Nanoemulsions of grape marc extract as natural additives to improve hazelnut paste shelf-life

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**ABSTRACT**

In previous studies the authors obtained a phenolic freeze-dried extract from red-grape marc by hydro-alcoholic extraction. Antioxidant properties of the extract could be maintained during long term storage, but the extract shows limited solubility both in aqueous and lipid systems. Since addition of the extract to hazelnut paste as natural additive to protect it against lipid oxidation improving its quality and shelf-life appears an interesting and profitable application, this study investigated the encapsulation of the extract into different nanoemulsion-based delivery systems. Three different delivery systems were fabricated at the nanoscale (around 200 nm) by high pressure homogenization for the encapsulation of the marc extracts. Formulation 1 was a sunflower-oil/water nanoemulsion with soy lecithin as emulsifier. Formulation 2 was a powder obtained by maltodextrin-assisted spray-drying of Formulation 1. Formulation 3 was an ethanol/solid-lipid nanoemulsion, produced by hot homogenization using soy lecithin as emulsifier. The original extract and its encapsulating formulations were homogenized into hazelnut paste at a concentration of 5000 ppm, resulting into different phenolics content, ranging from 1200 ppm for crude extract to less than 10 ppm for the delivery systems. An accelerated shelf-life test at 60°C was carried out and peroxides value (PV) of the lipid phase was periodically analyzed (until 100 days) to verify the protective effect of the extracts against oxidation. Results showed the efficiency of nanoemulsion formulation in improving solubility of the extract into the hazelnut paste. Addition of the extract could significantly improve the paste shelf-life by inhibiting its oxidation. Improved dispersion of nanoemulsion also increased the efficiency of the phenolic compounds, reducing the amount of extract required for the potential production of a natural preservative agent.

**Keywords:** Nanoemulsions; hazelnut paste; phenolic extract; shelf-life.

**INTRODUCTION**

In previous studies [1] we obtained a phenolic freeze-dried extract from red-grape marc (typical wine-making by-product) by hydro-alcoholic extraction. The extract could be stored for long times while maintaining good antioxidant properties. This extract offers potential applications as a natural-low-cost additive to improve quality and extend shelf-life of foods without the use of synthetic additives, such as BHA and BHT which have restricted use into foods for their toxicity. Particularly, a solution that would bring to a profitable phenolic extract is its employment for the development of innovative products and ingredients used for the production of many other foods. Actually, hazelnut paste is a food ingredient employed into the processing of different foodstuffs (ice-creams, confectionery and bakery products). Its lipid fraction, mainly unsaturated fatty acids, can easily undergo oxidation. Addition of the marc extract could be exploited to produce a more stable hazelnut paste and it has never been investigated.

However, the previously obtained freeze-dried extract exhibits a very low water and lipid solubility making its application into most food systems extremely difficult. While many investigations were carried out on the antioxidant, antimicrobial and pharmacological activity of phenolic extracts, little has been studied on technological implications that their addition into foods might have, even though for a real diffusion you should take into account important aspects related to miscibility, stability and interaction with the other food components [2].

Recently, nanotechnology significantly contributed to the development of nanometric delivery systems, capable of encapsulating the antioxidant compounds, of protecting them from undesired reactions, of minimizing the impact on the organoleptic properties of the product, as well as of enhancing their activity by
promoting the mass transfer rates to sites of action [3]. Different types of delivery systems exist, whose preparation requires an adequate formulation and opportune processing conditions. Among the nanoencapsulation systems, nanoemulsions and lipid nanoparticles appear particularly suitable for food applications. Nanoemulsions are very fine, kinetically stable emulsions, made of lipid droplets of nanometric size (50-200 nm), dispersed in aqueous phase, prepared by means of high pressure homogenization and the use of an adequate emulsifier at water/oil interface [4]. Lipid nanoparticles are systems very similar to nanoemulsions, with the difference that the disperse lipid phase is in solid state. The production is carried out similarly to nanoemulsion, with processing temperature of high pressure homogenization above the fusion point of lipid phase [5]. Depending on the emulsifier employed and the relative ratio of lipid and aqueous phase can be prepared, which are specifically suitable for the incorporation in foods with high lipid content. In particular, grape marc phenolics can be solubilised in an ethanol aqueous solution, which is subsequently nanodispersed in a lipid phase. Recently, the authors also proposed the use of physical processing methods (high-pressure homogenization), which reduce particle size and generate an amorphous state, to improve the dispersity of polyphenolic powder particles (i.e. curcumin) [6].

Based on these premises, this study investigated the exploitation of the protective effect of a grape-marc phenolic freeze-dried extract against the oxidation of a commercial hazelnut-paste by encapsulating the extracts into different nanoemulsion-based delivery systems with the aims of (a) improving the dispersion of the phenolic compounds into the hazelnut paste and (b) enhancing the antioxidant activity reducing the amount of marc extracts to be used.

**MATERIALS & METHODS**

Red-grape marc extract was obtained by solvent extraction with ethanol:water/60:40, at 60°C, followed by freeze-drying. The total phenols content was 24% (w/w) gallic acid equivalents GAE (evaluated according to the Folin-Ciocalteau analysis) [1].

Three different nanometric size delivery systems were fabricated. Formulation 1 consisted of an O/W nanoemulsion, where the grape extracts (0.25% wt) were first embedded in a sunflower oil emulsion (9% wt), stabilized in water by using soy lecithin (1% wt) as emulsifier and subsequently reduced to nanometric dimensions (200 nm) by high pressure homogenization (5 passes at 150 MPa). Formulation 2 consisted of a powder obtained by spray-drying Formulation 1. The nanoemulsion was dried out and entrapped in a micrometric vitreous maltodextrin structure. The final grape extract load was 0.7% wt. Upon rehydration, the emulsion retained a nanometric size (290 nm). Formulation 3 was an ethanol-oil nanoemulsion. The grape extracts (0.5%) were dissolved in ethanol (10%) and homogenized with a hot melt of stearic acid (4.4%) and peanuts oil (83.1%), using soy lecithin (2%) as emulsifier. The emulsion was further comminuted by high pressure homogenization (5 passes at 150 MPa). Antioxidant activity of final formulation was assessed by the DPPH radical assay and expressed as mg of ascorbic acid equivalents /ml (mgAAE/ml).

The original freeze-dried extract and its new formulations were homogenized into a fresh commercial hazelnut paste (containing emulsifiers) at a concentration of 5000 ppm (w/w), resulting therefore into different phenolics content. More specifically, the concentration of crude extracts (unencapsulated) in the hazelnut paste was of 5000 ppm, while the concentrations of the extracts in the hazelnut paste when encapsulated were significantly lower, namely of 12.5ppm, 35ppm and 25ppm in formulations 1, 2 and 3 respectively.

An accelerated shelf-life test was carried out keeping the samples and a paste blank into open vials in oven at 60°C. Peroxides value (PV) of the lipid phase extracted with hexane was periodically analyzed until 100 days. Antioxidant activity (assessed by the radical ABTS assay and expressed as percent AOP-antioxidant power) of hydrophilic fraction of the hazelnut paste samples was assessed on a methanol extract obtained from the degreased samples. Trials were carried out in duplicate.

**RESULTS & DISCUSSION**

A first trial was carried out adding the freeze-dried extract to the hazelnut paste at a concentration of 5000 ppm. The extract did not solubilise into the paste and over time it tended precipitating. Despite the non complete solubilisation, the extract showed a certain antioxidant effect. In fact, in the blank oxidation started after 14 days storage and peroxides formation followed an apparent first order kinetics (Fig. 1). In the paste added with the extract, oxidation was highly reduced, particularly after 40 days, but the reaction seemed not to follow any clear kinetic order. The maximum oxidation inhibition was at 60 days and equal to 65%.
In order to improve extract solubility into the paste, the different nanosized delivery systems described in materials and methods were tested.

Each formulation was homogenised into the paste at a concentration of 5000 ppm, but due to the different composition, final extract concentration was not the same, as reported in Table 1. In particular, the concentration in the hazelnut paste of marc extract when encapsulated in formulation 1 was 12.5 ppm, when encapsulated in formulation 2 was 35 ppm and when encapsulated in formulation 3 was 25 ppm. Furthermore, considering the total phenols content of the extract, it comes out that the phenols concentration in the paste was always lower than 10 ppm.

The antioxidant activities of the encapsulated extract were characterized in vitro before incorporation into the hazelnut paste by DPPH radical assay. The highest specific antioxidant activity (referred to the marc extract content) was exhibited by formulation 1, which was the oil-in-water nanoemulsion, where the large surface to volume ratio of the oil droplets (with average size of 200 nm) made the extracts readily available for exerting the antioxidant activity. The antioxidant activity of formulation 2 was lower than for formulation 1 when normalized for the marc extract content. Since it was prepared by drying nanoemulsion of formulation there might have been a thermal degradation effect during spray drying. Formulation 3, the ethanol solution-in-solid lipid emulsion, exhibited the lower antioxidant activity probably due to the lower availability of the marc extracts.

All the nanoemulsion formulations were homogenously dispersed into the hazelnut paste and did not give any precipitate.

The results of accelerated shelf-life tests showed again that paste oxidation started after 2 weeks (Fig. 2) and again the maximum inhibition effect of added extracts was at around 60 days, with a 58%, 43%, and 53% percent oxidation inhibition for formulation 1, 2 and 3, respectively.

After this maximum, the protective effect of polyphenols finished, probably due to their oxidation or to a residual antioxidant activity not able to prevent peroxides formation.

The more efficient formulation was the O/W nanoemulsion (formulation 1) which had also the lowest level of phenols, while the least efficient was formulation 3.

Remarkably, in comparison with the unencapsulated extracts, the encapsulation of the phenolics into the delivery systems allowed to obtain over 60 days similar inhibition effects on lipid oxidation, but with a
phenolic concentration that was from 150 to 400 times lower. This indicates that encapsulation could increase the dispersibility and hence the availability of the active compounds in the hazelnut paste. In order to prolong the inhibition over longer times, it is probably required to increase the initial concentration of the phenolics.

**Figure 2:** Influence of addition of nanoemulsion formulations on the oxidation of hazelnut paste.

Antioxidant activity of hydrophilic fraction of hazelnut paste was not influenced by extract addition (Fig. 3). Results indicate that the natural antioxidant activity of hazelnut paste is not involved neither diminished by lipid oxidation and that the polyphenols of grape marc extract, when encapsulated into nanoemulsions, remain into the lipid phase where they can exert their protective effect against oxidation.

**Figure 3:** Influence of addition of nanoemulsion formulations on the antioxidant activity of hazelnut paste.

**CONCLUSION**

This study showed the efficiency of nanoemulsion formulation in improving solubility of a grape marc extract into a high lipid content food such as the hazelnut paste. Addition of the extract could significantly improve the paste shelf-life by inhibiting its oxidation. Improved dispersion of nanoemulsion significantly increased the efficiency of the phenolic compounds, reducing between 140-fold and 400-fold the amount of extract required for the potential production of a natural preservative agent. Further research is needed to better understand the stability and action mechanism of the nanoemulsions and to optimise formulation (since the three tested formulations exhibited different efficiency) and dosing levels, from different points of view: costs, sensorial quality of the product and possibility of using the extract not only as preserving additive but also as an healthy-functional ingredient.
REFERENCES


