Innovative Use of Jenny Milk from Sustainable Rearing

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Abstract Thanks to its chemical composition and nutrient profile, jenny milk is usually recommended for the needs of newborn, heart and cholesterolemic patients. Nevertheless, the future use of this product is still to be defined. In the present, contributions of two promising ways of milk valorisation were discussed: natural cosmetics and new cheese productions. The results of our studies showed that face creams made with jenny milk allow a better skin hydration and moisturisation compared to conventional cosmetics. Moreover, the addition of small amounts of jenny milk is able to prevent late blowing defects in cow and in ewe cheese making. The provision of these findings to farmers may have important socio-economic and ecological implications.

1 Why a Growing Interest Towards Donkey Rearing?

The Pan-European Strategy on Biological and Landscape Diversity has encouraged the institution of new protected areas of regional and of national parks aimed to safeguard the extensive agriculture and the sustainable rearing of autochthonous breeds in order to reduce the abandon of rural areas. These areas, which include parks, reserves and marginal areas, have an economy mainly based on agriculture, livestock production and forestry. Among these activities, livestock production in general and the raising of autochthonous livestock breeds in particular contributed significantly to create traditional agricultural landscapes supporting a greater variety of plants and wild animals. Therefore, to maintain and valorise this biodiversity, it is fundamental to preserve and/or reintroduce autochthonous breeds into these areas. This, in turn, may have positive implications for protecting and conserving the natural heritage of these areas (Signorello et al. 2004).

In this context, the pastoral activity and semi-extensive farming of donkey, if properly managed, may exert a positive influence on biodiversity. Donkeys are

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grazers as well as browsers (Aganga and Tsopito 1998). Their teeth and lips allow them to graze close to the ground; thus, they can efficiently graze short vegetation. These animals are non-selective grazers (Aganga et al. 2000), preferring thistles, rushes and other coarse vegetation to more palatable grasses. Thus, donkeys are capable of controlling some invasive plant species and those with less appetite (Cosentino et al. 2012).

In recent years, there has been a renewed interest towards the products (milk and meat) and social practices (rural tourism, onotherapy, etc.) that this species can offer, although donkey's breeding is still poorly widespread (Vincenzetti et al. 2008). Concerning milk production, it is well known that jennies provide milk that shows the closest similarity to human milk (Malacarne et al. 2002). Thanks to its nutritional characteristics, jenny milk has several applications: in paediatric sphere, in patients affected by cow's milk protein allergy and intolerance, as best alternative to human milk in infant food and in geriatric field for the treatment of ageing diseases (Tesse et al. 2009; Caffarelli et al. 2010). According to the literature, this milk has low levels of casein allergens and high levels of lactose, of unsaturated fatty acids (linoleic and linolenic) and of lysozyme. This enzyme is practically absent in other species (cow, goat, sheep, human) (Miranda et al. 2004) and has important physiological functions like inhibition of certain microorganism growth, anti-inflammatory and antitumoural activity and increase of defence system in early childhood (Monti et al. 2007; La Torre et al. 2010; Nazzaro et al. 2010; Simos et al. 2011). Some jenny milk whey proteins (α -lactalbumin, in particular, and β-lactoglobulin) stimulate cytokine production with a considerable in vitro anti-proliferative activity (Erdmann et al. 2008; Criscione et al. 2009; Mao et al. 2009). Moreover, thanks to some of its characteristics (vasodilator function, high calcium content and low energy value), it is indicated in patients affected by heart disease, osteoporosis and atherosclerosis (Hernández Ledesma et al. 2006; Iacono and Scalici 2011).

Another promising valorisation strategy of jenny milk is the use of this product in cosmetic preparations. Nowadays, the cosmetic trade is mainly focused towards products made with natural ingredients, and it is oriented to a sustainable consumption without preservatives addition. This has led many companies to increase the use of natural ingredients in existing preparations or to create new products (Lundov et al. 2009; Parente et al. 2011). Because of their natural origin, milk components correspond in many fields to the needs of cosmetology. Proteins and other components of milk have a strong absorption capacity and water retention, encouraging a high degree of hydration of the skin and preventing the degradation of the epidermal cells (Temuujin et al. 2006; Simos et al. 2011). Moreover, milk proteins with a glycosylated part could be largely used in all products developed to fight against skin ageing. Among these, for example, lactoferrin, which has a high iron-chelating property, could prevent free radical production by the skin after long periods of exposure to the sun (Cotte 1991; Girardet et al. 2004).

In cosmetic preparations, jenny milk is often used as basic constituent. Minerals, vitamins, fatty acids, bioactive enzyme and coenzyme, lactose and whey proteins contained in jenny milk prevent skin-ageing process, thanks to the hydrating and

restructuring action of the dermal intercellular substance (Cosentino et al. 2011; Orsingher 2011). It is known that these properties are principally due to the high lysozyme and to the antioxidant action of fatty acids contained in jenny milk (Polidori et al. 2009a; Tesse et al. 2009; Simos et al. 2011; Al-Saiady et al. 2012; Cosentino and Paolino 2012; Cosentino et al. 2012).

With regard to donkey meat, recent studies (Polidori et al. 2008a, b, 2009b) demonstrated that this product may be used as an alternative to other red meats and for the sausages production. The donkey meat has an important chemical and nutritional value: besides, it has a low content of fat and of cholesterol, a high protein and unsaturated fatty acid contents and high amounts of potassium, phosphorus, sodium and magnesium. Maniaci et al. (2009) showed a high acceptability for sausage made from only donkey meat compared to sausage made with only Sicilian pig meat and mixed. Marino et al. (2009), in a study on donkey dried beef, found a low percentage of saturated fatty acids and a high content of polyunsaturated fatty acids, with higher amounts of ω -3 compared to dried beef obtained with cow meat.

Another important aspect related to the raising of donkeys is the possibility of using this species in important social practices, such as pet therapy. Thanks to its quite temperament, donkey can play an important role for the treatment of certain personality disorders (Patti and Gaziano 2007). To exploit this important "ability", in Italy, in recent years, the number of rehabilitation centres and social farms considerably increased (Cirulli and Alleva 2007; Rossaro 2009).

2 Main Characteristics of Donkey Farms in Basilicata Region

2.1 Donkey Population

In the early of the twentieth century, Italy was the second European country with the largest number (almost one million) of donkeys (Cosentino et al. 2010). This result was due to the creation of new farms raising donkeys as well as to the numerical consolidation of the pre-existent rearing.

The number of donkey farms has historically been higher in south of Italy, where the species has played an important role in the rural life being a useful travelling companion between rural and urban residence. This is particularly true for the Basilicata region, where the species is quite spread, but a well-defined autochthonous genetic type is still absent. Until the 1920s, the Pugliese was the most common breed of donkey. Indeed, it included several subraces (such as Martina Franca, Marchigiana, Romagnola, Calabrese and Lucana) sharing many morphological characters, including the dark colour of coat (Baroncini 1987). Although some of these subraces were appreciated for their rusticity, a reduction of their consistency occurred over the years in Basilicata. However, in recent years, following a national

trend, there has been a renewed interest towards this species, especially thanks to some regional policies aimed at promoting the reintroduction of autochthonous breeds and the adoption of extensive and sustainable rearing systems, in order to prevent the abandonment of rural areas and to preserve the naturalistic heritage of the region (Signorello et al. 2004; Regione Basilicata 2011).

The study of Cosentino et al. (2010) provided important information relating to the different aspects of farms raising donkeys in Basilicata. According to the authors, most of the farms are small sized and family owned, with the farmer playing a key role in the defence and government of rural areas (Mauri 2007). A total of 660 donkeys (1.3 % of the national population) are reared in the farms localised in Basilicata. About 90 % of the farms are situated in the mountainous areas of the region (ISTAT 2002, 2011), where there is the greatest number of donkeys (82 %). Over the past 10 years, there was a reduction of the number of subjects (from 830 to 660), with the concurrent increase of the average number per farm (from 2.7 to 4.68). The highest number of donkeys has been recorded in the province of Potenza, where 403 donkeys are reared in 104 farms. In the province of Matera, instead, there are 257 subjects reared in 37 farms. The farms can be divided into the following classes: equestrian with mares (64), reproduction (9), meat with mares (21) and work (47).

With regard to protected areas of Basilicata, about 50 % of the donkeys are reared in the Regional and National Parks (National Park of Appennino Lucano Val d'Agri Lagonegrese, 130 subjects; Pollino National Park, 68 subjects; Regional Park Gallipoli Cognato, 78 subjects; Regional Park of Matera Chiese Rupestri, 43 subjects). A high number of donkeys were found in some municipalities of the Parks, such as Calciano (49), Lauria (40), Matera (36), Anzi (33) and San Martino d'Agri (28).

2.2 Morphological and Biometrical Characteristics of Local Population

In the past decades, a number of studies on morphological and biometrical characteristics of the species have been carried out to establish for the Italian breeds the standards for the anagraphic registers (Cecchi et al. 2007). Other studies, instead, investigated the genetic relationship among the Italian populations (Di Rosa et al. 2007).

An important contribution to the definition of the morphometric characteristics of donkeys reared in Basilicata is that of Cosentino et al. (2010). In their study, the authors recorded some morphometric and biometric characteristics of 52 donkeys reared in semi-extensive farms. Their results showed significant gender differences in terms of withers height, depth and width of chest, shin circumference and sternum height. In most of the cases, the colour of coat was blackish, whereas in others, it was grey mouse with both shoulders stripe and bay dark.

The local population of donkeys had a small size with the cephalic region more accentuated than trunk. The biometric indexes and the zoometric measurements indicated a longilinear or dolichomorphic type. These are probably due to the little interest of small farmers or owners of one or few donkeys in applying rigorous techniques of breeding or controlled mating according to morphology and genealogy (Jordana and Folch 1998).

Overall, the local population can be considered suitable to the milk production, pet therapy and transport in the mountain paths, just as other breeds like Martina Franca, Amiata and Ragusana (Monti et al. 2007; Conte and Passantino 2008).

3 Qualitative Characteristics of Jenny Milk

Producing jenny milk may be an interesting, profitable and alternative activity for farmers, mainly in southern marginal areas. However, in Basilicata, there are still few farmers that produce jenny milk. This is mainly due to poor farm consistency, which, in turn, makes it difficult to start this type of production. Moreover, the regional land conformation does not help the market of the milk, and the cost of production is much higher than cow milk for limited daily production. Overcoming these kinds of difficulties is fundamental to exploit all the commercial opportunities that jenny milk can offer.

Considering its unique nutrient profile, jenny milk may be easily employed to satisfy the nutritional requirements of newborn, heart and cholesterolemic patients. In particular, cow milk allergy, heart ageing and cholesterolemic diseases are considered to be increasing problems, and, for these reasons, the acquisition of new knowledge on jenny milk is very important in clinical and nutritional aspects. However, little is known about qualitative and quantitative characteristics of jenny milk. Although the species is a seasonal polyestrous, in south of Italy, jennies foal every season, probably because of small photoperiod oscillations between different seasons, and, consequently, milk is available all year (Giosuè et al. 2008). During lactation, this species has a low but constant daily production (Malacarne et al. 2002; Polidori et al. 2009a). Production level is influenced by several aspects, such as stage of lactation, milking technique, presence of the foal and foaling season (Dell'Orto et al. 1993; Oftedal et al. 1983). In particular, during milking, the presence of foal and the stage of lactation influence fat and protein content. Moreover, lactose content is constant during lactation, being independent of breed, milking time and stage of lactation (Guo et al. 2007). Jenny milk composition is similar among subjects from different continents, except for fat content (Blasi et al. 2008; Ivanković et al. 2009).

Cosentino et al. (unpublished data) evaluated some aspects of donkey rearing that include the influence of lactation stage (30, 90 and 150 days from foaling) and of foaling season (spring and summer) on some qualitative aspects of jenny milk, in a farm situated at an altitude of 700 m a.s.l. in a protected area of Appennino Lucano National Park. The research was carried out on 23 jennies belonging to a

local population and aged between 7 and 10 years. These jennies foaled in two different periods: spring and summer (12 and 14 jennies, respectively). Milk samples were collected monthly, in the periods March–August in spring group and July–November in summer group. During the trial period, jennies were confined in boxes with a large paddock, in order to avoid the effect of grazing on qualitative characteristics of milk. Animals were offered a diet consisting of ad libitum oat hay and of an integration of 3 kg of concentrate, characterised by the following mixture: 37 % flaked corn, 30 % oats, 9 % locust bean crushed, 8 % wheat bran, 8 % dehydrated alfalfa, 6 % beet pulp dried and 2 % minerals and vitamin supplement. Diet was dispensed twice a day: during milking and in the evening. From the second month after delivery, jennies were milked by mechanical milking (40 kPa vacuum level, 60 pulse per min), at 11:00 am. From 8:00 am to the end of milking, foals were separated from mares, but were kept in the adjacent box maintaining the visual and the acoustic contact.

Immediately after collection, on milk individual samples were measured: pH and titratable acidity, protein, fat, lactose, dry matter and ash content. In addition, somatic cell count (SCC), expressed as somatic cell score (SCS, $\log_{10}n \times 1,000/$ mL), was determined.

The groups showed a milk production trend similar to that observed in literature by other authors (Giosuè et al. 2008; Santos and Silvestre 2008). Milk production, protein, ash, SCS and pH resulted highest in summer at 30 days; protein and pH resulted highest in summer also at 90 and 150 days, respectively (Table 1). Ash content in tendency decreased during lactation. The highest mineral content, at the start of lactation, is particularly important for the first growth stage of the young foal (Csapó-Kiss et al. 1995). During lactation, pH values varied significantly between groups. SCS values observed from other authors ranged from 3.94 to 4.34 (Beghelli et al. 2009; Finocchiaro and Conte 2009; Ivanković et al. 2009). In spring group, protein content was high at the start of lactation, decreased to a minimum at 90 days and increased at the end of lactation, as observed by Guo et al. (2007); instead, summer lactation showed two peaks at 30 and at 90 days. Protein content resulted lower than the values reported by other authors that studied the effects of seasons on jenny milk characteristics: 19.3 g/L in spring and 18.1 g/L in summer in Sicilian breeds (Giosuè et al. 2008) and 16.5 g/L in spring and 14.3 g/ L in summer in Croat breed (Ivanković et al. 2009). Fat content was significantly highest in summer group at the end of lactation. The average fat content of jenny milk was similar to mare milk and was much lower than other mammals; other authors observed values in the range 0.01-1.8 %. Lactose content and dry matter resulted significantly the lowest in summer only at 30 days. In the other periods, lactose was not influenced by the considered factors, as reported in literature (Oftedal and Jenness 1988; Santos and Silvestre 2008); dry matter content was in agreement with values reported in the literature for equine milk (Malacarne et al. 2002; Miranda et al. 2004); Ivanković et al. (2009) observed in Croat jenny values that ranged from 8.61 to 9.13 %. Titratable acidity (SH°) resulted highest in summer lactation at 90 and at 150 days, value lower than in cow's milk, attributable to the low content in casein and in phosphate (D'Auria et al. 2005; Guo et al. 2007).

Table 1 Jenny milk parameters (mean \pm SE)

	Days post-foaling					
	30		06		150	
Parameters	Spring	Summer	Spring	Summer	Spring	Summer
Milk production (L/day)	$1.05 \pm 0.03^{\mathrm{a}}$	1.47 ± 0.12^{b}	1.38 ± 0.15	1.13 ± 0.18	0.91 ± 0.11	0.93 ± 0.12
Protein (g/L)	12.65 ± 0.18^{a}	$13.76 \pm 0.84^{\rm b}$	12.00 ± 0.18^{a}	12.90 ± 0.18^{b}	12.50 ± 0.09	12.70 ± 0.09
Fat (g/L)	4.00 ± 1.30	4.10 ± 0.7	2.10 ± 0.20	1.60 ± 0.10	2.30 ± 0.28^{A}	$4.80 \pm 0.65^{\mathrm{B}}$
Lactose (g/L)	$60.60 \pm 0.37^{\mathrm{A}}$	$50.20 \pm 1.30^{\mathrm{B}}$	58.50 ± 1.20	58.50 ± 0.70	59.10 ± 0.28	58.00 ± 0.37
Dry matter (g/L)	$81.09 \pm 1.20^{\mathrm{A}}$	$72.60 \pm 3.50^{\mathrm{B}}$	74.90 ± 1.20	77.00 ± 0.28	77.75 ± 0.50	79.30 ± 1.80
Ash (g/L)	3.80 ± 0.09^{A}	$ 4.60 \pm 0.18^{\rm B}$	3.80 ± 0.20	3.90 ± 0.20	3.80 ± 0.10	3.90 ± 0.10
Hd	6.80 ± 0.03^{A}	$6.95 \pm 0.05^{\mathrm{B}}$	$6.86 \pm 0.04^{ m A}$	$6.70 \pm 0.05^{\mathrm{B}}$	6.77 ± 0.02^{A}	$7.02 \pm 0.01^{\mathrm{B}}$
TA, SH $^{\circ}$	2.48 ± 0.16	2.32 ± 0.15	$2.09 \pm 0.12^{\rm a}$	$2.32 \pm 0.14^{\rm b}$	1.86 ± 0.12^{a}	$2.32\pm0.20^{\mathrm{b}}$
SCS, $\log_{10}n \times 1,000 \text{ (mL)}$	$3.31 \pm 0.03^{ m A}$	$ 4.37 \pm 0.06^{\mathrm{B}}$	3.62 ± 0.03	$\boxed{4.28\pm0.08}$	3.90 ± 0.04	$ 4.30\pm0.02$
Means within periods with different superscripts significantly differ: a, $\mathbf{b} = P < 0.05$; A, $\mathbf{B} = P < 0.01$	rent superscripts signi	ficantly differ: $a, b = I$	P < 0.05; A, B = $P < 0.05$	0.01		

3.1 Cosmetics Made with Jenny Milk

The first representation of the donkey species was found in Egypt in a bas-relief of 2500 BC, and from the time of Herodotus (V century BC), donkey began to be appreciated for the therapeutic properties of its milk (Melani 1998; Paolicelli 2005). Some historical texts, such as the *De Materia Medica* of Dioscoride and the *Naturalis Historia* of Pliny the Elder, describing various uses of milk in cosmetics, and Ovid also, in his *Medicamina Faciei Femineae*, suggest beauty masks made with donkey milk (Virgili 1989).

Today, thanks to the properties of milk components, there are several products made from milk of different species in the cosmetic market. Cow milk preparations (face and body creams, cleansing milk and tonic) are the most known by consumers. However, there are cosmetics made from other ruminants like camel (Kalejman 2011), sheep (Drader 2005) and goat (Ribeiro and Ribeiro 2010) or from monogastric species like horse and donkey (Medhammar et al. 2012; Song 2012; Cosentino et al. 2013a). Mare's milk is considered as an ingredient in Mongolian cosmetics because of its high content of polyunsaturated fatty acids, which make it readily absorbed by human skin (Temuujin et al. 2006). It is known that jenny milk properties are principally due to the high lysozyme and to the antioxidant action of fatty acids contained in jenny milk (Tesse et al. 2009; Al-Saiady et al. 2012; Cosentino and Paolino 2012; Cosentino et al. 2012).

Notwithstanding the beautifying benefits of the jenny milk have been historically acclaimed (since Cleopatra), few studies have been conducted on its perceived quality in cosmetics or on its actions about skin-ageing process hydrating and restructuring the dermal intercellular substance (Orsingher 2011; Paolino and Cosentino 2011).

Cosentino et al. (2014) preliminarily evaluated whether the use of a face cream made from milk jenny affected the perception of some sensory aspects. The test was conducted on 80 regular female consumers of cosmetic cream, subdivided according to their skin type: dry (25), normal (30) and oily (25). Consumers were given two types of creams: a control and a treated cream, with the latter created by adding pasteurised jenny milk (30 % on total weight). Both creams were packaged in 50-mL containers and given to consumers with a ballot consisting of 11 questions about attributes of appearance, fragrance and effectiveness and the overall satisfaction of each cream. Consumers tested both face creams at home, for a period of 15 days; they were asked to apply the face cream every evening and to rate the attributes presented in the questionnaire for each face cream at the end of trial. The results showed that treated cream resulted appreciated by dry skin consumers for the following sensory aspects: spreadability, total appearance, smoothness, moisturisation and total effectiveness (Fig. 1). The overall judgement also resulted highest for face cream made with jenny milk. The other consumers expressed a good acceptability for both tested creams. These results confirm that jenny milk could be a cosmetic component suitable for all skin types thanks to its balancing skin moisture (Salimei and Fantuz 2012). A recent study on a face cream containing

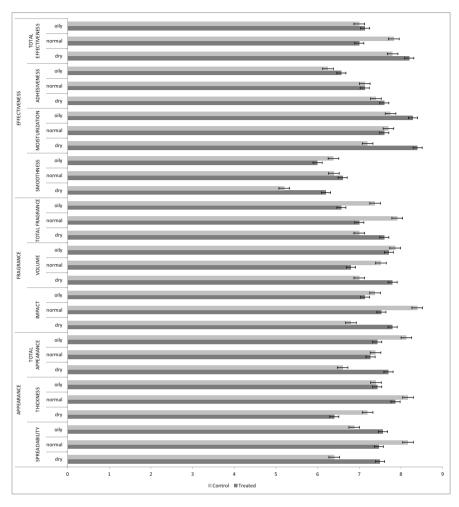


Fig. 1 Sensorial aspects of control and treated (made with jenny milk) face creams at the end of the trial (mean \pm SE)

lyophilised jenny milk (Orsingher 2011) showed a lot of benefits, such as wrinkle reduction, new collagen formation, restoration of the skin's natural defences against external agents, prevention of inflammatory processes in the case of dermatitis and increased elasticity. These results are probably related to the effectiveness of jenny milk components like proteins, minerals, vitamins, essential fatty acids and lysozyme, which allow the skin a balanced nourishment and a proper hydration (Guo et al. 2007). Lactose, particularly high in jenny milk, is an important emollient and moisturiser (Temuujin et al. 2006; Polidori et al. 2009a).

The beautifying properties in calming the irritation symptoms (Blasi et al. 2008; Vincenzetti et al. 2012) and in restructuring skin-ageing process (Nazzaro

et al. 2010; Al-Saiady et al. 2012) of its whey proteins (β -lactoglobulin and lysozyme) and of its essential amino acids are also well known. For this reason, it could be much more profitable if use in higher water content cosmetics (body and after-shave lotions, shampoos, hair conditioners and cleansing milk), in which a larger use of milk is possible.

These results clearly show that jenny milk could be a valuable and innovative ingredient for cosmetics. The marketing of cosmetics made with jenny milk could help to preserve local donkey genotypes and the marginal areas in which they are reared. A major challenge for the marketing of these kinds of products is the consumer's knowledge about jenny milk and sustainable farms. In this context, packaging may have an important communicative function thanks to iconic and textual elements (Topoyan and Zeki 2008). The measurement of packaging attributes is very difficult because a package generally induces a wide variety of stimuli: visual, tactile and even olfactory. For example, visual and tactile stimuli affect the consumer choices at time of purchase.

Cosentino et al. (2011) evaluated consumer knowledge on some qualitative and quantitative aspects of jenny milk cosmetics. Their main aim was to identify some packaging factors that influence consumer liking. The study was conducted using the quantitative method by interviews with a one to one questionnaire, consisting of 18 questions, that has been randomly administered to 450 people residing in the test area. The age of sample was in mean 40 years, since people in this age range are the most strongly concerned to counteract skin-ageing process and the appearance of wrinkles. The results showed that about 70 % of the surveyed consumers were willing to purchase a product labelled as "new generation cosmetics", probably because this cue refers to innovation and modernity in their imagination. These cosmetics could absorb a significant portion of the market for natural cosmetics based on milk. Descriptive survey put in evidence that customers knew jenny milk and expressed willingness to buy cosmetics made with this kind of milk in the future. Few consumers were already familiar with jenny milk cosmetics and had already bought this kind of product. In general, consumers showed a willingness to buy cosmetics in low price classes. However, many consumers were willing to pay more for these cosmetics if produced in sustainable rearing system and in the respect of animal welfare.

More recently, Cosentino et al. (2013a), in a study on the acceptability of different types of packaging for exalting the quality of cosmetics with jenny milk, found that consumers preferred the one evoking the concept of natural. A total of 300 consumers, aged in mean 33 years, evaluated the preferences by using a scale of values from 1 to 5. In order to identify the most preferred packaging, the naming, the type of packing paper and the communication aspect of packaging were studied (Fig. 2). The naming has been developed starting from the concepts of delicacy and of naturalness, with the direct representation of the product source, like the donkey with a specific soft brown tone coat. The name "Asinella" was the most preferred by a jury of 50 habitual cosmetic consumers; the farmers also identified it as the best choice (Fig. 2).

NAMING



TYPES OF PACKAGING PAPER





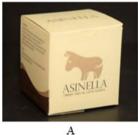






Matte

PACKAGING TEXT LABELS







В



C

Fig. 2 Types of packaging

Consumers were asked to evaluate different types of packing paper: matte, glossy and glossy embossed with texture (Fig. 2). Matte paper was the most preferred, because it evoked the perception of natural, more than the glossy paper, which is usually used by the competitors (Asilac, Milk drops and Dahl). In addition, consumers were more willing to buy cosmetics packaged with matte paper.

The communication aspect of packaging proposed was A, "Asinella"; B, "Asinella" + "Natural product"; and C, "Asinella" + "Natural product" + "Made in Basilicata" (Fig. 2). Consumers considered packaging information important to induce to purchase and judged the message of type B more persuasive than the other two communication forms of packaging, probably for the reliability of the product. They also considered the most persuasive combination in purchasing the name "Asinella", with the text label "Natural product". From the consumer perspective, naming is an important quality cue and makes it easier to infer quality. In addition

to this parameter, traceability systems, branding and labelling can help consumer's choice (Grunert 2002).

3.2 Jenny Milk as Inhibitor in Cheese Making

Late blowing defects on ripened semihard and hard cheese are an important problem with a high negative economic impact in dairy production. The causes depend on both technological and microbiological factors. Technological factors include milk quality, heat treatment, hygiene practices, manufacture technology, compositional parameters and ripening temperature/moisture, which influence cheese making and ripening (Bogović Matijaŝić et al. 2007). Microbiological factors are the most difficult to control, because undesirable microorganisms, such as coliforms, yeasts, heterofermentative lactic acid bacteria and spore-forming bacteria, may cause early or late blowing defects in cheeses (Little et al. 2008; Gómez-Torres et al. 2014). Late blowing defect in semihard and hard cheeses has been attributed to the outgrowth of strains of *Clostridium* spp. (mainly *C. butyricum* and C. tyrobutyricum), capable of fermenting lactic acid with production of butyric acid, acetic acid, carbon dioxide and hydrogen (Garde et al. 2012). Clostridium spores are ubiquitous, much more resistant to heat, chemicals, irradiation and desiccation than vegetative cells, and its growth in cheese is affected critically by different factors such as salt concentration, pH, ripening time and temperature as well as by the presence of other microorganisms (Garde et al. 2011).

Many studies have attempted to prevent late blowing by physical treatments (bactofugation or microfiltration prior to processing), or by the use of additives (nitrate or lysozyme), or by the addition of strains of lactic acid bacteria (LAB) producing bacteriocins, active peptides displaying a bactericidal mode of action towards specific Gram-positive bacteria (Wasserfall and Teuber 1979; Vissers et al. 2007; Martìnez-Cuesta et al. 2010; Schneider et al. 2010).

Among the above prevention methods, the use of lysozyme as a commercial additive is the preferred one since 1983. Lysozyme, which is typically extracted from hen egg white (HEW, 3.5 % of the egg white proteins), has been approved as a preservative (E1105) in the entire European Community, according to the Directive No. 95/2/EC (*quantum satis* in ripened cheese) (Pellegrino and Tirelli 2000; Scharfen et al. 2007; Schneider et al. 2011). In Italy, the use of lysozyme is quite widespread: this enzyme has been employed in the process of making several cheeses, such as Grana Padano cheese, grated hard cheese mixtures (Iaconelli et al. 2008; Panari and Filippi 2009) and semihard goat and ewe cheeses (Dragoni et al. 2011; Schneider et al. 2011). The content of lysozyme from egg in cheese ranges from 50 to 350 μ g/g of cheese, with the maximum of 400 μ g/g of cheese depending on the type of cheese and the production process (Pellegrino and Tirelli 2000; Ávila et al. 2014).

In recent years, the use of lysozyme from egg as a prevention agent has waned, since some studies have shown its allergenic effect in consumers allergic to egg,

due to its content in ovomucoid, ovalbumin and conalbumin (Frémont et al. 1997; Pérez-Caldero et al. 2007). In the last decade, a number of severe allergic reactions have been recorded due to the presence of lysozyme E1105 in semihard cheeses. Frémont et al. (1997) found patients allergic to eggs to show a severe reaction after eating Gruyere cheese. Kerkaert et al. (2010) reported that 5 out of 21 case studies of allergic reactions to eggs were attributed to the presence of this additive in cheese and that this additive was likely responsible for episodes of severe edema (Pérez-Caldero et al. 2007). For these reasons, in the recently changed EC legislation, the use of lysozyme as an additive has to be declared on the label (EC legislation in Europe 2003/89/EC, Directive 2000/13/EC).

Jenny milk may be a possible alternative to the use of lysozyme from egg during cheese making. In fact, jenny milk is characterised by a high lysozyme content, ranging from 1.0 to 3.7 mg/mL, according to the lactation stage and the production season (Zhang et al. 2008; Vincenzetti et al. 2012). The content of lysozyme in jenny milk is much higher than cow (0.13 μg/mL), ewe (0.20 μg/mL) or goat milk (0.25 µg/mL) (Fratini et al. 2006; Scharfen et al. 2007; Cosentino and Paolino 2012). Moreover, in jenny milk, lysozyme shows the highest activity at an optimum temperature of 37 °C and is stable up to a temperature of 50 °C, decreasing to 50 % of activity at 70 °C. Recently, Galassi et al. (2012) described the addition of jenny milk as a substitute for egg lysozyme to prevent late blowing in Grana Padano cheese. The authors found that the addition of 10 L of jenny milk in 500 L of cow milk reduced significantly physico-chemical and microbiological defects of cheese. Cosentino and Paolino (2012) studied the effect of lysozyme from jenny milk on blowing defects in artisanal ewe cheese caused by clostridia and coliforms, usually present in ewe cheese produced in traditional cheese factories. When adding jenny milk to ewe milk, no late blowing defect on cheese was observed (Fig. 3). Therefore, lysozyme contained in jenny milk was found to be an important inhibitor agent against coliform bacteria, although its addition to ewe milk did not affect the number of Clostridium butyricum spores (Cosentino and Paolino 2012; Cosentino et al. 2013b). The lower content of coliforms in treated ewe cheese was in agreement with results from the literature on reduced growth of C. butyricum in Grana Padano (Iaconelli et al. 2008; Dragoni et al. 2011) and Gouda cheese (Bester and Lombard 1990). Martinez-Cuesta et al. (2010) observed a higher contamination of Clostridium in Manchego control cheese compared with that treated with lysozyme HEW.

Cosentino et al. (unpublished data) also evaluated whether increasing additions of jenny milk to pasteurised cow milk reduced the late blowing defect in semihard cheese caused by *C. tyrobutyricum*. To verify this hypothesis, the authors made two types of cheeses, control and treated, with the latter being deliberately contaminated with approximately 3 log spores/mL milk of *C. tyrobutyricum* CLST01, in order to induce butyric acid fermentation and consequent blowing defect. Both control and treated cheeses were made by adding different aliquots of jenny milk to cow milk. The addition of jenny milk resulted in a sporostatic effect on both control and treated cheeses. Visual and odour inspections during ripening demonstrated that all cheeses contaminated with *C. tyrobutyricum* developed signs of late blowing

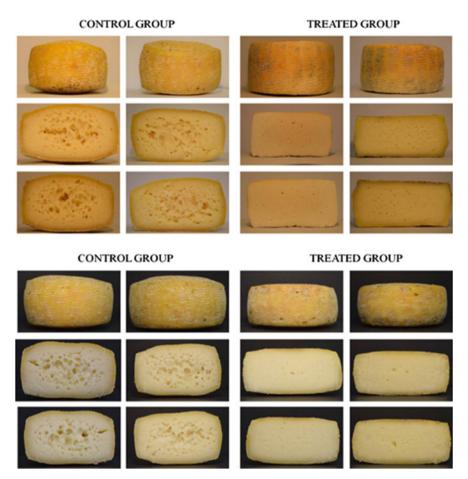


Fig. 3 Signs of late blowing defects in ewe cheese (from first to third row) and cow cheese (from fourth to sixth row) at the end of ripening

defect, except the cheese containing the highest aliquot of jenny milk. This product presented a uniform texture without cracks and splits (Fig. 3). The content of lysozyme was high in both control and treated cheeses (1.57 and 1.52 mg/kg, respectively). Ávila et al. (2014) found that 40 μ g/mL of lysozyme was the concentration required to completely inhibit the growth of vegetative cells of *C. tyrobutyricum* strains. Cosentino et al. (unpublished data) also found that the acceptability of cheeses was not affected by the addition of jenny milk, since consumers did not found differences between the products made with only cow milk and those made also with jenny milk. These results are in line with the findings of Galassi et al. (2012).

4 Conclusions

The 21st agenda item of World Summit carried out in Rio in 1992 emphasised the intrinsic value of biological diversity and its ecological, genetic and socio-economic components, recognising that the fundamental need for the preservation is the in situ safeguard of ecosystems and of natural habitats. In recent years, diversity preservation has become an important topic, as shown by different strategies and action plans, such as "Countdown 2010" and "Forests 2011" for European and national governments and for productive sectors.

In this context, the jenny rearing finds its ideal placement thanks to its peculiarities capable to fulfil the requirements of a wide range of consumers, from newborn nutritional needs to those of elderly people.

The present contribution provides a wide discussion of the above aspects, highlighting alternative ways of valorisation of this precious animal production. Among them, one of the most promising is the use of jenny milk for making innovative products (new cheese productions and natural cosmetics), which, in turn, may led to the expansion of donkey rearing for milk production, even in more vulnerable areas, such as Natura 2000 Network sites.

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