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Effects of freeze-drying and spray-drying on donkey milk volatile compounds and whey proteins stability

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ABSTRACT

In this work, the profile of the volatile compounds has been determined for the first time in fresh donkey milk and in donkey milk subjected to accelerated shelf-life test, freeze-drying and spray-drying processes. The effects of freeze-drying and spray-drying processes in donkey milk have been analyzed also on three representative whey proteins, β-lactoglobulin, α-lactalbumin and lysozyme, by determining the total amount, the enzymatic activity and the thermal stability. Considering the volatile compounds in fresh donkey milk, 2-heptanone, 1,3-bis (1,1-dimethylethyl)-benzene, nonanal, D-limonene, octanoic acid, and 1-octanol were detected. The accelerated shelf-life test revealed a constant increase of octanoic acid, 2-heptanone, and nonanal compared to fresh donkey milk ($P < 0.001$) and the appearance of ethyl octanoate, ethyl dodecanoate, 2-nonanone and 2-undecanone, indicating that these compounds may be highly eligible markers of donkey milk shelf-life. Freeze-drying and spray-drying affected β-lactoglobulin and lysozyme content in donkey milk. In particular, spray-drying process decreased significantly lysozyme enzymatic activity (58% of residual activity) and β-lactoglobulin content (6.43 mg/mL in fresh milk vs 5.51 mg/mL in spry-dried milk) due to the high temperature to which donkey milk is subjected.

1. Introduction

Donkey milk (DM) may be considered a good and safe replacer to other kinds of milk for some specific consumers, among all infants affected by cow's milk protein allergy (CMPA), especially if breastfeeding is not possible for these subjects (Carroccio, Cavataio, Montalto, D'Amico, & Alabrese, 2000). The main attribute of DM is that its chemical composition is very similar to human milk especially regarding the lactose and the protein content, and also the mineral composition (Salimei et al., 2004). However, the low lipid content must be considered when DM is used in infant's nutrition; a fat supplementation must be provided in order to supply the correct amount of calories for growing children (Iacono et al., 1992). DM is believed to be hypoallergenic because of its low content of αs1-and β-caseins and only trace levels of αs2-and κ-caseins (Chianese et al., 2010; Vincenzetti et al., 2008). The main problem in the use of DM is linked to its poor availability, that is responsible of the high cost per liter of this food. DM supply is limited to a few months per year since it depends on the ass's fertility which is related to the photoperiod (Polidori, Beghelli, Mariani, & Vincenzetti, 2009). Consequently, it is necessary to find long-term storage conditions for DM using thermal treatments such as lyophilization through a freeze-drying procedure or a spray-drying technique. However, heat treatment processes could determine undesirable reactions, described as thermal damage, which can affect the sensorial and nutritional properties (Wolf, Bergamini, Perotti, & . Hynes, 2013; Rattray, Gallmann, & Jelen, 1997).

Flavor, that results from both taste and aroma compounds, strongly depends on metabolite repertoires, including volatile compounds. These low molecular weight compounds (less than 300 Da) easily vaporize at 25 °C. As soon as they reach olfactory receptors, they stimulate an odor sensation (Belitz, Grosch, & Schieberle, 2009). They are often fat-soluble and easily bind to membrane receptors. Flavor compounds play a crucial role in product identification and consumer acceptance. Heat treatments influence the composition of milk volatile compounds

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as they may be responsible for the off-flavors development, due to the variations in the concentration of molecules such as aldehydes, methyl ketones and sulphur compounds (Contarini & Povolo, 2002). From an extensive literature search, it is clear that only a study investigated on the flavor of DM, finding that DM taste was sweet and pleasant, the aroma was milky, with a sweet flavor and no persistent aftertaste (Malissiova et al., 2016). However, there is a lack in the literature as regards the molecular fingerprint of the volatile compounds of DM.

For this reason, we decided to investigate the volatile profile of fresh DM, here determined for the first time, through solid phase micro extraction gas chromatography coupled to mass spectrometry (HS-SPME-GC-MS). Furthermore, the profile of the volatile compounds was determined in DM subjected to freeze-drying, spry-drying and to the accelerated shelf-life test, in order to determine potential markers of DM shelf-life.

It is known that milk whey proteins can be used as indicators of milk quality after a technological treatment (Morales, Romero, & Jimenéz-Peréz, 2000). Although in two previous works, the effects of some technological treatments on DM protein fraction and on vitamin C were evaluated (Polidori & Vincenzetti, 2010; Vincenzetti et al., 2011), the aim of the present work was a detailed study on the effects of freeze-drying and spray-drying treatments on DM whey protein fraction. At this purpose, three representative whey proteins, β-lactoglobulin, α-lactalbumin and lysozyme, have been analyzed in the DM samples (fresh DM, and both reconstituted freeze-dried and spray-dried DM) by determining the total amount, the enzymatic activity and the thermal stability. The latter was established by dynamic light scattering and differential scanning microcalorimetry experiments. The enzymatic activity of lysozyme has been performed since the activity assay of specific enzymes such as lactoperoxidase, alkaline phosphatase and lysozyme can represent a valuable index of the nutritional quality of heat processed milk (Claeys, Ludikhuyze, Van Loey, & Hendrickx, 2001; Addo & Ferragut, 2015). Finally, the solubility toward temperature of the three main whey proteins obtained from reconstituted freeze-dried and spray-dried DM has been assessed and was compared to those obtained from fresh DM.

2. Materials and methods

2.1. Donkey milk samples, freeze-drying and spray-drying processes

The bulk raw DM samples were collected from the morning milking of 16 Martina Franca breed pluriparous asses in midstage of lactation; the asses were routinely machine milked in a registered farm located in Introdacqua (AQ), Abruzzo Region, Italy. After milking, DM was transported in a refrigerated bag (+4 °C) to the laboratories to be analyzed or processed.

From each sampling, three aliquots were separated: sample A, fresh DM; sample B, freeze-dried DM; sample C, spray-dried DM. Furthermore, two other aliquots of fresh DM were stored at 20 °C (thermostated) for 48 h and 72 h (samples A2 and A3, respectively) in order to accelerate the milk deterioration process, and were used for the determination of potential shelf-life markers of DM (accelerated shelf-life test).

The freeze-drying process was performed by CRAB (Consorzio Ricerche Applicate alla Biotecnologia), Avezzano (AQ), Italy. A Beta 1-16 lyophilizer (Christ, Osterode am Harz, Germany), able to remove 10 kg/day of water was used. During this process, the following parameters were continuously monitored: temperature of the dish, temperature of the product and condenser temperature. The spray-drying process was also performed by CRAB, with a GEA Niro Spray Dryer, Model SD-6.3N (NIRO SOAVI, Parma, Italy), the inlet air temperature was 190 °C, the outlet temperature was 90 °C. All subsequent experiments performed using DM samples A, A2, A3, B and C were carried out in triplicate. The chemical analysis aimed to determine DM volatile compounds have been carried out in the laboratories of Montani Institute of Fermo, while all the other chemical analysis have been carried out in the laboratories of the University of Camerino.

Before being analyzed, 0.9 g of powdered DM from both samples B and C, were reconstituted in 10 ml of distilled water. Both fresh milk (sample A) and the reconstituted milk samples (B and C) were skimmed through centrifugation at 3000 × g for 30 min at 15 °C. DM whey proteins were separated from caseins by adjusting the pH of skimmed DM to 4.6 with acetic acid (10mL/100 mL) and centrifuging again at 3000 × g for 10 min in order to obtain a supernatant of whey proteins and the isoelectrically precipitated caseins. The protein concentration was determined on each DM sample, following the method of Bradford (1976).

2.2. Profile of donkey milk volatile compounds

Milk samples (A, A2, A3, B and C) were weighted (2.0000 ± 0.0010 g) in 5 mL headspace vials and closed through a PTFE/silicone septum. Volatiles in the headspace were measured through solid phase micro extraction gas chromatography coupled to mass spectrometry (HS-SPME-GC-MS). The experimental design was run in triplicate.

SPME fibers were obtained from the Supelco Company (Bellefonte, PA). The fiber was divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) 50/30 μm and was conditioned before use, as recommended by the manufacturer. Before extraction, the stabilization of the headspace in the vial was reached by equilibration for 30 min at thermostated temperature (20 ± 0.1 °C). Volatiles were then adsorbed for 12 h. After extraction, injections provided the fiber thermal desorption into a Hewlett Packard GC-MS, G1800C GCD Series II (Palo Alto, CA) equipped with a HP-5MS column 30m × 0.25 mm I. D. x 0.25 μm film thick (Hewlett-Packard). The mass spectrometer was tuned before the analyses via a reference gas (perfluorotributhylamine) across the full mass range.

The fiber was left in the injection port (equipped with a 0.75 mm i.d. inlet liner) for 4 min. The injector was set at 270 °C and operated in the splitless mode for 1 min. Before sampling, the fiber was reconditioned for 5 min at 270 °C and blank runs were done periodically during the study to reveal possible carryover. The carrier gas was helium; constant flow 1 mL/min; the oven temperature was held isothermal at 30 °C for 15 min, then programmed from 30 to 260 °C at 10 °C/min and then held isothermal (1min). Mass spectra were acquired in the electron impact mode (70 eV), using full scan with mass analysis in the range 30–400amu. The detector temperature was set at 270 °C. As the SPME technique is a solventless one, no solvent delay was needed and in this way, we could detect also the earliest eluting analytes. The identification of the constituents was based on the comparison of the retention times with those of authentic samples obtained from Sigma Aldrich (Milan, Italy), if available. The identification was also based on computer matching against commercial (NIST 1998) libraries. In the absence of the commercial standard (8-nonen-2-one and 3,7-dimethyl-undecane) the identity of the spectra at 98% was needed for identification of compounds (Cecchi & Alfei, 2013). Volatile compounds were also identified by comparison of their linear retention indices relative to n-alkanes, calculated using straight-chain alkanes mixture C6-C19, with the averaged values reported in the bibliography for chromatographic columns similar to that used (Van Den Dool, & Kratz, 1963).

Absolute peak areas were recorded in area counts. In each case, samples were spiked with 1.33 μg/mL chlorobenzene (internal standard in refined oil) that was used to normalize peak areas. Only compounds with a signal to noise ratio higher than 5 were considered.

2.3. Solubility studies on fresh, freeze-dried and spray-dried donkey milk samples

Solubility studies toward temperature have been performed on the three major DM whey proteins: β-lactoglobulin, lysozyme and α-