Enrichment of Extra Virgin Olive Oil for the Development of Functional Oil for Special Consumers

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The aim of this PhD research project was to evaluate the impact on the shelf life evolution and bioactivity of functional olive oils obtained by EVOO enrichment with selected matrices by using two different technological approaches: malaxation and infusion. The obtained flavoured virgin olive oils (FVOOs) were found to be pleasant from a sensorial point of view and with marked health properties during shelf life.

Funzionalizzazione di un olio extravergine di oliva per la creazione di prodotti destinati a categorie speciali

Lo scopo del progetto di dottorato è stato quello di valutare l'impatto sull’evoluzione della shelf life e la bioattività di oli di oliva funzionalizzati ottenuti dall'arricchimento di EVOO con matrici selezionate, utilizzando due diversi approcci tecnologici: gramolatura e infusione. Gli oli di oliva vergini aromatizzati (FVOOs) ottenuti sono risultati gradevoli dal punto di vista sensoriale e con spiccate proprietà salutistiche durante la shelf life.

**Key words**: Functional olive oil; antioxidant activity, enzymatic activity; health properties.

# **1. Introduction**

In accordance with the PhD thesis project previously described (Custureri, 2021), in this oral presentation the following aspects were analysed:

A1) EVOO enrichment with selected matrices by using two different technological approaches: malaxation and infusion;

A2) EVOO and FVOOs physical-chemical and sensorial analysis;

A3) EVOO and FVOOs UHPLC analysis;

A4) Determination of antioxidant activity by multi-target approaches and enzyme inhibitory assays related to health status;

A5) Evaluation of EVOO and a selection of FVOOs aroma profile by the optimization of a SPME-GC-MS method;

A6) Statistical analysis.

Extra-virgin olive oil (EVOO) is an essential condiment used by the population of the Mediterranean basin.

Several published studies document that most of the health effects of the Mediterranean diet can be ascribed to EVOO. In fact, consumption of EVOO is related to a reduction in the oxidation process of biomolecules such as lipids and DNA, a reduction in insulin-resistance and an improvement in the lipid profile etc. These effects protect from both metabolic disorders and cardiovascular disease (Buckland and Gonzalez, 2015). Recently, an increasing amount of scientific evidence has revealed that phenolic compounds which represent only ~2% of EVOO may also contribute to the healthy features of EVOO (Jimenez-Lopez et al., 2020). The chemical composition of the EVOO varies according to the olive cultivar, the harvesting period and geographical origin, the pedoclimatic conditions of growth (Giuffrè, 2017). Italy is the second largest EVOO producer with protected designation of origin (PDO) and protected geographical indication (PGI) mark. Many of these cultivars are located in Calabria, a region of southern Italy, due its favourable microclimate conditions (Marra et al., 2013). Among them the Ottobratica cultivar represent one of the most cultivated (Sicari at al., 2009). Spices are widely used to increase food palatability (Issaoui et al., 2016). Moreover, they provide some biological effect and extend the shelf life of food (Opara and Chohan, 2014). These actions are due to their phytochemical content of polyphenols, terpenoids and carotenoids (Wahyuni et al., 2013). The addition of herbs and spices to EVOO has become more popular in recent years, due to consumer demand of “gourmet oils” (Clodoveo et al., 2016). The addition of spices or other flavourings means the resulting oil no longer satisfies the European Union Commission definition for extra virgin olive oil, but can be defined as a Flavoured Olive Oil (FVOO). Several research papers have investigated the effect of the addition of herbs and spices on oil nutritional quality, oxidative stability, and sensorial characteristics. Clodoveo et al. (2016) compared the impact of different FVOOs production techniques (infusion of herbs into the oil, addition of herbs to the crushed olives before malaxation and application of ultrasound before the olive paste malaxation) on the quality of the FVOO.

In this context, our work examines the effect of two different technological approaches: malaxation and infusion.

The following plant matrices were used for enrichment: Turmeric (*Curcuma longa* L.), Ginger (*Zingiber officinale* R.), Mace (*Myristica fragrans* Houtt.), Spirulina (*Spirulina* sp.), Bergamot (*Citrus bergamia* Risso & Poiteau) and Goji berries (*Lycium barbatum* L.).

# **2. Experimental procedures**

Olive fruits (*Olea europea* L.) of Ottobratica variety were harvested in Reggio Calabria province (Italy). The oil extraction was performed by a mini-pressing apparatus. The parts of plants and spices were purchased at a local supermarket, except for the bergamot fruit and goji berry puree (purchased from Calabrian producers). All the matrices were added as a powder with the exception of bergamot fruits and goji berries, which were cut into slices and used as a puree, respectively. For the addition during malaxation (1%), the extraction was performed at room temperature and malaxation lasted for 40 minutes. With the aim of making a comparative analysis, the enrichment was also done by infusion (2%). The oil was infused for 30 days in the dark and under constant agitation. The enrichment matrices were not added directly to the oil, but small bags, similar to tea bags, were created with sterile gauze. Bergamot and goji berry puree, before the addition by infusion, were freeze-dried. The additions were made with single matrix or in combination with two or three matrices to evaluate any antagonistic or synergistic effect. The obtained FVOOs were filtered and packaged in amber glass bottles with a capacity of 100 mL with threaded screw cap with drip catcher. The analyses were made for EVOO and FVOOs at different pre-established times to evaluate stability throughout one year of storage. Samples were analysed in triplicate. The analysis of variance (one-way ANOVA) was conducted by applying the post hoc Tukey test at p < 0.05 (SPSS software, 21.0 version, Armonk, NY, USA).

# **3. Results and Discussion**

In this discussion, data concerning the enrichment with Turmeric (T), Mace (M) and Ginger (G), produced both by infusion (INF) and by malaxation (MAL) are examined.

The unflavoured olive oil during the storage suffered a slight oxidation, but it could be considered an extra virgin olive oil (EVOO) due to the values of free acidity (FA) ranging from 0.68 to 0.84 % at T0 and T360, respectively and peroxide values (PV) from 9.45 to 17.89 mEq O2 kg-1 at T0 and T360, respectively. Among the FVOOs, FA and PV naturally increased during the storage, but did not greatly affect the quality of the oils. Only T-MAL and T-INF, showed a lower level in term of FA than the EVOO, even after the storage. A fundamental parameter for consumer acceptability is colour, and the addition of spices can greatly alter this. In EVOO there was a significant decrease in chroma (C\*) (from 7.24 to 2.24). For all the FVOOs, a decrease was found with values approximately four-times lower than T0 after 360 days of storage, too. The evaluation of total carotenoids content (TCC) highlighted a great variability among the FVOOs. The values of EVOO ranged from T0 6.15 to T360 4.80 mg kg-1. Although the content of carotenoids is highly variable, at the end of storage all the samples had a greater content than the EVOO, except for M-MAL. The different procedures applied led to different results and the infusion allowed a major recovery and stability of these compounds (Figure 1). The total phenolic content (TPC) trends are described in Figure 2. EVOO showed a TPC value of 418.51 mg kg-1 and an increase in phenols was observed in all the FVOOs. The highest values were discovered in the first 30 days, probably for a solubilisation of these compounds, followed by a natural decline after 60 days. Regarding FVOOs-MAL, M at the end of storage presented the upper level of TPC (557.82 mg kg-1). However, regarding the FVOOs-INF, all the samples presented higher values in comparison to the control and about two-times higher than the FVOOs-MAL. These results could be explained both by the inhibition of β-glycosidase activity and by the hydrolysis/partition phenomena toward lipid and water phases of biophenols during the malaxation and centrifugation steps (Sacchi et al. 2017). On the contrary the addition by infusion of herbs and spices to EVOO lead to FVOOs richer in TPC. The combination of the plant matrix with olive paste does not seem to be useful for the equilibrium of these molecules, presumably by the partitioning phenomena towards the lipidic and aqueous phases and by phenomena of antagonism. Among the goals of the present study there is the evaluation of the α-tocopherol content. The starter values were similar for all the FVOOs, with the exception of the mixtures with T that possessed the lowest values. At the end of storage, in all the FVOOs, the content of α-tocopherol remained higher than the control; in particular G-MAL and M-MAL showed the greatest protection against α-tocopherol loss, maintaining the highest values (101.16 and 97.21 mg kg-1, respectively) (Figure 3).

**Figure 1** *Total carotenoid content of EVOO, FVOOs-MAL (****a****) and FVOOs-INF (****b****). Data are expressed as means ± S.D. (n= 3). Differences within and between groups were evaluated by one-way ANOVA followed by Tukey’s test: \*\*p < 0.01. Results followed by \*\* differ significantly at the same time of analysis; results followed by letters differ significantly during the storage.*

**Figure 2** *Total phenolic content of EVOO, FVOOs-MAL (****a****) and FVOOs-INF (****b****). Data are expressed as means ± S.D. (n= 3). Differences within and between groups were evaluated by one-way ANOVA followed by Tukey’s test: \*\*p < 0.01. Results followed by \*\* differ significantly at the same time of analysis; results followed by letters differ significantly during the storage.*

**Figure 3** *α-tocopherol content* *of EVOO, FVOOs-MAL (****a****) and FVOOs-INF (****b****). Data are expressed as means ± S.D. (n= 3). Differences within and between groups were evaluated by one-way ANOVA followed by Tukey’s test: \*\*p < 0.01. Results followed by \*\* differ significantly at the same time of analysis; results followed by letters differ significantly during the storage.*

Ottobratica EVOO showed a promising 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity with IC50 values from 12.33 to 29.54 μg mL-1 for T0 and T360 samples, respectively. A similar situation was observed also with 2,20-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) test. A loss of antioxidant activity, in terms of protection from lipid peroxidation was observed using the β-carotene bleaching test (IC50 values from 48.72 to >100 μg mL-1 for T0 and T360 samples, respectively) (Figure 4). Data from ferric reducing antioxidant power (FRAP) assay revealed that regardless of the storage time, the EVOO results were lower than the butylated hydroxytoluene (BHT), used as positive control (from 25.01 to 4.31 μM Fe(II) g-1 for T0 and T360, respectively) (Figure 5). The antioxidant potential of dry spice extracts showed that mace exhibited the highest DPPH radical scavenging potential (IC50 16.56 μg mL-1) whereas turmeric was the most active in the ABTS test (IC50 3.14 μg mL-1). T-MAL sample (at T0) showed a promising radical scavenging effect with IC50 values of 9.49 and 3.47 g mL-1 for the DPPH and ABTS tests, respectively. A ferric reducing ability power better than those reported for the positive control BHT was observed only for G-MAL sample (86.42 vs 63.42 μM Fe (II) g-1) (Figure 5). This biological property is positively correlated with the α-tocopherol content (*r*=0.97). Storage time reduces the protection from lipid peroxidation measured by the β-carotene bleaching test. Notable results were obtained with T and G in both enrichment procedures (Figure 4). In particular, in the β-carotene bleaching test, G-MAL was positively correlated with TCC (*r*=0.99). Moreover, in both the FRAP and β-carotene bleaching tests, after 12 months of storage G-MAL and T-MAL samples, are characterized by a good antioxidant potential, better than those found for G-INF and T-INF.

The evaluation of bioactivity includes, also the inhibition of carbohydrate hydrolysing enzymes and lipase.

Ottobratica EVOO exhibited IC50 values from 269.02 to 289.32 μg mL-1, and from 137.34 to 778.23 μg mL-1 at T0 and T360, for α-amylase and α-glucosidase, respectively. IC50 values from 143.46 and 312.97 μg mL-1 at T0 and T360 were recorded against lipase. From the analysis of data, it emerges that sample M is characterized by a good pancreatic lipase inhibitory activity (with IC50 value of 83.6 μg mL-1). Promising results were obtained also with M-INF that showed IC50 values ranging from 62.25 to 138.66 μg mL-1 at T0 and T360, respectively whereas values from 63.45 to 195.96 μg mL-1 at T0 and T360 were recorded for G-INF.

By UHPLC analysis, the trend is highly variable among the FVOOs.

With regard to sensorial analysis, the FVOOs were tested by a group of expert panellists. They scored different overall acceptability and are listed below in descending order for both approaches: mace, ginger and turmeric (Figure 6 and 7).

**Figure 4** *β-carotene bleaching test of EVOO, FVOOs-MAL (****a****) and FVOOs-INF (****b****). Data are expressed as means ± S.D. (n= 3). Differences within and between groups were evaluated by one-way ANOVA followed by Tukey’s test: \*\*p < 0.01. Results followed by \*\* differ significantly at the same time of analysis; results followed by letters differ significantly during the storage.*

**Figure 5** *FRAP assay of EVOO, FVOOs-MAL (****a****) and FVOOs-INF (b). Data are expressed as means ± S.D. (n= 3). Differences within and between groups were evaluated by one-way ANOVA followed by Tukey’s test: \*\*p < 0.01. Results followed by \*\* differ significantly at the same time of analysis; results followed by letters differ significantly during the storage.*

The assessors were not able to identify the enrichment matrices for all the FVOOs. The EVOO was characterized by the presence of a slight “muddy” and “sludge” defects. Starting from the most appreciated, M-MAL and M-INF, a “citrusy” note appeared and there was an increment in the “vegetable” and “green fruity” notes whereas in M-MAL sample the attribute “sweet” significantly increased. In G-INF emerged a strong rise in the “pungent” note and resulted the most “spicy” FVOOs moreover the panellists asserted that in G-INF the “muddy” defect was not covered differently to G-MAL. The most characteristic note of T-MAL and T-INF was obviously the colour, which became a bright yellow. They are also characterized by a high “ripe fruity” and “spicy” attributes. They differed from each other because in T-MAL the defects of the starting oil were covered and its flavour was more balanced than T-INF, in fact T-MAL sample resulted the most sweet and equilibrated FVOOs in the gustatory sensations. To evaluate the volatile profile, FVOOs were subjected to different storages to simulate either producer’s bottling conditions (with no headspace) or domestic consumption (with 50% headspace). Solid-phase microextraction followed by gas chromatography coupled with mass spectrometry (SPME GC-MS) analysis, under previously optimized conditions (*T*=44°C, *teq*=10 min, *text*=60 min), showed no significant changes for most volatiles stored with no HS. However, storage under domestic conditions favoured the loss of a few volatiles, this effect being more evident for extended storage. Among the FVOOs, mace samples possessed the richest volatile profiles.

# **4. Conclusions and Future Perspectives**

The obtained results confirmed the complexity and the variability of the EVOO aromatization process. In fact, the addition of spices does not always improve either the shelf life of the olive oil nor its quality. Frequently the low lipophilic character of the bioactive compounds deriving from the flavouring matrices limits their transfer to the FVOO.

The blend with turmeric, in infusion or in malaxation, produces FVOOs with the lowest level in free acidity. FVOOs obtained by malaxation lead to products characterized by a high and stable α-tocopherol content and a better antioxidant activity, but the infusion approach generates results higher in term of total phenolic content. FVOOs obtained by the addition of ginger and mace are characterized by a promising pancreatic lipase inhibitory activity. Concerning biological activity, infusion gives the most advantageous results. However, a significant reduction of bioactivity was observed during shelf life. The malaxation enrichment process promotes the covering of any defects in the starting olive oil; moreover, enriching an olive oil with slight defects by malaxation could be favourable thanks to a masking effect on the negative attributes, enhancing those of the spice. The FVOOs-MAL possess the richest volatile profiles. Finally, it could be stated that there is no best enrichment procedure valid for all matrices.

**Figure 6** *Olfactory sensations of EVOO, FVOOs-MAL and FVOOs-INF.*

**Figure 7** *Gustatory sensations of EVOO, FVOOs-MAL and FVOOs-INF.*

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