**Application of functional molecules recovered from bergamot by-products: development and improvement of food systems**

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In the last years, bergamot (*Citrus bergamia Risso*) has shown great interest due to its beneficial effect on human health due to the high content of phenolic compounds. The aim of the work is to valorise bergamot pomace recovering this fraction through a selected extraction method (among conventional, ultrasound and microwave extraction) and application in food systems. The use of the obtained liquid extract rich in phenols, was tested in an edible coating to study its effect strawberries shelf life. Moreover, the extract was microencapsulated investigating its impact during storage of enriched lipophilic and hydrophilic food matrices.

**Applicazione di molecole funzionali recuperate da pastazzo di bergamotto: sviluppo e miglioramento di sistemi alimentari**

Negli ultimi anni, il bergamotto (*Citrus bergamia Risso*) ha riscontrato grande interesse per i suoi effetti benefici sulla salute umana grazie all'elevato contenuto di composti fenolici. Lo scopo del lavoro è quello di valorizzare il pastazzo di bergamotto recuperando questa frazione attraverso un metodo di estrazione selezionato (tra estrazione convenzionale, ultrasuoni e microonde) e l'applicazione in sistemi alimentari. L'uso dell'estratto liquido ottenuto, ricco di fenoli, è stato testato in un rivestimento edibile per studiare il suo effetto sulla shelf life delle fragole. Inoltre, l'estratto è stato microincapsulato per studiarne l'impatto durante la conservazione di matrici alimentari lipofiliche e idrofiliche arricchite.

**Keywords**: Bergamot by-product; phenolic extraction; edible coating; microencapsulate; functional foods

# **1. Introduction**

One of the most important citrus fruits grown in Calabria is Bergamot, which represents a valuable source of active molecules that contribute to antioxidant, anti-inflammatory, and cholesterol reduction capacities (Da Pozzo *et al.*, 2018; Schwingshackl *et al.*, 2020). After its industry processing, waste is an industrial problem to manage. Nevertheless, the high amount of antioxidants, especially flavonoids, allow to consider it as source of natural additives.

In accordance with the PhD thesis project, this oral communication reports the main results of the following activities:

A1) Selection of the best extraction to recovery bioactive compounds from bergamot pomace;

A2) Application of the best extract (AE) to edible coatings for strawberries’ shelf life extension;

A3) Effect of microencapsulated AE in lipophilic and hydrophilic food systems.

# **2. Materials and Methods**

## **2.1 Extraction of antioxidant compounds**

Bergamot pomace (BP) represented by skins, pulp and seeds, was subjected to dehydration (at 50°C) to reduce the moisture content (up to 12%) and powdered by mean of a laboratory mill to facilitate the extraction process. In order to obtain an extract with high antioxidant power, different techniques were carried out. Three extraction methods were tested: conventional maceration (C), ultrasound (UA) and microwave (MA) assisted extraction; water and ethanol/water mixture (50:50, v/v) were considered as solvents (food grade). For C and UA methods the extraction was carried out with different combination of temperature (25 and 70 °C) and time (30 and 60 min), while for MA the extraction time was different (5 and 15 min). The best extraction conditions were evaluated referring to the main physicochemical characteristics and antioxidant activity and constituents, such as total phenols and flavonoids, total antioxidant activity, individual flavonoids and limonoids (UHPLC-DAD), as reported by Gattuso *et al.* (2023). The obtained extracts were characterized based on dry weight of BP (dw: 17%). Later, the best selected extract obtained by conventional maceration at 70°C for 30 minutes in an hydroalcoholic mixture (AE), was prepared using 200 g of BP and 800 mL of solvent and was applied in the formulation of an edible coating solutions and for the preparation of the microencapsulated.

**2.****2 Edible coating formulation and application on strawberries**

The coating was prepared following the method reported by Tahir *et al.* (2018) using a concentration of arabic gum of 2%. AE and BHT solutions were added and heated at 40 °C for one hour under continuous stirring. Subsequently, 1% glycerol (v/w) was added as plasticizer to improve the strength and flexibility of the coating solutions. The concentrations of AE and BHT added to the coatings were: 100 ppm of BHT (sample B); 1% AE (sample C); 2.5% AE (sample D); 5% AE (sample E). Additionally, a control sample (A) was prepared. Strawberries were dipped in the different coating solution for 3 min and the excess of the coating was drained and air-dried (under UV and at room temperature to prevent environmental contamination). The fruit samples were packaged into hinged food containers (PET) in normal atmosphere and stored at 4 °C. Shelf-life study was conducted monitoring changes in strawberries (3, 7, 10, and 14 days).

## **2.3 Encapsulation of AE and application in sunflower oil and apple juice**

For the preparation of the microencapsulate, maltodextrin at concentrations of 20% was added and the sample was lyophilised as reported by Ballesteros *et al.* (2017). After, 2% of microencapsulate (MD20) was used to enrich apple juice (EJ) and sunflower oil (EO), this concentration was choice after different tests to verify the best response in food. For each product three darkened containers for each monitoring time were prepared and stored at 25°C until further analysis.

## **2.4 Food products characterization**

Organic acids, microbiological counts, texture in strawberries and characterization of the phenolic profile of MD20, EJ and EO were carried out as reported by De Bruno *et al.* (2023). To investigate the effect of MD20 in EO during storage time an oxidative stress test was conducted by mean of OXITEST system (Imeneo *et al.*, 2021).

## **2.5 Statistical analysis**

Results of the present study were expressed as mean ± SD of three measurements (n = 3), except for strawberries’ firmness in which ten measurements were conducted. Appropriate test statistics, One-way ANOVA with Tukey’s post-hoc test, and t-test were at p < 0.05 were performed by SPSS Software (Version 15.0, SPSS Inc., Chicago, IL, USA).

# **3. Results and Discussion**

## **3.1** **Characterization of the best selected extract**

The best selected extract (AE) was obtained by conventional maceration at 70°C for 30 minutes, using as solvent a hydroalcoholic mixture (H2O/EtOH 50:50 v/v). Ethanol is environmentally friendly and allows to obtain a good extraction of polyphenols (Gil-Martín *et al.*, 2022). The extraction procedures provide to convert citrus waste in a source of value-added products to use in functional foods as widely demonstrated in the literature (Andrade *et al.*, 2023). As reported in Table 1, the selected extract exhibited a phenolic content of 26.30 mg gallic acid equivalent (GAE) g-1 dw and 6.18 mg catechin equivalent (CE) g-1 dw. The total antioxidant activity level was investigated with DPPH and ABTS assays, in which it showed values of 1.6 and 16.93 mmol Trolox equivalent (TE) g-1 dw, respectively. The main individual flavonoids and limonoids analysed by chromatographic analysis (UHPLC-DAD) were reported in Table 1. Neoeriocitrin, naringin, neohesperidin, brutieridin and melitidin were the main flavonoids detected in BP, as also reported by other authors (Di Donna *et al.*, 2020; Gorinstein *et al.*, 2001).

The best selected method was chosen due to its high content of bioactive compounds. For TPC, AE and the extract obtained with the same method prolonged for 60 minutes, showed similar content (26.30 and 26.06 mg GAE g−1 dw, respectively), although UA also led to a good recovery of TPC (about 23.64 mg GAE g−1 dw extract at 25°C for 30 min with H2O/EtOH). Compared to the other types of extraction tested, AE showed 60% higher extraction than MAE performed at 25°C for 5 min with H2O/EtOH. In total antioxidant activity the ABTS assay was also considered in the selection of the method. The assay displayed values that ranged between 3.22 and 16.93 mM TE g−1 dw. The best extraction in terms of total antioxidant activity was obtained by applying the hydroalcoholic solution (H2O/EtOH) as extraction solvent and assisted by C at 25°C 60 min and in AE confirming the highest recovery of bioactive compounds in the last one. The choice was established also comparing the concentration of the major flavonoids detected. The main abundant flavonoids (neoeriocitrin, naringin, neohesperidin and brutieridin) were always found in the highest concentrations in AE and in the extract obtained for C at 70°C for 60 min with H2O/EtOH mixture, as was the case for TPC. Also, for the extraction of limonoids, AE showed the highest quantity recovered. In the selected extract, limonin was higher almost 20% compared to all extractions, reaching more than 90%. For nomilin lower differences were found in the extracts resulting by the use of hydroalcoholic mixture and with UA for a time of 30min and 25°C, and the extract in C at 25°C for 60 min, respectively 13.10% and 14.25% but more than 80% in others. In conclusion, data highlighted that for the parameter considered, the conventional maceration extraction system produced the greatest extractability of bioactive compounds. As observed previously in other food matrices, the hydroalcoholic solvent is the best choice to obtain the maximum yield of antioxidant compounds, when combined to 70°C of temperature (Chemat *et al.*, 2017).

\*mg g-1 dw

**Table 1** Antioxidant characterization of AE.

## **3.2 Effect of edible coating on strawberries**

Generally, minimally treated fresh fruit have a short shelf-life (4–7 days), which is very important to preserve the freshness of the fruit and avoid excessive losses due to the reduction in their quality. In Table 2, the microbiological results are reported. The Total Bacterial Count (TBC), yeasts and molds were revealed already since the 1st day of storage in samples A (control) and B, while the other samples did not show any contamination. The use of AE in edible coatings provide advantages to preserve their high sensibility to microbial decay. The obtained microbiological values fall within the acceptable limits of a maximum aerobic plate count of 5 × 107 cfu/g at the end of shelf life for different fresh-cut vegetables as reported by Fan and Song (2008). During the storage, increases in all analysed microbiological parameters were observed, particularly at 14 days of storage. This grow could be also related to humidity conditions developed inside the containers. All the samples showed an increment of yeasts and molds during the storage time. After 14 days, for yeasts the highest concentration was found in A (7.0 Log10 CFU g−1), while for molds, in A and B samples. These values showed that the application of edible coatings is useful to improve and extend the quality of strawberries.

**Table 2** *The microbiological counts of minimally treated strawberries (Log10 CFU g−1).*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | *TBC* |  | *Yeasts* |  | *Molds* |  |
|  | **1st** | ***7th*** | **14th** | **Sign.** | **1st** | 7th | 14th | **Sign.** | 1st | 7th | 14th | **Sign.** |
| *A* | 1.8 aC | 2.9 aB | 3.4 bA | \*\* | 1.1 aC | 4.9 bB | 7.0 aA | \*\* | 3.0 aB | 3.5 aB | 5.3 aA | \*\* |
| *B* | 1.0 bC | 2.0 bB | 4.1 aA | \*\* | 1.1 aB | 6.1 aA | 6.4 bA | \*\* | 2.1 bB | 2.1 bB | 5.3 aA | \*\* |
| *C* | 0 cC | 2.5 abB | 3.7 abA | \*\* | 0 bB | 5.8 aA | 5.2 cA | \*\* | 0 cB | 0 cB | 4.4 bA | \*\* |
| *D* | 0 cB | 2.9 aA | 2.4 cA | \*\* | 0 bB | 4.9 bA | 6.4 bA | \*\* | 0 cC | 2.1 bB | 4.8 abA | \*\* |
| *E* | 0 cC | 1.3 cB | 3.1bA | \*\* | 0 bC | 1.5 dB | 4.7 cA | \*\* | 0 cB | 0 cB | 3.2 cA | \*\* |
| **Sign.** | \*\* | \*\* | \*\* |  | \*\* | \*\* | \*\* |  | \*\* | \*\* | \*\* |  |

The fruit firmness was analysed on the coated samples during the storage time (Table 3), because it represents one of the essential parameters to determine fruit quality. The softening is a natural physiological effect of fruit ripening with cell wall changes and the dissolution of the middle lamella, which in turn causes loss of cell-to-cell adhesion (Chen *et al.*, 2011; Villarreal *et al.*, 2016). At time 0, the firmness of the fruit was 10.1 N, while after seven storage days, a decreased was observed, both in the uncoated and coated samples with lowest value found in the control sample (3.21 N), similar to sample B (3.62 N). The highest firmness values were recorded in sample D at 6.32 N. Additionally, after 14 days of cold storage, samples A and B showed the lowest firmness values, 1.54 N and 3.22 N, respectively. The results were in accordance with those of Tahir *et al.* (2019), in which the retention of flesh firmness of blueberries was achieved by the combined effect of African baobab pulp extract and arabic gum; while Kahramanoğlu *et al.* (2022) reported that uncoated strawberries over time showed a significant decrease in firmness than in those coated. As also demonstrated by statistical analysis, at the end of the storage time, D and E samples showed the highest values of firmness among the samples.

**Table 3** *Firmness in strawberries samples during storage.*

|  |
| --- |
| FIRMNESS (N) |
|  | 1st | 3rd | 7th | 10th | 14th | Sign. |
| A | 10.10A | 6.32cdB | 3.24cC | 2.69dD | 1.54cE | \*\* |
| B | 10.10A | 7.01cB | 3.62cC | 3.58cC | 3.22bD | \*\* |
| C | 10.10A | 8.63aB | 4.31bC | 4.03bCD | 3.85abD | \*\* |
| D | 10.10A | 9.61aB | 6.32aC | 5.95aC | 4.18aD | \*\* |
| E | 10.10A | 8.02bB | 4.43bC | 4.28bD | 4.08aD | \*\* |
| Sign. | n.s. | \*\* | \*\* | \*\* | \*\* |  |

Ascorbic acid (AA), or vitamin C, is one of the major components of strawberries, and its content is an indicator of quality relevant to define freshness of fruits (Cordenunsi *et al.*, 2023). Many authors have reported that decrease of AA during storage is caused by its oxidation (Atress *et al.*, 2010) and by respiration rate of the fruit (García *et al.*, 1996). The use of a coating promotes protection against both effects. As is possible to see in Table 4, the results obtained confirm this effect. The control sample (A) showed significant variation of the AA content values during the storage period (p < 0.01), with the lowest value being shown at 14 days. The initial AA content was 33.01 mg 100 g−1, and after seven days it decreased to 28.35 mg 100 g−1, while at 14 days it was 27.02 mg 100 g−1 (the lowest detected value) After 14 days, also the sample B showed a low content (29.07 mg 100 g−1), while the samples coated with AE solution showed the highest values.

Citric acid is the predominant organic acid in strawberries. The data detected during this experimentation process are reported in Table 4. There were great losses (highly significant, p < 0.01) of this organic acid, particularly in the control sample (A), from 692.5 mg 100 g−1 at the beginning to 402.9 mg 100 g−1 at the end of the shelf life (14 days). Regarding the trend shown during the storage period, the CA contents varied significantly only in two samples, A and B (p < 0.01); all other samples meanwhile samples treated with AE showed no significant differences (p > 0.05). All coatings enriched with AE highlighted the good stability of this acid over time.

**Table 4** Ascorbic and citric acid content in strawberry samples (mg 100g-1).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Ascorbic Acid** | ***A*** | ***B*** | ***C*** | ***D*** | ***E*** | ***Sign.*** |
| 1st | 33.0 ± 0.5 a | 33.0 ± 0.5 a | 33.0 ± 0.5 a | 33.0 ± 0.5 a | 33.0 ± 0.5 a | n.s. |
| 7th | 28.4 ± 0.1 bC | 27.3 ± 0.4 bC | 32.6 ± 0.1 aA | 29.6 ± 0.8 bBC | 31.4 ± 1.02 abAB | \*\* |
| 14th | 27.0 ± 0.2 cB | 29.1 ± 0.3 bB | 30.3 ± 0.1 bA | 31.5 ± 1.4 abA | 29.8 ± 0.5 bA | \* |
| Sign. | \*\* | \*\* | \*\* | \* | \* |  |
| **Citric Acid** | ***A*** | ***B*** | ***C*** | ***D*** | ***E*** | ***Sign.*** |
| 1st | 692.5 ± 26.5 a | 692.5 ± 26.5 a | 692.5 ± 26.5 | 692.5 ± 26.5 | 692.5 ± 26.5 | n.s. |
| 7th | 604.1 ± 42.0 aB | 669.1 ± 2.2 aB | 720.5 ± 10.2 A | 745.7 ± 2.1 A | 698.0 ± 12.3 A | \* |
| 14th | 402.9 ± 3.3 bC | 583.5 ± 56.0 bB | 711.1 ± 20.3 A | 727.5 ± 32.1 A | 676.8 ± 17.9 AB | \* |
| Sign**.** | \*\* | \*\* | n.s. | n.s. | n.s. |  |

**3.3 Evaluation of microencapsulate and enriched food products (EJ and EO)**

In accordance with the above, in Table 5 are shown the main flavonoids identified in MD20. Flavonoid concentrations in enriched juice during monitoring times displayed statistical differences (Table 6) for all of them. In EJ storage at 25°C the major flavonoids, neoeriocitrin, naringin and neohesperidin followed the same trend with an increment after 90 days. The other compounds followed a decrease at the 90th day of monitoring. Specifically, eriocitrin, narirutin and brutieridin exhibited a stable trend after 45days with values comparable to T0, and a significative (p<0.01) reduction at the last monitoring time. Melitidin presented a small decrement after 45 days passing from 19.94 mg L-1 (T0) to 17.18 L-1 (T45) reaching 5.37 mg L-1 at T90. These different trends could be due to a gradual and different release of single compounds. However, the overall content increased over time, showing a gradually release of phenolic compounds. This effect was also observed by Wyspiańska *et al.* (2019), who found a gradually increasing of isoflavonones after a degradation over time of maltodextrin capsules with a release of phenolic molecules in the solution.

**Table 5** *Flavonoids composition of MD20.* **Table 6** *Flavonoids**Flavonoid composition of Enriched Juice.*

|  |  |  |  |
| --- | --- | --- | --- |
| MD20 (mg g-1) |  |  | EJ (mg L-1) |
|  |  | **T0** | **T45** | **T90** | **Sign.** |
| *Eriocitrin* ***(1)*** | 0.2 ± 0 |  | **1** | 2.29 ± 0.1a | 2.31 ± 0.1a | 1.23 ± 0.29b | \* |
| *Neoeriocitrin* ***(2)*** | 7.32 ± 0.09 |  | **2** | 88.18 ± 1.45b | 90.41 ± 2.08b | 107.16 ± 1.99a | \*\* |
| *Narirutin* ***(3)*** | 0.11 ± 0.01 |  | **3** | 0.85 ± 0.04a | 0.87 ± 0.03a | 0.11 ± 0.01b | \*\* |
| *Naringin* ***(4)*** | 8.55 ± 0.1 |  | **4** | 104.38 ± 1.47b | 111.54 ± 3.02b | 129.81 ± 0.63a | \*\* |
| *Neohesperidin* ***(5)*** | 4.75 ± 0.19 |  | **5** | 57.44 ± 1.21b | 63.18 ± 1.93b | 94.41 ± 0.71a | \*\* |
| *Melitidin* ***(6)*** | 1.27 ± 0.08 |  | **6** | 19.94 ± 0.56a | 17.18 ± 0.2b | 5.37 ± 0.31c | \*\* |
| *Brutieridin* ***(7)*** | 3.2 ± 0.11 |  | **7** | 41.12 ± 0.42a | 41.23 ± 1.79a | 4.68 ± 0.08b | \*\* |

The microencapsulate in sunflower oil showed a different behaviour that in juice as reported in Table 7. Except for melitidin (6), differences of flavonoids content where found during the storage of EO. Eriocitrin (1), neoeriocitrin (2) and brutieridin (7) evidenced the same trend with a maximum level at 45th day and a subsequently slightly decrease (p<0.05). Narirutin (3) increased in the second monitoring time to decrease below the initial value at the end. High statistical differences (p<0.01) were also found in naringin (4) content which revealed an initial value of 47.1 mg L-1, and higher values at T45 (58.89 mg L-1) and T90 (56.26 mg L-1). Moreover, neohesperidin (5), after an initial increase maintained constant concentration. The gradually release of encapsulated antioxidants in oil phase was also observed by Mohammadi *et al.* (2016). The different behaviour of the microencapsulate in EJ and EO could be due to the hydrophilic nature of maltodextrin, which is hydrophilic and high soluble in water, permitting an easy release of compounds from the capsules (Hermanto *et al.*, 2016). This could also explain a faster solubilization of the microencapsulate in EJ founding a higher amount of phenolic compounds just from the beginning of the experimentation.

In edible oil the oxidation control is a parameter that defines its quality and safety for human health. It depends on several factors intrinsic and extrinsic, and the enrichment with natural antioxidants is a good strategy for its preservation (Fadda *et al.*, 2022). In this study is reported the oxidative stability analysed with OXITEST, which was expressed as induction period (IP). IP represents the time needed to get to the point where oxidation begins. It was analysed in order to evaluate the resistance of fat matrix to oxidation (De Bruno *et al.*, 2021). As expected, the presence of phenolic compounds added with MD20, resulted in a significantly grown in IP (Figure 1) after 45 days at the storage temperature of 25°C, reaching 14:12 (h:m) starting from 13:16 (h:m) with a consequently enhancement due to the antioxidant effect of bioactive compounds as observed by De Bruno *et al.* (2022). After 90 days, the IP of EO showed lower value compared to IP of T0 due to different mechanism reactions given to the natural irreversible oxidation combined with the storage temperature.

**Table 7** Changes in flavonoids in Enriched Oil during storage.

|  |
| --- |
| **EO (mg L-1**) |
|  | **T0** | **T45** | **T90** | **Sign.** |
| **1** | 1.03 ± 0.02b | 1.27 ± 0.03a | 1.13 ± 0.05ab | \* |
| **2** | 37.77 ± 1.25b | 46.71 ± 2.04a | 43.31 ± 1.13ab | \* |
| **3** | 0.97 ± 0.04b | 1.28 ± 0.08a | 0.39 ± 0.02c | \*\* |
| **4** | 47.1 ± 0.66c | 58.89 ± 0.4a | 56.26 ± 0.65b | \*\* |
| **5** | 25.37 ± 0.62b | 32.4 ± 1.61a | 30.32 ± 0.14a | \* |
| **6** | 8.84 ± 0.03 | 10.42 ± 0 | 9.53 ± 1.41 | n.s. |
| **7** | 18.01 ± 0.79b | 22.87 ± 0.77a | 20.4 ± 0.48ab | \* |



**Figure 1** Oxidation curves at T0, T45 and T90 of EO.

# **4. Conclusions and Future Perspectives**

These studies have proposed strategies for the valorisation of bergamot pomace, highlighting its high content of antioxidant compounds recoverable through food grade extractions, and its use applicable to the food industry in the formulation of edible coating, and the enrichment of juices and oils. This study also made possible to highlight the natural preservative effect to extend shelf life of strawberries, and on the other hand to supply antioxidant compounds to poor foods such as apple juice or seed oil, improving, in the case of oil also the oxidative stability. Overall, the use of this by-product has made possible to obtain foods with high added value, contributing to the reduction of citrus waste. The results obtained are very promising and the future research could focus on the purification of the extract evaluating the effect of the single compounds, and to set-up in vivo trials to confirm their functionality.

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