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Full Length Research Paper

Effect of different physical treatments on antioxidant activity of jenny milk

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The shelf life of food can be extended by employing several physical treatments. Among them, pasteurization and condensation are widely adopted for prolonging the shelf-life of milk by reducing the microbial load. However, these treatmens could affect the antioxidant activity of the product. The aim of the present study was to investigate the effect of pasteurization and condensation on the Total Antioxidant Capacity (TAC) of raw and pasteurised jenny milk. Using 2,2'-azinobis (3-ethylbenzthiazoline-6-acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays. TAC was also measured after condensation at 40 and at 20% of the initial volume. ABTS assay was found to be more accurate and reproducible than DPPH. Pasteurisation reduced significantly (P<0.05) the total antioxidant capacity from 84.15to 82.09% (ABTS test). The TAC decreased with the increase of condensation in raw and pasteurised milk. Overall, the TAC was reduced by only 3 and 4%, in pasteurised and in raw milk, respectively (ABTS test). Therefore, both pasteurization and condensation are recommended as physical treatments to prolong the shelf-life of jenny milk.

Key words: Jenny milk, antioxidant activity, condensation, pasteurization, ABTS, DPPH.

INTRODUCTION

Heat treatment of milk is widely adopted by the dairy industry to prolong the shelf-life and to improve the quality of this complex biological fluid by reducing principally the microbial load (Raikos, 2010). Heat treatment is also employed to improve the organoleptic properties of some dairy formulations by manipulating the functionality of milk proteins (del Angel and Dalgleish, 2006). One of the most common milk thermal treatments is pasteurization. Pasteurised milk has an extended shelf life because of the consequent inhibition of microbial population growth, both pathogenic and spoilage (Walkling-Ribeiro et al., 2011). Microbial growth and metabolism shorten the shelf life of milk by producing undesirable changes in aroma and taste attributes influencing the consumer acceptability in products (Fromm and Boor, 2004). Nevertheless, some variations induced by thermization may alter some physical and chemical characteristics of milk. An overview of the milk changes caused by heating, is given by Walstra and Jenness (1984). Some important ones are a decrease of pH, precipitation of calcium phosphate, denaturation of whey proteins and interaction with casein, lactose isomerisation, Maillard browning, and modifications to the casein micelle. The overall effect is the altering of the sensory characteristics, i.e. overall appearance, colour, flavour, texture and the nutritional value as well as making it safe and improving its keeping quality (Lewis and Deeth, 2009).

In order to increase the milk shelf life, thermic treatments, such as lyophilization or pulverization, are

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obtained by removing the water (Ribeiro and Ribeiro, 2010), that is the main cause of acidification by hydrolysis of fats into free fatty acids (Breckenridge and Kuksis, 1968). Vincenzetti et al. (2011) observed that the pulverization, obtained at the temperatures next to 200 °C, as well as reduces the content of some nutrients, inactives significantly lysozyme (reduced by 70% at 90 °C) and almost all enzymes and vitamins of the milk (Polidori and Vincenzetti, 2013). Moreover, farmers should support high production costs to acquire instruments for freeze-drying and spray dry.

To transform milk in dairy products (yogurt, soft cheeses, etc…) an alternative method is the condensation. Condensed milk is the product obtained by evaporating part of the water contained in whole or in skimmed milk. Condensed milk is a dairy product that may be utilized as an intermediate material for evaporated, sweetened condensed and dried milk productions or as a final product for the consumer both in foods and in cosmetics. Low water content in condensed milk limits milk acidification and bacterial growth (Scott, 1957). In literature studies on effect of condensation are scarce. Water removal causes a reduction in the spacing between particles. Vélez-Ruiza and Barbosa-Cánovas (1998) evidenced that the major interactions between cow milk solids, such as proteins and lipids, and the physicochemical changes within components could seriously affect characteristics of milk. On the contrary, McLaughlan et al. (1981) observed that heat treatment may have no serious harmful effect on nutritional value apart from some destruction of heat-labile vitamins. In the present study, the effects of phisical treatments, such as pastorization and condensation, on total antioxidant capacity of jenny milk were assessed. Jenny milk may incur quickly to souring due to its water content, which is higher than cow milk (90.8 vs. 87.8%, respectively) (FAO 2013). Jenny milk has recently stimulated scientific interest for its attractive nutritional characteristics that make it fit to use in thepediatric sphere, in patients affected by Cow Milk protein Allergies (CMA) and intolerance, as the best alternative to human milk in infant food, and in the geriatric field for the treatment of aging diseases (Nazzaro et al., 2010). Moreover, jenny milk is commonly used in cosmetics for its emollients and antioxidants properties (El-Agamy, 2007). Jenny milk is rich in omega 6, omega 3, lysozyme, vitamins and minerals, and it is renowned for its moisturizing and antioxidants properties (Cosentino et al., 2012 and 2013).

MATERIALS AND METHODS

The research was carried out on bulk milk of 15 pluriparous Martina Franca breed, in mid-stage of lactation (180 days after foaling), ageing between 7 and 10 years. Jennies were fed on *ad libitum* oat hay with an integration of 1 kg head/dayof concentrate, characterized by the following mixture: 37% flaked corn, 30% oats, 9% locust bean crushed, 8% wheat bran, 8% dehydrated alfalfa, 6% beet pulp dried, 2% minerals and vitamins supplement.

After milking, milk aliquots were immediately refrigerated at +4 °C and transported to the laboratory, where, by Milkoscan FT 6000, the following chemical composition was determined (mean percent ± SD): protein (1.72±0.03), fat (0.16±0.03), lactose (6.42±0.01), and dry matter content (12.74±0.05).

The effect of different levels of concentration on the antioxidant scavenging activity of jenny milk was evaluated on Raw (R) and on Pasteurised (P) milk. Pasteurization was obtained by heating raw jenny milk at +63 °C for 30 min. Milk was condensed by rotary vacuum evaporation using a system RV8, IKA®-Werke (GmbH and Co. KG, Germany) equipped with a vacuum pump model PC 3001Vario (Vacuumbrand GmbH, Wertheim, Germany), under the following conditions: water bath temperature, +35 °C; pressure 40 mBar; processing time, 2and 3 h for condensation at 40%(R_{C40} and P_{C40}), and at 20%(R_{C20} and P_{C20}) of the initial volume, respectively. Before determining antioxidant activity, in order to reconstitute the initial water volumes, 6 ml of bidistilled water were added to 4 ml of R_{C40}or P_{C40}, and 8ml of bidistilled water were added to 2 ml of R_{C20} or P_{C20}.

As showed by Frankel and Meyer (2000), the determination of antioxidant capacity in food is affected by the type of assays, as well as by the substrate and the mode of inducing oxidation. To the best of our knowledge there is no study describing Total Antioxidant Capacity (TAC) in jenny milk. In the present study, two tests were performed, ABTS and DPPH. These two methodics were previously employed on human milk by Martysiak-Żurowska and Wenta (2012). In this trial, these assays were used to determine the Radical Scavenging Activity (RSA%) of raw and of pasteurised milk (R_{C0} and P_{C0}) at 40% (R_{C40} and P_{C40}), and at 20% (R_{C20} and P_{C20}) utilizing the following formula: Radical Scavenging Activity (RSA%) = $(1 - A_i / A_0) \times 100\%$, where A_i is the absorbance of sample and A_0 is the absorbance of colorimetric radical substance without sample. *DPPH assay***-** The stock radical solution of the DPPH was prepared by dissolving 20 mg of 2,2-diphenyl-1-picrylhydrazyl (DPPH) in 15 ml of methanol. Thereafter, 1 ml of the stock DPPH solution was diluted with 29 ml of methanol. A fixed ammount of milk (50 µL) was then added to 950 µL of DPPH solution and left in dark for 30 minutes. After centrifuging (5 min, 8000 rpm), absorbance was measured at 515 nm against the reference sample (methanol) by using spectrophotometer UV-Vis (LKB Biochrom 4050 Ultrospec II). Mesurement of absorbance was carried out in triplicate (Martysiak-Żurowskaand Wenta, 2012).

ABTS assay - This assay is based on the reduction of $ABTS^{\bullet+}$ radicals by antioxidants of tested milk. The stock

Table 1. Radical Scavenging Activity (%) of jenny milk.

Sample[1]	ABTS			DPPH		
	Average	SD[2]	CV[3]	Average	SD[2]	CV[3]
R_{C0}	84.15[a]	0.94	1.12	45.24[a]	4.73	10.46
R_{C40}	82.09[b]	1.72	2.10	37.50[b]	3.43	9.14
R_{C20}	80.40[c]	1.24	1.54	29.61[c]	2.37	7.99
P_{C0}	82.23[b]	1.34	1.63	44.19[a]	2.71	6.12
P_{C40}	80.62[c]	1.12	1.39	37.18[b]	3.12	8.40
P_{C20}	79.66[c]	1.91	2.40	28.08[c]	3.50	12.46

[1]Samples are combinations of raw (R) and pasteurised (P) jenny milk at different levels of condensation (0, 40, and 20% of the initial milk volume).
[2]SD = standard deviation
[3]CV = coefficient of variation.
Means in the same column with different letters are significantly different (P<0.05) according to the Tukey's HSD test.

solution of the ABTS radical was prepared by dissolving 38 mg of 2,2'-azinobis (3-ethylbenzthiazoline-6-acid) (ABTS) in 10 ml of a sodium persulphate solution (2.45 mM), and the mixture was kept in dark for 12-16 hours. For the analysis, 1 ml ofstock $ABTS^{\bullet+}$ solution was diluted with 29 ml of methanol. A fixed amount of milk (20 µL) was added to 980 µL of $ABTS^{\bullet+}$ solution. Samples were then incubated for 2 h in dark and at room temperature. After centrifuging (5 min, 8000 rpm), absorbance was measured at 734 nm against the reference sample (methanol) by using spectrophotometer UV-Vis (LKB Biochrom 4050 Ultrospec II). The solutions were prepared fresh for the analysis and all determinations were carried out in triplicate (Re et al., 1999).

To assess the effect of thermic treatment (2 levels: R and P) and condensation (3 levels: C0, C40, and C20) on antioxidant capacity of jenny milk, data from both ABTS and DPPH assays were subjected to two-way ANOVA, and the means were compared by Tukey's HSD at P<0.05.

RESULTS AND DISCUSSION

Two-way ANOVA revealed that both the factors 'thermic treatment' (P<0.001) and 'condensation' (P<0.001) had a significant effect on TAC of jenny milk measured by ABTS assay. Concerning DPPH assay, only condensation was significant (P<0.001) on TAC. In both assays, the interaction thermic treatment× condensation was never significant.

Both assays evidenced the lowest antioxidant activity of P milk, even if this difference was significant (P<0.05)only in ABTS assay (Table 1). This test is considered the most sensitive method, since it is characterized by higher repeatability, detectability and sensibility than DPPH assay (Martysiak-Żurowska and Wenta, 2012). Our results seem to confirm the difference in sensitivity between the assays. Moreover, the Coefficient of Variation (CV) values were the lowest when measuring TAC by ABTS (Table 1). In this regard, Marinova and Batchvarov (2011) evidenced that DPPH determinations may be affected by several aspects, such as the type of solvents (methanol, ethanol, etc.), concentration of DPPH working solutions, ratio between volumes of sample/reagent, duration of reaction, wave length of absorbance measurement, standard solutions, and equations for calculation of the results. Other authors (Frankel and Meyer, 2000; García-Herreros et al., 2010). higligthed the difficulty to compare TAC results from ABTS and DPPH assays in food and in biological systems because of their oxidative deterioration. For this reason, in this paper only the ABST data were commented.

As shown in Table 1, pasteurisation reduced the antioxidant capacity of raw milk from 84.15 to 82.09%.TAC also decreased at growing levels of condensation: from 80.60 (R_{C40})to 80.40% (R_{C20}). Similarly, in pasteurised milk, the TAC was reduced from 82.09 (P_{C40}) to 79.66%(P_{C20}). In each stage of condensation, antioxidant activity of raw milk resulted greater than in pasteurized milk (Table 1); these differences were always significant (P<0.05), with the exception for the comparison R_{C20}- P_{C20}.

Jenny milk is rich in vitamins, which have a great antioxidant activity (Polidori and Vincensetti, 2013). Previous studies showed that vitamins could be thermally degraded also at temperatures below 30 °C (Vikram et al., 2005). Moreover, Alyaqoubi et al. (2014) put in evidence that the capability of the pasteurized milk samples to scavenge and to destroy the free radical chain was weaker than that of the unpasteurized fresh

samples. A previous study (De Feo et al.,2006) reported that pasteurization negatively affects the total polyphenol concentration because of the possible denaturation of phenol content and amino acid catabolism. These differences in antioxidant capacity can be attributed to the higher serum proteins content and to the lower content of albumin in pasteurized than in raw milk. In milk, several components are active, and lipids contribute to a large proportion of the scavenging capacity (Castillo et al., 2013). According to Chen et al. (2003), a major $ABTS^{+}$scavenger in milk is probably casein, because it has a high content of potentially antioxidative amino acids, such as tyrosine, tryptophan, histidine, lysine and methionine (Uchida and Kawakishi, 1992). It was previously reported that casein and its hydrolysates can scavenge DPPH (1,1-diphenyl-2-picrylhydrazyl) and hydroxyl radicals (Suetsunaet al.,2000), and inhibit enzymatic and non-enzymatic lipid peroxidation, probably due to a free radical scavenging mechanism (Rival et al., 2001). Lysozyme, in so high concentration in jenny milk (Vincensetti et al., 2007), could also contribute to increase the antioxidant capacity, because it is rich in lysine, amino acid that shows an antioxidative activity (Ahmad et al., 1996).

In conclusion, both pasteurization and condensation affected the TAC of jenny milk, although %RSA decreased by only few percentage points. Further studies are needed to identify the components of jenny milk responsible for its antioxidant activity, and to quantify them after different physical treatments.

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