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Effects of Phenolic Enrichment on Antioxidant Activity of Mayonnaise

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The aim of this work was to evaluate the effect of phenolic extract addition on the oxidative stability and antioxidant activity of mayonnaise. The mayonnaise was enriched with different concentrations (50, 150 and 300 mg kg^{-1}) of Olive Leaf phenolic commercial extract and stored at 30°C for 30 days. Its quality was monitored for microbiological and sensory parameters, individual phenolic content, oxidative stability, antioxidant parameters. The analysis quantitative showed that all added phenolic compounds were transferred to the oil fraction., particularly a high concentration of hydroxytyrosol acetyl derivative (Hy-Ac) was detected (0.88 g 100 g^{-1}). The addition of 150 mg kg^{-1} of extract allowed to obtain a mayonnaise more stable to oxidation, as demonstrated by a constant induction period over time. Hydroxytyrosol and hydroxyl acetyl derivate functionalized and improved the antioxidant activity of the analyzed samples as confirmed by their correlation with DPPH assay. Enriched mayonnaises showed good pH values and no microbial contamination was revealed. Furthermore, for the production of enriched mayonnaise is better to use lower concentration of phenolic extract (50 mg kg^{-1}). An excessive use of extract can change the taste of the final product, making it unacceptable for the consumer.

1. Introduction

Mayonnaise represents one of the most widespread sauces in the world (Gavahian et al., 2013). It is an oil-in-water emulsion prepared with three different ingredients: 70-80% oil (the dispersed phase), vinegar (the continuous phase), and egg yolk as an emulsifier at the interface (Ghorbani Gorji et al., 2016). The low pH of mayonnaise (pH 4) causes breaking of the iron bridges among egg yolk proteins and releasing of the iron which is able to participate in lipid oxidation promoting reactions with unsaturated lipids, forming lipid radicals or leading to degradation of peroxides (Honold et al., 2016, Sorensen et al. 2010). Therefore, as for all foods with high oil content, mayonnaise is susceptible to deterioration due to the oxidation of the unsaturated fats in its oil fraction. The rate of lipid oxidation in the emulsion is also influenced by several factors including the molecular structure of lipids, heat, light, physical characteristics of emulsion droplets, and processing conditions (Kiokias et al. 2009). The use of antioxidants allows to delay or inhibit the lipid oxidation reactions improving the oxidative stability of the emulsion: in a multiphase system, such as mayonnaise, they can partition into the aqueous phase, the oil phase, and the interface between oil and water, but their partition is influenced by the hydrophilic and lipophilic character of the specific antioxidants (Jacobsen et al. 1999). In recent years, particular attention to the use of natural antioxidants particularly phenolic compounds have been given, both for their healthy effect and for food preservation properties (Romeo et al., 2020).The obtainment of natural phenol compounds by agro-industrial residues as olive mill water and pomace (Romeo et al., 2019) or olive leaves, nowadays still undervalued (Flamminii et al., 2019), is a valid way to transform industrial scraps into a resource obtaining from these healthy and functional ingredients, with positive economic and environmental effects. In addition, natural extracts are produced industrially with the aim of being marketed for food, cosmetic and pharmaceutical applications, given that the consumers are increasingly interested to purchase healthier foodstuff and/or foods containing functional components (Rojas et al., 2019). The aim of this work was to test the efficacy of natural antioxidants obtained from olive leaves both to improve

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mayonnaise conservation and to formulate a new functional product. In particular, the effect of the enrichment with phenolic extract on oxidative stability and antioxidant activity of mayonnaise samples was evaluated.

2. Material and methods

2.1 Samples preparation

Mayonnaise was produced in the laboratory of Instituto de la Grasa, Sevilla (Spain) according to Honold et al. (2016) with some modifications. The preparation of about 600 mL of mayonnaise consisted in 1mL of lactic acid (45%), 4.3 mL of glacial acetic acid, 20 mL of lemon juice, 0.6 g of potassium sorbate, 10 mL of water, 4 eggs, 3.6 g salt (0.6 %) and 550 mL of sunflower oil. A hydrophilic Phenolic Olive Leaves extract (PE) powdered, (Biomaslinic S.L. Escúzar, Granada-Spain), was used as natural antioxidant for enrichment of mayonnaise samples, due to its potential health effects and for improving the oxidative stability of the emulsion. The PE enrichment was performed of mayonnaise at different concentrations: 50,150 and 300 mg kg^{-1} (Hydroxytyrosol equivalent). These samples were named respectively M50, M150 and M300, and were compared to the control sample represented by the mayonnaise without extract. The samples were stored and monitored for 30 days.

2.2 Microbiological analysis

The viable populations of the principal groups of microorganisms were determined on mayonnaise samples, at production day and at the end of storage, by plates inoculation and incubation at 32 °C up to 3 days before counting the colonies in the following selective media: total mesophilic bacteria in Plate Count Agar (Plate Count Agar, Conda-Pronadisa, Spain), lactic acid bacteria in MRS Agar (LAB) (Oxoid), yeasts and moulds in OGYA (Oxoid).

2.3 Evaluation of antioxidant activity of mayonnaise

Mayonnaise samples were submitted to the extraction method described by Chatterjee et al. (2015), appropriately modified: 0.5 g of the mayonnaise was dissolved in 5 mL of methanol; then the solution was centrifuged at 9000 rpm for 5 min at 10 °C (SIGMA Laborzentrifugen Model 1K15 Micro Centrifuge) and filtered through a 0.2-mm pore size nylon filter. The obtained extracts were evaluated for antioxidant activity by measurement of DPPH and ABTS radical scavenging activity according to De Bruno et al. (2018) with some modifications.

For *DPPH (2,2-diphenyl-1-picrylhydrazyl) a*ssay: 50 µL of sample were added to 2950 µL of DPPH solution in a cuvette and the decrement of absorbance was evaluated spectrophotometrically at 515 nm using a Cary 1E UV-vis spectrophotometer (Varian, Mulgrave, Australia) against methanol. The results were expressed as percentage of inhibition and calculated by applying the following formula:

$$\% \text{ Inhibition} = 100 * \frac{(At0 - Ate)}{At0}$$

where Ate is the value of absorbance measured after 70 min while At0 is the value of absorbance of DPPH_solution at the initial time.

For *ABTS (2,2' -azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) assay*: the reaction mixture was prepared by mixing 2950 µL of ABTS and 50 µL of sample. The decrement of absorbance was determined spectrophotometrically at 734 nm against ethanol. The quenching of initial absorbance was
plotted against Trolox concentration (from 1.5 to 24 µM) and the results were expressed as TEAC values (mmol TE g^{-1} of sample).

2.4 Phenolic quantification of lipid phase – HPLC analysis

The quantification of phenolic compounds was performed on the oil separated from mayonnaise. Samples of mayonnaise were frozen at -80°C for 8 h to separate the emulsion. Afterward, frozen mayonnaise was thawed leaving for 4 h at 5°C. Due to these operations; oil was separated from aqueous phase and then collected in an Eppendorf tube (50 mL of capacity).

The quantification of phenolic compounds was performed on the oil separated from mayonnaise, following the method reported by Romero et al. (2016). The final extract was filtered through a 0.22 µm pore size nylon filter and injected into the HPLC system (Waters Inc. Mildord, MA). Separation was achieved using an elution gradient, where the used solvents were: A: acidified water (pH 2.7 with phosphoric acid) and B: methanol; with a flow rate of 1 mL/min. Internal standard (syringic acid, Extrasynthèse) were used for the quantification and results were expressed as mg kg^{-1} of the lipid fraction.

2.5 Oxidative stability of mayonnaise oil

Oil stability index was determined at 100 °C and 20 mL air/h using a 6709 Rancimat apparatus (Metrohm, Herisau, Switzerland) following the AOCS Method (1994). 2.5 g of oil were weighed in each reaction vessel, the reaction vessel attachment was introduced into the narrow glass tube through which air enters. The Rancimat apparatus was used to evaluate induction period (time corresponding to the inflection point in the oxidation curve) measured as hours.

2.6 Sensory analysis

A triangle test was applied following ISO 4120:2004 and choosing α=0.2, β=0.05, Pd =40% and 8 panelists (males and females, from 25 to 60 years old, recruited among researchers and technicians of the Instituto de la Grasa, Sevilla - Spain) for sensory evaluation. The sensory evaluation was carried out on enriched samples of mayonnaise versus control. Six different combinations were distributed in randomized order to the panelists and the forced choice procedure was used. The study of perception of "negative sensation" (abnormal flavor and bitter) was also performed.

2.7 Statistical analysis

All the experimental results were expressed as mean values ± standard deviation (SD) of four measurements (n= 4). In these single-factor experiments, significant differences (P<0.05) among treatment means were determined by One-way analysis of variance (ANOVA) and *post-hoc* Tukey's test. The influence of time and temperature of storage were analysed by multivariate analysis. SPSS Software (Version 15.0, SPSS Inc. Chicago, IL, USA) was used for data processing.

3. Results

Olive Leaves phenolic extract, a by-product from the olive oil industry, was used as natural antioxidant for enrichment of mayonnaise samples, due to its potential health effects and for improve the oxidative stability of the emulsion. Through chromatographic analysis (HPLC), the main phenolic compounds present in PE were highlighted and the results were reported in Table 1.

Table 1: Individual phenolic compounds of Olive Leaf phenolic extract

Phenolic compounds (g 100 g^{-1})	
Hydroxytyrosol	12.28±0.05
Tyrosol	2.38±0.05
Hydroxytyrosol acetyl derivate (Hy-AC)	0.88±0.05
Vanillic acid	0.07±0.01
Hy-glycol	0.20±0.01
Total	15.81±0.75

Table 2: Individual phenolic compounds of lipid phase of mayonnaise samples during storage

mg kg^{-1}	day	Hydroxytyrosol	Tyrosol	Vanillic acid	Hy-AC	TPC
M50	1st	2.18±0.38	1.37±0.18	0.26±0.11	12.16±0.57	15.97±1.11
	30th	0.74±0.05	1.46±0.02	0.23±0.01	6.66±0.23	9.09±0.28
	Sign	**	n.s.	**	*	**
M150	1st	7.08±0.44	4.40±0.18	1.01±0.59	41.12±1.39	53.61±2.01
	30th	5.14±0.11	4.68±0.05	0.74±0.08	28.92±0.94	39.47±1.09
	Sign	**	*	**	**	**
M300	1st	13.59±0.85	8.64±0.18	2.01±0.29	77.01±8.30	101.80±10.07
	30th	12.28±0.54	8.99±0.19	1.54±0.11	59.92±0.91	82.93±1.92
		*	n.s.	*	*	*

*Significance at P<0.05; ** Significance at P<0.01; n.s. not significant (by Tukey HSD^a test)

The main compounds detected were: hydroxytyrosol and tyrosol and, in lower amounts hydroxytyrosol acetyl derivate vanillic acid, and hydroxytyrosol glycol. As reported by Apicella et al; 2019 the content of phenolics

compounds as well as the antioxidant activity of extracts depend of different factors among which its physicochemical characteristics (dry matter) and raw materials (olive pomace, wastewaters or olive leaf). Jaski et al; 2019 the interaction between the various constituents of olive leaf extract determine a good antioxidant activity of extract ranged from 29.3 and 45.2 µg mL. The activity of PE phenolic compounds is linked to three different mechanisms: a) chain-breaking antioxidants, b) hydroperoxide destroyers and c) metal chelators (Altunkaya et al., 2013). The addition of PE to mayonnaise involved the transfer of phenolics from hydrophilic to the lipophilic phase, proportionally to the used concentration: the obtained results are illustrated in Table 2.
The main compound which was transferred from PE to the lipidic phase of mayonnaise was the hydroxytyrosol acetyl derivative (Hy-Ac). This result was in agreement with the data reported by Lisete-Torres et al. (2012) regarding the distribution of hydroxytyrosol and Hy-Ac in oil-water emulsion. They showed that hydroxytyrosol is oil-insoluble while acyl derivatives are both oil and water-soluble with a partition constant of 0.6. Among the revealed individual phenolic compounds, only the tyrosol in M50 e M300 samples did not show significant differences during storage, while all the others showed a lower concentration at the end of storage (P<0.05). The Rancimat test was used to determine the oxidative stability of mayonnaise during the storage period, and the obtained results (Figure 1) showed better stability (higher induction period) due to the PE enrichment.

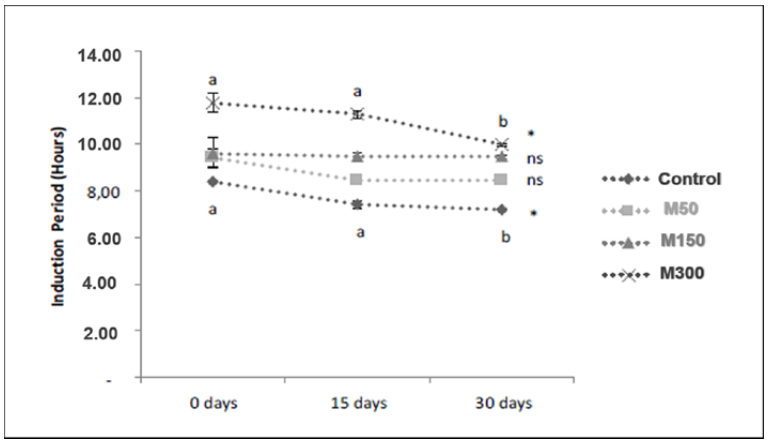

Figure 1: Oxidative stability of mayonnaise during storage period

On 1st day of production, M300 sample revealed a longer induction period (11.8±0.4 h) compared to M50 and M150, but the stability of this sample already decreased after 15 days of storage, until reaching an induction period close to the other two samples. Indeed, M50 and M150 demonstrated to be the most stable with a constant induction period during storage time. This work showed in general that the enriched mayonnaise presents a good oxidative stability value compared with results obtained by other authors (Raikos et al. 2016).
DPPH and ABTS assays were performed in order to evaluate the total antioxidant activity (TAA) of mayonnaise samples and the obtained results were reported in Table 3.

Table 3: Evaluation of antioxidant activity (DPPH and ABTS assays) of Mayonnaise samples during storage

		M50	M150	M300
DPPH (% of inhibition)	1st	27.5±0.9	42.7±1.4	70.3±2.1
	30th	18.8±0.7	33.5±0.4	50.1±0.8
	Sign	**	**	**
TEAC (mmol TE g^{-1})	1st	5.96±0.35	5.73±0.71	6.34±0.05
	30th	4.49±0.20	6.62±0.17	7.68±0.34
	Sign	**	**	**

*Significance at P<0.05; ** Significance at P<0.01; n.s.not significant (by Tukey HSD[a] test).

The antioxidant activity measured with both assays showed significant differences among samples (P<0.01). TAA measured by DPPH test allowed to highlight that all samples showed a higher value at production day, with values that ranged between 27.5 and 70.3 (% of inhibition), with values proportionally correlated to the concentration of extract added, but during the storage period (30 days) there was a reduction of TAA. The

reduction of % of inhibition during the storage period can be because of the positive effect of antioxidants (PE) on the mayonnaise samples, namely the added phenols had donated hydrogen atom to the oxidable substrate, promoting the stability of the sample. A different trend was revealed by ABTS assay, for which the values ranged from 5.96 to 6.34 mmol TE g^{-1} at time zero in all the samples.Although ABTS. assay is more accurate for the determination of antioxidant capacity of lipophilic and hydrophilic compounds compared with DPPH test, due to the fact that ABTS test is not affected by ionic strength (Prior et al. 2005), the obtained results did not show a great variability of antioxidant activity proportional to the amount of extract added.
The high antioxidant activity of samples could be explained considering the effect of hydroxytyrosol and HY-Ac as demonstrated by Bouallaugui et al. (2011) that showed a comparable effect of these compounds on % of inhibition The efficacy of enrichment with PE on the antioxidant stability was established by their high correlation with DPPH value (r=1.00 for hydroxytyrosol and r=0.99 for hydroxyl acyl derivate).
The multivariate analysis showed that the time of storage and the treatments as well as their combination have significant effect on the antioxidant activity ($P<0.01$).
Safety, quality and antioxidant activity of foods are important aspects to consumers. To evaluate the potential application of enriched mayonnaise, all samples were subjected to microbiological analysis. During the storage time (30 days), the samples differently treated did not show measurable mesophilic aerobic microorganism colonies, yeast, and lactic bacteria (<1 cfu/mL). After this time, approximately around the 45^{th} day of storage was observed microbial spoilage, which determined the conclusion of experimentation. . At same time, the samples were subjected to pH analysis, that represents another important parameter for the safety of the stored foods and showed pH <4.2. Due to the low pH, high fat and acetic acid level, mayonnaise is commonly considered as resistant to microbial spoilage (Depree & Savage, 2001). In the meantime, these results also suggest that PE may be used as a natural preservative agent to improve the microbiological quality of mayonnaise.
Another important aspect considered by the consumer is the sensorial characteristics of the final product. For this reason, a group of panelists evaluated the main parameters, through a triangular comparison test, in order to detect differences among the samples. 3 out of 8 panelists recognized Control and M50 samples different for the flavour character. Moreover, it was evident that the addition of higher concentrations of phenolic extract altered the flavour of “conventional” mayonnaise, so it was not appreciated by the panellists. Based on the results of the triangle test the sensorial analysis of an eventual “negative perception” (abnormal flavour and bitterness) was also performed. For all samples no tester detected abnormal flavour while a light bitterness sensation was detected for enriched mayonnaise (M50). Even though all samples were recognized as different from conventional mayonnaise, the addition of a low concentration of phenolic extract did not seem to negatively alter the taste of the final product.

4. Conclusions

The results of this work showed that the enrichment of mayonnaise with different concentrations of a commercial olive leaf phenolic extract significantly enhanced the oxidative stability of mayonnaise during the storage period. The study of the stability of mayonnaise confirmed also the polar paradox about the role of lipophilic compounds in an oil-water emulsion. In fact, the higher antioxidant activity in enriched mayonnaise couldbe related to a higher content of lipid-soluble fractions of Hy-AC. Despite the samples enriched with the highest concentration of phenolic extract (300 mg L^{-1}) showed high antioxidant activity and a good oxidative stability, the addition of 150 mg L^{-1} of the extract resulted in a more stable mayonnaise, so a positive result by an intermediated concentration, among those tested, and therefore a reduction in production costs. Furthermore, for the formulation of an enriched product, another important aspect to consider is the sensory properties, indeed a high concentration of added phenolic extract changed the taste of the final product, making it unacceptable for the consumer.

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