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Original Article

# Fortification of bovine milk with natural polyphenols extracted from pomegranate peels

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**Summary.** Polyphenols were extracted from Pomegranate peels and then encapsulated by ionic gelation. The aqueous extract and the microbeads were used for the preparation of fortified milk. All beverages were kept in refrigerator and the shelf life of milk in terms of polyphenols content was studied. Results showed that 62.23% of polyphenols remained stable for 11 days in milk fortified with alginate-pectin (2:1) microbeads, while only 38.13% and 5.47% of polyphenols remained stable in milk fortified with sodium alginate microbeads and aqueous extract, respectively. Results also suggest that the formation of milk protein-polyphenol complexes compromises the antioxidant potential of the beverage.

**Key words:** antioxidant, fortified milk, microbeads, polyphenols, pomegranate peels, shelf life

## Introduction

Functional foods provide health benefits over and above normal nutrition (1). They must generally be made available to consumers in forms that are consumed within the usual daily dietary pattern of the target population group. Consumers expect functional foods to have good organoleptic qualities and to be of similar qualities to the traditional foods in the market (2). New functional food products launched in the global food and drinks market have followed the route of fortification or addition of desirable nutrients and bioactives including antioxidants. Those ingredients are prone to degradation and interaction with other components in the food matrix, leading to loss in quality of the functional food products. To overcome these problems, the added antioxidant should be isolated from environments that promote degradation or undesirable interactions. This may be accomplished by the use of microencapsulation where the sensitive antioxidant is packaged within a secondary material for delivery into food products.

Different types of phytochemicals have been identified in various parts of the pomegranate tree (*Punica granatum*), including fruits, seeds and peels. The major class of pomegranate phytochemicals is the polyphenols that predominate in the fruit (3). Epidemiological studies strongly support a role for polyphenols in the prevention of cardiovascular diseases, cancers, osteoporosis, diabetes mellitus, arthritis and neurodegenerative diseases, which are associated to oxidative stress and chronic inflammation (4, 5). Polyphenols produce their beneficial effects by scavenging free radicals. The beneficial effects of polyphenols depend on the amount consumed, their bioavailability and the biological activities of the formed conjugates (6). Direct interactions between polyphenols and some components of food, such as binding to proteins, fat, polysaccharides or alcohol, can occur, and these interactions may affect absorption (7, 8).

It has been considered that no less than 1–2 g of polyphenols should be consumed daily (9) whereas, in an average diet consumed in Poland, they are only 0.032 g daily (9). In Japan and in the USA, they are estimated to be 0.068 g and daily 1.1 g/daily, respectively (10, 11).

Functional dairy products account for 42.9% of the functional food market (12). Dairy products have

been the most popular delivery vehicles for a number of functional and healthy ingredients, from vitamin and mineral fortification to addition of bioactives to promote health benefits. As milk and dairy products are a normal part of our daily diet, in all life stages, any new product launched can be expected to gain some market share.

Although in literature some reports on milk enriched with polyphenols can be found (13, 14), there is -in our knowledge- no one developed using polyphenols extracted from pomegranate peels. In light of this, the aim of this study was the development of a new milk product fortified with natural polyphenols extracted from pomegranate peels. These peels are usually discarded as waste even a significant portion of polyphenols are often present in high concentrations in the outer parts of fruits. A simple and fast method for the extraction of polyphenols was described in our previous work (15). The gelled microbeads containing polyphenols extracted from pomegranate peels were formed by ionic gelation using a single polymer (sodium alginate) (16) and a combinations of sodium alginate and pectin at a ratio of 2:1 (17). These microbeads beads can entrap polyphenols in sufficient amount and also protect their antioxidant activity. They can also successfully deliver them in gastro-intestinal fluids (17). Stability of microbeads and the antioxidant activity of the resulting milk beverages were investigated during two weeks of cold storage.

## Materials and methods

*Chemicals and reagents*

Sodium alginate, pectin, pepsin and Folin-Ciocalteu reagent 2N were purchased from Sigma-Aldrich (Switzerland), 2,2-Diphenyl-1- picrylhydrazyl (DPPH) (Sigma-Aldrich, USA), calcium chloride and tri-sodium citrate were purchased from (Carl Roth, Germany), methanol (Sharlau, Spain), bovine milk was purchased from local markets.

*Equipments*

Micropipette 100-1000 µl (Iso lab, Germany), sensitive balance (Sartorius, Germany), ultrasonic bath, electric stirrer and heater, centrifuge (Shanghai surgical instruments factory, China), centrifuge Megafuge 2.0 R, (Heraeus instruments GmbH, Germany), spectrophotometer (Jasco V-530, USA), dissolution apparatus with paddle (Pharmatest, Geramny).

*Beads preparation*

The polyphenols extraction from pomegranate peels was performed as described by zam et al. (15). Beads were obtained by mixing 100 ml of the aqueous pomegranate extract with 100 ml of the sodium alginate solution 3% or a combination of 2% sodium alginate with 1% pectin solution. Once homogenized, 100 ml of calcium chloride solution 0.05 M was added to the alginate solution and was cured for 20 min at 25°C. The beads formed in this process were maintained in the gelling bath to harden for 15 min. Then, they were centrifuged at 4,000 rpm and 4°C for 15 min as preceded by Zam et al. (16, 17).

*Milk fortification and stability of polyphenols as a Function of Time (Shelf Life)*

Three different milk beverages fortified with 150 mg of polyphenols per serving (250 ml) were prepared. The first beverage was prepared by adding 200 ml of aqueous extract to 800 ml of milk (sample 1). The second was prepared by suspending microbeads of polyphenols prepared with sodium alginate (sample 2) and the last one was prepared by suspending microbeads prepared with a combination of sodium alginate and pectin (sample 3).

All beverages were kept in refrigerator and the shelf-life of milk beverages in terms of polyphenols content and radical scavenging activity quality was studied.

*Polyphenol content*

Polyphenol content in microbeads was estimated after centrifugation of fortified milk, the precipitated microcapsules were dissolved in sodium citrate (10% w/v) during 20 min in a shaker at 37°C and 125 rpm as reported by Deladino et al. (18). The amount of polyphenol content was determined by Folin-Ciocalteu ac-

cording to the International Organization for Standardization (19).

*DPPH radical–scavenging activity*

The antioxidant activity was measured in term of hydrogen donating or radical scavenging ability using the stable DPPH method according to the method proposed by Brand-Williams et al. (20).

To ensure that any effects noted were not simply due to dilution, pomegranate peels' aqueous extract (200 mL) with 800 ml of milk added was compared to a standard 200 mL of extract added to 800 ml of cold water. The total antioxidant activity was followed over 14 days.

*In vitro digestibility assay*

The method consists on a pepsin/HCl digestion with shaking to simulate gastric conditions. 315 units per mL of pepsin was added to the fortified milk (sample 3) and then the pH was adjusted to 1.7 with HCl (21). The digestion was carried out for 2 h at 37°C with shaking. After the 2 h, the antioxidant activity of the mixture was quantified.

## Results and discussion

*Shelf-life of milk beverages*

There has been evidence that polyphenols possess a high binding affinity for proteins (22, 23), particularly proline-rich proteins such as caseins (24). This affinity is strongly influenced by the structural differences of dietary polyphenols (25). Results demonstrated in Figure 1 were in agree with this evidence, as a rapid decrease of polyphenols content in milk fortified with the direct addition of pomegranate peels aqueous extract occurred. After 2 days of storage, 49.37% of polyphenols remained stable in fortified milk, and only 5.47% of polyphenol remained after 11 days of storage.

As noticed in Figures 2 and 3, the addition of polyphenols' microbeads was more effective for the fortification of milk beverages compared with the addition of pomegranate peels aqueous extract.

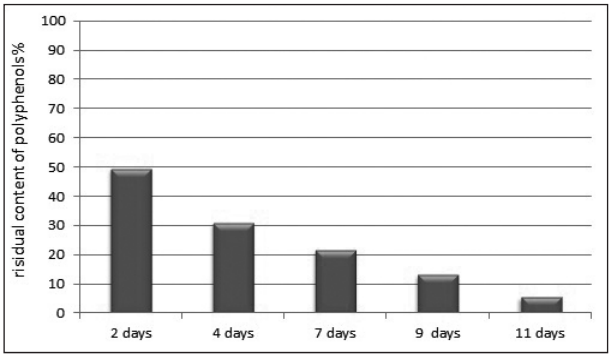


**Figure 1.** Residual content of polyphenols in milk beverage (sample 1).

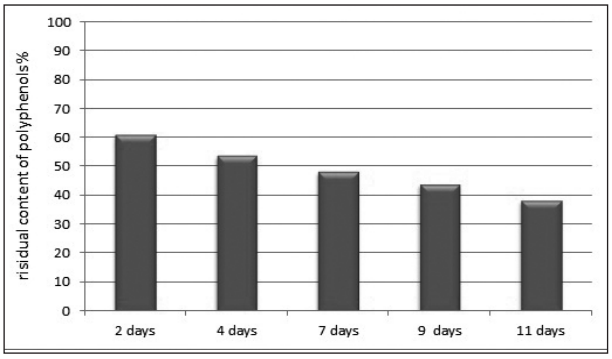


**Figure 2.** Residual content of polyphenols in milk beverage (sample 2).

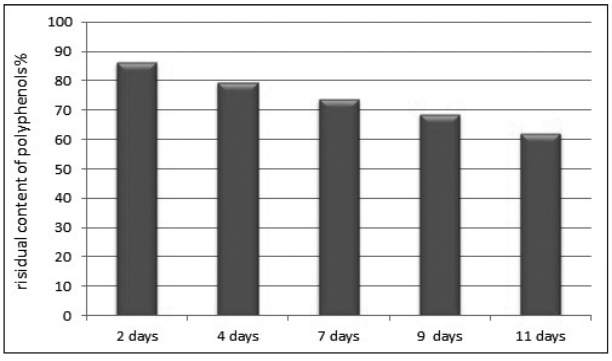


**Figure 3.** Residual content of polyphenols in milk beverage (sample 3).

Results also indicated that the stability of microbeads was less when prepared with a single type of polymer in comparison of the beads prepared with two types of polymers (sodium alginate and pectin). As only 38.13% of polyphenols remained stable in sample 2, while 62.23% of polyphenols remained stable in sample 3, after 11 days of cold storage. This is due to

the presence of two types of protective layers in beads, one of calcium pectinate and other one of calcium alginate, which prevented the diffusion of polyphenols more effectively than a single type of layer only.

*Antioxidant activity of polyphenols-milk beverages*

The antioxidant activity of polyphenols in the body is well established, but the effect of the addition of milk on the bioavailability of antioxidant polyphenols still very contradictory. Van het Hof et al. (26) showed that consumption of black tea was followed by a rapid increase of the total polyphenols concentrations in blood. However, they noticed that milk did not impair the bioavailability of tea polyphenols. Green et al. (27) noted an increase in polyphenols recovery from teas formulated with 50% bovine milk after an in vitro digestion procedure. Hollman et al. (28) found that the increase in plasma concentrations of polyphenols after black tea consumption was also not affected by the addition of milk to tea. These results do not support the findings of Arts et al. (29) who reported that the interaction between flavonoids and proteins affects their antioxidant activity in vitro. Serafini et al. (30) also reported that the increase in antioxidant activity of plasma after black tea consumption was attenuated by the addition of milk.

Our results are in agree with those of Arts et al. (29) and Serafini et al. (30) as a decrease in the total antioxidant activity occurred directly after the addition of milk to the aqueous pomegranate peels extract compared with the diluted standard. As noticed in Figure 4, the total antioxidant activity decreased to 11.00% in fortified milk while the antioxidant activity of the diluted extract was 45.02% after 7 days of storage in refrigerator. And only 1.86% of the antioxidant activity remained after 14 days in fortified milk compared with 43.37% for the diluted extract.

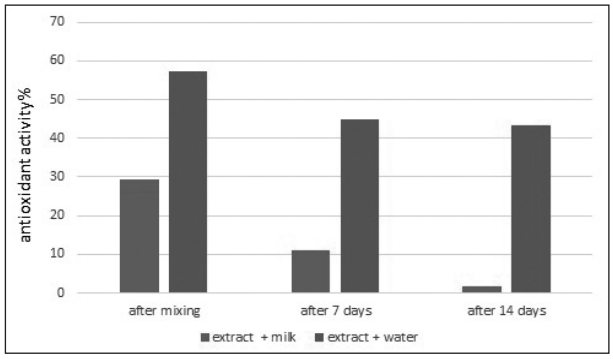


**Figure 4.** Change in total antioxidant activity of aqueous pomegranate peels extract mixed with milk or water over 14 days.

*In vitro digestibility assay*

As observed in Table 1, the antioxidant activity of polyphenols decreased directly from 58.48% to 27.67% after being mixed with the milk sample. After digestion, the antioxidant activity raised again to 40.56% which could be attributed to the partial degradation of complex and the release of polyphenols.

From Table 1 it is possible to observe that after digestion the antioxidant activity of polyphenols is less of its activity initially present in the extract. This means that some complexes formed are more resistant to the digestion conditions performed in this study. Soares et al. (31) have already demonstrated that complexes formed with the lowest polymerized tannins (monomers, dimers and trimers) could be disrupted by gastric digestion leading to the release of tannins whereas the complexes formed by tannin tetramers and pentamers were significantly more resistant to stomach conditions. These results could explain the findings of our study.

## Conclusion

Functional milk beverages fortified with polyphenols (150 mg/250 ml) extracted from pomegranate peels were manufactured.

Results showed that polyphenols' microbeads prepared by ionic gelation using a combination of sodium alginate and pectin at a ratio of 2:1 was suitable for preparation of functional milk beverages.

**Table 1**. Antioxidant activity of the extract and the fortified milk (sample 3) before and after digestion.

| Sample | Antioxidant activity |
|---|---|
| Extract | 58.48 |
| milk + extract | 27.67 |
| milk + extract after digestion | 40.56 |

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