164,963	2,260	0	3,980,000
Non coliforms	<5,000	111,544	360	0	3,000,000

(by Heinrichs J. and Jones C., 2011, modified) - [4] On 55 samples, in 8 (15%) *Salmonella* spp was isolated.

the most important component of colostrums: the IgG amount has very high amount; IgM, IgA, IgD and; IgE follow (Sgroiforossi *et al*., 2009)

Usefulness of bovine colostrum for humans

Colostrum properties could be fully exploited if its components reach as such in human gut; in fact, the majority of pathogens act by clinging to the intestinal mucosa. Colostrum ingestion contributes to the improvement of health status and wellness in healthy humans and it assists the healing of subjects with an immune deficiency status.

Furthermore, it contributes the healing during flu, Candidiasis, diarrhea and gastro-enteric illness with bacterial, viral and parasitic origin. *Clostridium difficile*, *Salmonella* sp, *Shighella* spp., *Campylobacter* spp., *Vibrio colerae* etc; rotavirus, *Cryptosporidium parvum*, *Entamoeba histolytica*, *Giardia lamblia* seem to be sensible to colostrum. It is also useful against Herpes virus I and II and against gastric ulcer caused by *Helycobacter pylori* 4lifeitalia.org/images/colostro.pdf). Some colostrum antibodies are active against the virulence factors produced by enterohaemorrhagic strains produced by *Escherichia coli*, particularly Shiga – like toxin, intimin, haemolysine (Huppertz *et al*., 1999). The administration of colostrum would be useful also during some autoimmune pathologies, such as rheumatoid arthritis, some type of asthma and allergies, lupus eritematosus, multiple sclerosis, fibromyalgia. During Chronic Fatigue Syndrome, Epstein- Barr viral pathology, colostrum could have a positive effect for its immuno stimulating action and because it is active against viruses. The usefulness of colostrums is documented also for athletes, due to the presence of IGF- I and IGF II. In fact, the amino acids sequence of these substances is very similar to insulin sequence. IGF- I and IGF II could be considered as proinsulin hormones, with anabolic effects (Valla and Campus, website: *4lifeitalia.org/images/colostro.pdf*).

Colostrum and pathogens

Colostrum could be a vehicle of some pathogens for animals and/or human health, such as *Salmonella* (S.) dublin and *S. typhimurium* (Palmer and Mudd, 1974). As a consequence of a cross contamination or after an inadequate stockage it can contain Mycobacterium (M) bovis, *Mycobacterium avium* subsp. paratuberculosis, *Mycobacterium californicum*, *Escherichia coli*, Salmonella spp., Listeria. monocytogenes and others could be contaminants of colostrum. Pasteurization at 72°C for 15 seconds could reduce the IgG content, as a consequence of protein denaturation (Heinrichs and Elizondo, 2009). Recently, Heinrichs e Jones (2011) referred the results of microbiological examination of colostrum samples (Table 2).

Materials and Methods

Colostrum samples were collected from farms of dairy cows, mainly Italian Frisona breed. The farms, both with semi-intensive and intensive breed, were located in Sicily, in the provinces of Palermo, Siracusa and Ragusa and in Calabria, in the province of Catanzaro (Italy). Thirty individual samples were collected after 2-3 days postpartum; then they were placed in sterile pots and carried at laboratory at refrigeration temperature. The laboratory tests included the measurement of the following physical and chemical parameters:

- pH, by potentiometric method (pHmeter 330i/set, WTW, Germany).
- percentage of fat (GRS), total nitrogen (PRT), lactose (LTS) and dry matter (DM), by Lactostar (Funke Gerber, Germany).

Hygienic and sanitary criteria were also considered: samples were examined for somatic cell count (SCC) by DeLaval cell counter DCC (De Laval, Italy). Microbiological counts were performed with the following procedure:

- preparation of serial dilution with sterile buffered peptone water (1:9 ratio)
- determination of standard plate count at +30°C (SPC),
- total psychrophilic aerobic count (PAC) and agar plates incubation at +7° C for 10 days
- total and fecal coliforms, by MPN (Most Probable Number)
- coagulase-positive (BP +) and coagulase-negative (BP-) staphylococci count with Baird Parker.

The analysis were performed according to the official methods of analysis APHA (American Public Health Association). Sulphite-reducing bacteria (SR) were performed as follows: preparation of serial dilution of colostrum sample with Reiforced Clostrial Medium (RCM, Oxoid) (1:9 ratio); pour plate technique was applied for analysis, using Sulphite Polymixin Sulfadiazine medium (SPS medium Oxoid); plates were incubated in anaerobic atmosphere at +37°C for 48 hours; samples collected in Calabria farms were not examined for SR. *Salmonella* spp. was determined according to the official methods of analysis APHA (American Public Health Association); Rappaport Vassiliadis broth (Oxoid) and Selenite Cystine broth (Oxoid) for the selective enrichment were used, with an incubation at +37°C and +42°C, respectively. Enrichment streaking onto XLD Agar (Xylose Lysine Desoxycholate Agar, Oxoid) was performed, followed by incubation at 35° ±37°C for 24 hours. After the examination of plates, the tests for the identification of any strain of Salmonella spp. were arranged. Statistical analysis of data was performed by Spearman's rank correlation coefficient; for bacteriological criteria, the correlation was applied to the data from SPC, PAC and SCC .

Results and Discussion

The results of chemical-physical evaluations are presented in Table 3. The results were grouped upon some ranges of values for their better understanding. It was not found a great variability of pH values. In fact, the pH mean values was in agreement with the data reported for the so called milk "of the first milking" (Agraria.org; website: *http://www.agraria.org/industrie/marlatte.htm*). Colostrum pH values, lower than milk values, were related to the high proteins content, that is usually revealed in colostrum obtained 2-3 days after birth. Statistical analysis showed a significant negative correlation ($p < 0.01$) between pH, PRT and LTS amount. This has encouraged the assumption about the influence exerted by proteins also on the pH values even for colostrum. As for milk, even for the colostrum, acid groups could be carried mainly by casein.

The negative correlation for LTS was explained with the fermentative potential of this disaccharide. GRS, PRT and DM percentage showed a moderate variation between samples; whereas for LTS content this trend was less noticeable. Moreover, mean values for GRS and PRT were mostly similar to the data referred to the colostrum samples collected in farms in Pennsylvania (Heinrichs and Jones, 2011). According to the data reported in the present work, and to statistical results, no correlation was showed between the two parameters. Mean, minimum and maximum values for LTS (Table 4) were quite variable, with rather low amounts. A para-physiological status of mammary gland, during a particular physical stressing event, as birth, affecting the gland secretory

Table 3. Range of values for physical and chemical parameters of colostrum

pH	Samples n. (Sn)	GRS (%)	Sn	PRT (%)	Sn	LTS (%)	Sn	DM (%)	Sn
5.0-5.97	8	2.7-5.83	13	7.75-9.96	11	1.28-1.84	17	4.28—5.91	15
6.0-6.33	15	6.19-6.9	4	10.03-11.75	7	1.93-2.88	11	6.08-8.28	7
6.37-6.7	7	7.48-8.47	6	16.01-21.85	12	2-67-3.59	2	8.6-11.99	8
		10.39-18.40	7						
Total S.	**30**		**30**		**30**		**30**		**30**

Table 4. Mean, minimum and maximum of physical and chemical parameters of colostrum samples

CRITERIA	*MEAN*	*MINIMUM*	*MAXIMUM*	*±S.D.*
pH	6,056	5,03	6,55	2,13
GRS (%)	7,86	2,55	16,09	4,43
PRT (%)	12,20	8,85	21,85	5,70
LTS (%)	2,04	1,46	3,19	0,90
DM (%)	6,96	4,28	12,05	3,18

function, could have caused a LTS content reduction. The correlation between LTS and PRT was highly significant ($p<0.001$). Both substances are synthesized in the mammary gland; therefore its dysfunction had an impact on both parameters. In addition, the average PRT percentage were considered mostly comparable to the values reported by Montuoro (2005/2006); on the contrary, the GRS content in all samples was higher than values indicated by the same author. It useful to underline that high levels of total nitrogen were attributed to a strong immunoglobulins increase in colostrum samples. A highly significant negative correlation ($p <0.001$) between DM, PRT and LTS contents was found. PRT and LTS have a certain influence on DM values, particularly the LTS with its very low amount.

Comparing the overall picture under study, a relationship between the various parameters was not easy, due to the differences between several factors, as for example animals breed, diversified farms management and other intrinsic and extrinsic additional factors relating to the colostrum sampling.

In Table 5 bacteriological results are showed; they are summarized as for physical and chemical results. Standard plate count was very high in 7 samples (7 log10 CFU/ml), although mean values were 6.87 log10 CFU/ml. This situation was probably the consequence of a lack of hygiene conditions during colostrum production or to rearing system in some farms. Maximum and minimum (tab. 6) values were consistent with the results reported by Heinrichs and Jones (2011) for colostrum samples tested in Pennsylvania (Heinrichs and Jones, 2011); the results also partially agreed with the results by Sgoiforossi *et al*. (2009) . Psychrophilic aerobic bacterial count was very high (6 log10 CFU/ml – 7 log10 CFU/ml) in 6 samples; mean values were high (6.51 log10 CFU/

Table 5. Range of values for physical and chemical parameters of colostrum samples

(log_{10}CFU/ml)					(log10 MPN/ml)		
SPC	Samples n. (Sn)	PAC.	Sn	BP (C+)	Sn	BP (C-)	Sn
2.43-4.23	9	<1-3.77	8	<1	17	<1	13
4.8-5.73	12	3.9-4.8	10	3.11-4.17	3	2.3-3.60	7
6.3-7.84	9	5.53-7.60	9				
		n.d.	3				
Total s.	30		27		30		30

(log10 MPN/ml)				(log10 MPN/ml)	
TC	Sn.	FC	Sn.	SCC	Sn.
0-7	9	0-4	9	3.47-5.05	6
20-40	3	20-90	6	5.27-5.88	12
150-500	5	200-500	9	6.04-6.55	10
>1100	12	>1100	2	n.d.	2
n.d.	1	n.d.	4		
Total S.	**30**		**26**		**28**

Table 6. Mean, minimum and maximum values of hygienic and sanitary criteria of colostrum samples

	SPC	PAC	BP (+)	BP (-)	TC	FC	SCC
	(log_{10}CFU/ml)				log10 MPN/ml		log10 cells/ml
MEAN	6,87	6,51	2,60	2,82	2,71	2,34	2,95
MIN	2,43	1	1	1	0	0	3,47
MAX	7,84	7,60	3,34	4,17	3,04	3,041	5,57
±s.d.	1,630	2,138	0,77	0,88	520,95	310,47	0,79

ml), although the samples were not refrigerated for a long time. High PAC rates could show a lack of hygiene in the farm environment. It is not negligible to consider, moreover, that psychrophilic bacteria can have a detrimental effect during the refrigerated storage of the product; this would be a significant factor when colostrum is frozen, as Regulation (CE) No. 1662/2006 stated. Therefore, thawing before colostrum processing would require the implementation of specific prevention, due to the ability of these microorganisms to produce proteolytic and lipolytic enzymes. Furthermore, some psychrophilic bacteria could be foodborne pathogens; in this case if a raw product is ingested it could be potentially harmful. Acceptable values for coagulase positive staphylococci was considered a positive factor; in 3 samples *Staphylococcus aureus* were isolated at very low values; in 1 sample *Staphylococcus epidermidis* was isolated. This report, therefore, did not give particular concern. The same consideration may be valid for coagulase negative staphylococci.

A low staphylococci count could be related to the colostrum defense activity, as underlined also by Kleinsmith (2011). This author considered this activity also against *Escherichia coli*, *Salmonella* spp., and *Mycobacterium paratuberculosis* (Kleinsmith, 2011).

A high variability in the number of total coliforms, as for fecal coliforms was observed; the bacterial loads were frequently fairly high. From these results it was assumed that samples were subjected to an environmental contamination; they were also subject to a fecal contamination, that was frequently found in some samples. This condition can be explained by poor implementation of good manufacturing practices.

A high SCC in the majority of examined samples was found; this arose a great doubt, although these levels were comparable to the data reported by Sgoiforossi *et al*. (2001) These count was low in a very few samples examined for the present study. The high SCC could have been caused by a stress situation during cows birth, as a consequence of increased susceptibility to the udder infection, especially in the animals whose birth was difficult, even in situations with no particular risk (Sgoiforossi *et al*., 2009).

This hypothesis was not supported by the analysis for mastitis diagnosis for the present study. The absence of *Salmonella* spp. and sulphite-reducing anaerobic bacteria was a positive factor; a potential risk related to the presence of pathogens and / or environmental contaminants spores were excluded, even if environmental spore - producing bacteria are usually very common in the farms environment. Statistical analysis showed a highly significant correlation ($p < 0.001$) between SPC and PAC bacterial loads. This has been easily understood because these bacterial groups could have a common origin. The absence of correlation between SPC and SCC, already reported in the literature (Ying *et al*., 2002), would have been the consequence of different characteristics of the colostrum and it could have been related to the different samples origins.

Conclusions

The data presented in this work were not easily compared with the indices reported in the literature, due to the different and variable management conditions between farms.

Despite these difficulties, this study has enabled to define some interesting aspects about bacteriological picture of the examined samples. Furthermore, qualitative findings reported by other authors were referred to colostrum that was used for calves feeding after birth. Nevertheless, this work revealed some interesting aspects. The first consideration concerned the bacterial loads for SPC, PAC and for SCC because their amount was often very high. These values were different from those indicated by Catellani *et al*. (2011) for water buffaloes colostrum; the authors showed mean values 1.4×10^4 CFU/ml for SPC; medium total coliforms were 30.4x10 CFU/ml and fecal coliforms were 2.3×10^2 CFU/ml. Some critical aspects showed in the present study must lead to a greater accuracy in the qualitative controls of raw colostrum by the competent authorities. The farms in which these discrepancies were found need some interventions aimed at optimizing all management aspects. Therefore, colostrum collection, its handling and stockage would require a good care in view of its destination both for calves feeding and for human. Kleinsmith (2011) stated that a good quality colostrum should be collected in very strict conditions. This assertion is fully share. In the opinion of this author, these conditions require that: i) the colostrum is used only when collected only from cows that have given birth two or three times to maximize the bioactive substances quality and to ensure a broad range of antibodies; ii) the colostrum is not derived from breasts affected by inflammation; iii) the product should not contain blood, mucus, aggregates of somatic cells or filamentous material, foreign materials and it should not have discoloration. In addition, the colostrum should be frozen immediately after collection and transferred as soon as possible to the processing plant. In fact, if colostrum is held as liquid product the growth of some spoilage bacteria, and probably also high levels of pathogens, would be encouraged. The application of heat treatment would not safe for a colostrum with bad microbiological conditions to improve its status. It is useful to note that the pasteurization process that is applied according the parameters cited in the Regulation (EC) No. 1662/2006, would be able to eliminate a lot of pathogens, as also Heirinchs and Elizondo-Salazar (2009) reported ; nevertheless the conditions for a complete health quality of raw colostrum must be absolutely guaranteed. Based on the above mentioned references, concerning the colostrum pasteurization process, the effect colostrum immunoglobulins denaturation must be considered when it is pasteurized (as indicated in the above EC Regulation), with a consequent considerable reduction of the beneficial effects expected when the product is used as a food or as a food component. Since no data are available on the quality control of bovine colostrum to be used for human consumption, the parameters of proximate composition and hygienic aspects presented in this study largely agree with the indexes reported by other researchers. The same data could be a first reference available in Italy for the qualitative controls of bovine colostrum to be used for humans. This study provided some preliminary indications of the colostrum conditions produced in some areas of South Italy. Further insights will be helpful for implementation studies on the topic, with the aim to provide some elements for the rigorous quality checks of the product during different phases: primary production, manifacturing chain, marketing during products shelf-life as probiotics or nutraceutical products. As Regulation (EC) No.1662/2006 also indicated, the promulgation of legislative guidelines on the criteria for the control of bovine colostrum for human consumption should be necessary. These guidelines should be provided promptly, given the wide spread, with special reference to electronically marketing (by internet), of nutraceuticals containing colostrum. In fact, these products are consumed not only by adults, but also by children and elderly. A large input from the business, academic and legislative authorities is needed to enhance the popularity of these products, with a special regard to the innovative functional food, such as colostrum and colostrum based products.

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