Traceability of Functional Bioactive Compounds in Fresh and Pasteurized Milk Obtained from Goats Fed with Orange Pulp

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ABSTRACT
Traceability is the ability to identify and trace the history, distribution, location, and application of products, parts, and materials. A traceability system records and follows the trail as products, parts, and materials come from suppliers and are processed and ultimately distributed as end products (Prache et al., 2002). In this work, were studied the bioactive compounds (total vitamin C, ascorbic acid, total phenols, flavonoids, carotenoids, vitamin A and vitamin E) and antioxidant activity of goat fresh milk and pasteurized one. The goats were fed with a standard diet (control diet) and then with a diet that incorporates orange pulp. The control diet (CD) corresponded with a standard ration (a ration which provide the energetic and proteic values), daily food for milking animals. From that ration, the Department of Animal Science, from Politechnic University of Valencia replaced the different proportions of the ingredients for incorporating orange pulp diet (OPD). The results of the present study show that the citrus pulp silage mixture used can be fed to goats without any negative effects on the performance of the animals. Results of this study indicate that citrus pulp silage can replace part of the conventional ration of goats, thus lowering the cost of production. The first aim of this study was to compare the two types of goat diets: a standard diet and a diet with orange pulp, by analyzing the bioactive compounds in fresh and pasteurized milk. The results demonstrate that all the bioactive compounds are bigger in the orange pulp diet than in the control diet. The second objective of this study was to analyze the bioavailability and traceability of bioactive compounds in fresh milk.

Keywords: functional bioactive compounds, goat milk, orange pulp.

INTRODUCTION
Citrus pulp is the residue of citrus juice canning industry. The common raw material for juice industry, particularly in countries around not only the Mediterranean basin but also elsewhere in the world, is orange fruit ‘Citrus sinensis’. Citrus pulp is a widespread by-product used mainly in ruminant diets (Gohl, 1981).

Since citrus pulp is a cheap raw material widely available in Mediterranean countries, its feeding value should be fully investigated with sheep and goats, the dominant farm animal species in the area. The conservation of fresh citrus pulp is very important for semiarid areas, since in this way roughage is made available during the dry summer, when feed resources from pastures become scarce (Volanis et al., 2004).

The valorization of residues requires knowledge of their chemical composition. Orange waste contains 16.9 g 100 g⁻¹ soluble sugars, 9.21 g 100 g⁻¹ cellulose, 10.5 g 100 g⁻¹ hemicelluloses, and 42.5 g 100 g⁻¹ pectin, which is its most important component. Due to its composition being rich in soluble and insoluble carbohydrates, this by-product shows great potential for use in products with high benefit obtained through
chemical or enzymatic hydrolysis and subsequent biological conversion (Rivas et al., 2008).

The soluble sugars present in orange peel are glucose, fructose, and sucrose. The insoluble polysaccharides of the cellular wall of the orange peel are composed of pectin, cellulose, and hemicelluloses. Pectin and hemicelluloses are rich in galacturonic acid, arabinose, and galactose, and also contain small amounts of xylose, rhamnose and glucose (Torado et al., 2011).

The orange juice industry uses approximately 50% of the fruit, while the other 50% is peels, seeds and albedo, which can reach 60% of the total byproducts (Tainara et al., 2013).

In the year 2008, the most recent year with available data, Spain had a caprine population of 2,959,300 head, of which 1,403,850 were milking goats (FAO, 2010). One of the major strengths of the Spanish goat industry is the general presence of indigenous breeds (‘Murciano-Granadina’, ‘Malagueña’, ‘Florida’, ‘Payoya’, ‘Palmera’, ‘Majorera’, ‘Tinerfeña’) that show an acceptable productivity of high-quality milk (Sidonia Martinez et al., 2011).

The Murcia-Granada is well adapted to the hot and dry conditions of the semiarid areas of southeastern Spain. It is the most productive domestic animal in this climate because of its ability to maintain a high milk production under less than ideal conditions.

Goat milk differs from cow or human milk in having better digestibility, alkalinity, buffering capacity and certain therapeutic values in medicine and human nutrition (Park and Chukwu, 1988). Sheep milk has higher specific gravity, viscosity, refractive index, titratable acidity, and lower freezing point than average cow milk (Haenlein and Wendorff, 2006).

Goat milk also exceeds cow milk in the provision of mono-unsaturated, poly-unsaturated fatty acids and medium chain triglycerides – all of which are well known to be beneficial for human health, especially for cardiovascular conditions (Haenlein, 2004).

Many studies over the last 10 years have demonstrated that milk fat may offer health benefits compared to some common sources of dietary fats (MacRae et al., 2005).

If a human infant fed solely on goat milk, the infant is oversupplied with protein, Ca, P, Vitamin A, thiamin, riboflavin, niacin and pantothenate, in relation to the FAO-WHO requirements (Jenness, 1980).

**MATERIALS AND METHODS**

In a pre-experimental period of 14 days, 24 goats of the breed Murciano Granadina, in the sixth month of lactation, from the farm of small ruminant from the Politechnic University of Valencia were fed with a control diet and then, the goats had been divided in two groups taking into account their productive characteristics and composition of their milk. From the point of energetic and protein view each group were randomly provided with different nutritionally equivalent diets.

Animal Science Department from UPV, started to feed the goats with a control diet (CD) similar to a standard ration food for milking animals. From that standard ration, the Department started to replace different ingredients proportions with the objective to include orange pulp diet (OPD).

**Tab. 1. The ingredients used for the control diet and the orange pulp diet**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>g/animal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfalfa</td>
<td>1000</td>
</tr>
<tr>
<td>Nutriment Biona</td>
<td>1300</td>
</tr>
<tr>
<td>Straw</td>
<td>200</td>
</tr>
<tr>
<td>Alfalfa</td>
<td>1500</td>
</tr>
<tr>
<td>Nutriment Biona</td>
<td>100</td>
</tr>
<tr>
<td>Straw</td>
<td>200</td>
</tr>
<tr>
<td>Orange pulp</td>
<td>2500</td>
</tr>
<tr>
<td>Soy</td>
<td>360</td>
</tr>
</tbody>
</table>

The first 25th days, the first one was fed with CD, and the second groups was fed with OPD.

To be sure that the results are with a high accuracy, the diet was changed: the second group became the first group, and the first group the second one (who was eating orange silage).

**Tab.2. Standard diets from goats groups**

<table>
<thead>
<tr>
<th>Cycle</th>
<th>From the first day to 25th day</th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycle 1</td>
<td>CD</td>
<td>OPD</td>
<td></td>
</tr>
<tr>
<td>Cycle 2</td>
<td>From 26th day to 46th day</td>
<td>OPD</td>
<td>CD</td>
</tr>
</tbody>
</table>
In this study was analyzed total vitamin C, ascorbic acid, total phenols, flavonoids, carotenoids, vitamin A, vitamin E, and antioxidant activity of the animal food composition.

Ascorbic acid (AA) and total vitamin C (ascorbic acid + dehydroascorbic acid) were determined by HPLC (Jasco, Italy), according (Xu et al., 2008a). Samples were homogenized and the mixture was centrifuged (Selecta Medifriger-BL) at 2630×g for 10 min at 4 °C. 1 mL supernatant aliquot was extracted with 9 mL 0.1% oxalic acid for 3 min. Afterwards, the same procedure as that used for the ascorbic acid method was performed.

Total phenols (TP) were analyzed by using the method reported by Selvendran and Ryden (1990) and Benzie and Strain (1999) based on the Folin–Ciocalteu method, which involves the reduction of the reagent by phenolic compounds with the concomitant formation of a blue complex. The homogenate was centrifuged (2630×g, 10 000 rpm, 10 min, 4 °C) to obtain the supernatant which was filtered by a 0.45 μm membrane filter.

The samples were mixed and allowed to stand for 8 min in darkness before 3.75 mL of 7.5% sodium carbonate aqueous solution was added. Water was added to adjust the final volume to 25 mL.

Samples were allowed to stand for 2 h at room temperature before absorbance was measured at 765 nm in a UV–visible spectrophotometer (Thermo Electron Corporation, USA). The total phenolic content was expressed as mg of gallic acid equivalents (GAE) (Sigma-Aldrich, Germany) per g of sample.

The extraction of flavonoids was carried out in the same way as that of total phenols but using bidistilled water instead of HCl.

Total flavonoids were determined using 1 mL of extract obtained from the total phenols to which were added 1 mL of AlCl3 methanolic solution (20g/L).

This mixture was left in the dark for 30 minutes and then measured the absorbance at a wavelength of 430 nm, using a UV-visible spectrophotometer (Thermo Electron Corporation, USA).

Nowadays, antioxidants have gained more importance because of their positive involvement as health promoters. In this context, several epidemiological studies have associated the consumption of phenol compounds with lower risks of different types of disorders such as cancer and cardiovascular disease (Hooper et al., 2008). Besides, when added to foods, antioxidants minimize rancidity, retard the formation of toxic oxidation products, maintain nutritional quality, and increase shelf life of food products (Jadhav et al., 1996).

Antioxidant capacity was assessed using the free radical scavenging activity of the samples evaluated with the stable radical DPPH, as described by Sánchez-Moreno et al., 2003.

Briefly, milk was homogenized and centrifuged (Selecta Medifriger-BL) at 2630×g for 10 min at 4 °C. 0.1 ml of supernatant diluted in methanol was added to 3.9 ml of DPPH (0.030 g/L, Sigma-Aldrich, Germany) in methanol.

A Thermo Electron Corporation spectrophotometer (USA) was used to measure the absorbance at 515 nm at 0.25 min intervals until the reaction reached a plateau (time at the steady state).

In the case of vitamins A and E, the milk was homogenized (T25 Janke and Kunkel Turrax). Ethanol (4 mL) was added to 2 g homogenate milk and the mixture was centrifuged (Selecta Medifriger-BL) at 2,000 rpm for 3 min at 4°C.

The supernatant was filtered through a Whatman no. 1 filter paper and 0.5 mL of n-hexane was added to the filtrate and mixed. Vitamins A and E were extracted twice in the hexane phase and the collected extract was dried under a stream of liquid nitrogen. The dried extract was solubilized in 0.2 mL methanol.

HPLC conditions were as follows: mobile phase, methanol/acetonitrile/chloroform (47:42:11, v/v/v); volume injection, 20 μL; flow rate, 1 mL/min; detection at 326–296 nm at 25 °C.

The carotenoids present in the samples were extracted following the methodology of Olives et al., 2006. To this end, 5 ml of milk were placed in a test tube protected from light, and mixed with 50 mL of hexane/acetone/ethanol (50:25:25, v/v/v) extraction solvent. The mixture was magnetically stirred for 30 min.

Distilled water (15 mL) was added and an upper layer aliquot of 0.6 mL was dried under a stream of liquid nitrogen. The residue was dissolved with tetrahydrofuran/acetonitrile/methanol solution (15:30:55, v/v/v) to a final volume of 1 mL. The spectrophotometric reference method of AOAC (2000) was used for quantification. Sample absorbance was measured at 446 nm and at 415 nm in a UV-visible spectrophotometer (Thermo
Electron Corporation, USA). The total carotenoid content was expressed as milligrams of β-carotene per 100 g of dried solids. Standard of β-carotene was provided by Fluka-Biochemika (USA).

RESULTS AND DISCUSSION

First of all, were analyzed total vitamin C, ascorbic acid, total phenols, vitamin A and vitamin E, antioxidant activity of the ingredients used in animal food (Table 3).

The average between ascorbic acid from the superficial orange pulp and intermediate orange pulp is 22.8 mg and is smaller than the average between vitamin C from superficial orange pulp and intermediate orange pulp.

After analyzing the ingredients bioactive compounds, were analysed the bioactive compounds from fresh and pasteurized goat milk, after the diet (first day) and in the third and seventh day of the diet.

Flavonoids amount in fresh milk is low (0.0035mg/100g) and the recovery in pasteurized milk does not exist.

Carotenoids amount in fresh milk is low and the recovery in pasteurized milk doesn’t exist.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Ascorbic acid (mg)</th>
<th>Vitamin C (mg)</th>
<th>Total phenols (mg)</th>
<th>Antioxidant activity (mmol Trolox)</th>
<th>Vitamin E (mg)</th>
<th>Vitamin A (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superficial orange pulp</td>
<td>23.31</td>
<td>28.62</td>
<td>44.97</td>
<td>22.41</td>
<td>0.00</td>
<td>0.04</td>
</tr>
<tr>
<td>Intermediate orange pulp</td>
<td>22.29</td>
<td>23.31</td>
<td>56.27</td>
<td>10.24</td>
<td>0.00</td>
<td>1.20</td>
</tr>
<tr>
<td>Alfalfa</td>
<td>5.27</td>
<td>5.33</td>
<td>74.49</td>
<td>2.67</td>
<td>0.03</td>
<td>0.41</td>
</tr>
<tr>
<td>Straw</td>
<td>0.00</td>
<td>0.00</td>
<td>108.33</td>
<td>0.66</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Nutriment Biona</td>
<td>0.00</td>
<td>0.00</td>
<td>274.24</td>
<td>23.53</td>
<td>0.36</td>
<td>0.07</td>
</tr>
<tr>
<td>Soy beans</td>
<td>2.87</td>
<td>3.24</td>
<td>81.00</td>
<td>0.33</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Naranja Intermedia</td>
<td>22.29</td>
<td>22.29</td>
<td>75.42</td>
<td>17.33</td>
<td>0.00</td>
<td>0.06</td>
</tr>
<tr>
<td>Alfalfa</td>
<td>5.37</td>
<td>5.20</td>
<td>88.06</td>
<td>9.77</td>
<td>0.04</td>
<td>0.17</td>
</tr>
</tbody>
</table>

![Fig.1](image1.png)  
**Fig.1.** Total vitamin C content from the first and the second group – fresh milk

![Fig.2](image2.png)  
**Fig.2.** Total vitamin C content from the first and 2 second group - pasteurized milk
Traceability of Functional Bioactive Compounds in Fresh and Pasteurized Milk from Goats Fed with Orange Pulp

**Fig. 3.** Ascorbic acid content in fresh milk

**Fig. 4.** Ascorbic acid content in pasteurized milk

**Fig. 5.** Total phenols content in fresh milk

**Fig. 6.** Total phenols content in pasteurized milk

**Fig. 7.** Total flavonoids content in fresh milk

**Fig. 8.** Antioxidant activity content in fresh milk

**Fig. 9.** Antioxidant activity content in pasteurized milk

**Fig. 10.** Vitamin A content in fresh milk
CONCLUSION

The citrus pulp silage can replace part of the conventional ration of goats, thus lowering the cost of production. All the bioactive compounds are bigger in the orange pulp diet that in the control diet. As was noticed, total vitamin C content from the diet is recovery by 40% in fresh milk, ascorbic acid by 30% and total phenols by 60%. In pasteurized milk, total vitamin C amount is almost the same that in fresh milk, ascorbic acid has the same amount and total phenols recovery is 70%. Flavonoids amount in fresh milk is low (0.0035mg/100g) and the recovery in pasteurized milk does not exist. The carotenoid content from the orange pulp diet is recovered in milk by 20%. Carotenoid concentration and colour in dairy products can be rapidly and efficiently controlled by dairy cow feeding management (Noziere et al, 2006). To conclude, diet orange silage is useful from two points of view: economically one and due to the supplementation of bioactive compounds in fresh and pasteurized milk.

REFERENCES

6. FAO (Food and Agriculture Organization), 2010. Official statistics. Rome


