



Effectiveness of natural antioxidants on oxidative stability of margarines

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ABSTRACT

Lipid oxidation has been identified as the main deterioration process of margarines and in which the addition of antioxidants is necessary. Consumers are increasingly demanding that natural preservatives replace chemical antioxidants in foods. The main aim of this study was to improve the oxidative stability of eight commercial margarines without antioxidants, selected by Principal Component Analysis and grouped into three clusters by the addition of six natural ground herbs: *Curcuma longa*, *Illicium verum*, *Rosmarinus officinalis*, *Taraxacum officinale*, *Thymus piperella* and *Thymus vulgaris*. A preliminary assay showed that the margarines with higher total fat and saturated fatty acids presented higher Induction Periods. When adding spices and herbs to margarines, *Rosmarinus officinalis* increased the oxidative stability value by 73.17% with respect to its baseline values, *Curcuma longa*, *Thymus vulgaris* and *Thymus piperella* between 17 and 26% and *Taraxacum officinale* together with *Illicium verum* reduced it by 19% and 34% respectively. The most effective species shown by chemical and statistical analysis were those containing a high content of highly lipophilic apolar phenolic compounds. A multiple linear regression model showed that Induction Period has a positive correlation with saturated and polyunsaturated fatty acids, $\omega-3$ acids, the monounsaturated/polyunsaturated fatty acid ratio and the antioxidant activity of the added herbs. The oxidative stability of margarines is influenced by various factors and compounds present in their formulation, but the use of plants and spices could be an alternative to improve this emulsion.

1. Introduction

Margarine is a popular plant-based alternative to butter, commonly used for cooking and baking. It consists of a water in oil emulsion containing liquid vegetable oils, water, emulsifiers, and other minor ingredients (Silva, Barrera-Arellano, & Badan Ribeiro, 2021). The most common vegetable oils used are corn, cottonseed, safflower, sunflower, rapeseed, soy, and palm oils due to their affordable prices and spreadability (Zhang et al., 2024).

However, these polyunsaturated fatty acid rich oils are prone to oxidation, which negatively impacts product quality through rancidity development (Fruehwirth, Egger, Flecker, et al., 2021). Lipid oxidation

is initiated by free radicals which damage polyunsaturated fatty acids, producing a variety of volatile oxidation products (Wang, Xiao, Lyu, Chen, & Wei, 2023). Primary oxidation compounds include hydroperoxides, while secondary products consist of aldehydes, ketones, alcohols and short chain fatty acids (Shahidi & Zhong, 2010).

Factors influencing margarine oxidation include: fatty acid profile, metals, light, oxygen, and temperature (Silva, Barrera-Arellano, & Ribeiro, 2021). Margarines with higher polyunsaturated fatty acids levels are more susceptible to oxidation than those containing more saturated and monounsaturated fatty acids (Shahidi & Ambigaipalan, 2018). This deterioration process gives the food a rancid or off-flavour, reduces its nutritional value and shortens its shelf life (Geng, Liu, &

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Zhang, 2023).

To increase shelf life and prevent lipid oxidation, synthetic antioxidants like butylhydroxyanisole (BHA) (E-number 320, food additive) and butylhydroxytoluene (BHT) (E-number 321, food additive) and others, are commonly used in margarines (Boon, McClements, & Weiss, 2022). The low cost of these products, their stable chemical structures, their thermal stability and their efficiency, together with the possibility of being produced in large quantities, mean that they are widely used in the food industry (Xu et al., 2021, Gonçalves-Filho & De Souza, 2022).

But these products have been shown to be potentially harmful. Several studies have shown that BHA could cause growth and metabolic disorders, thyroid damage, carcinogenesis and neurotoxicity due to mechanisms such as endocrine disruption, genotoxicity, altered energy metabolism, oxidative stress, signalling pathways and imbalances in calcium homeostasis (Zhang, Diao, & Zhang, 2023). While the toxicity of BHT is still debated, evidence of side effects, including carcinogenicity, has been reported (Nieva-Echevarría, Manzanos, Goicoechea, & Guillén, 2015). As a result, there is growing interest in developing effective natural antioxidant alternatives from plant sources to promote food safety and human well-being (Almajano & Gordon, 2020).

Species from the genera *Rosmarinus*, *Thymus*, *Curcuma*, *Taraxacum* and *Illicium* and their extracts have traditionally been used for culinary and medicinal purposes. Antimicrobial, anti-inflammatory, antidiabetic, antihypertensive, hypoglycaemic and other properties have been attributed to them (Alan Jiang, 2019).

These herbs and spices have a high phenolic content which gives them a high antioxidant potential. Both *in vitro* and in food studies have demonstrated these protective effects. In a study carried out with different natural ground herbs (black pepper, ginger, turmeric, rosemary and oregano) in vegetable oils, it was found that rosemary was the most effective antioxidant among the ones tested (Redondo-Cuevas, Castellano, & Raikos, 2017). The methanolic extract of rosemary had a higher antioxidant capacity than oregano when studied in fish oil (Bale et al., 2007). In another trial, a phenol-enriched olive oil, prepared with a phenolic extract composed of 60% olive and 40% thyme, showed significantly higher oxidative stability compared to the control (Rubí, Motilva, Macià, Ramo, & Romero, 2012). Some studies have demonstrated that extracts of rosemary exhibited very strong antioxidant activity, in natural sunflower oil stored at 60 °C and rapeseed oil (Chen et al., 2014; Guo et al., 2023, Sahunie, 2024). The thyme extract obtained by ethanolic extraction showed the ability to prevent the formation of primary and secondary lipid oxidation products in oil in water emulsions consisting of various proportions of wheat and almond oils for a period of 10 weeks at 37 °C (El-Guendouz et al., 2019). Emire et al. found that thyme leaf and flower powder at a concentration of 0.1% increased the induction period when tested in soybean oil and in a butter sample. Thyme extract significantly ($P < 0.05$) increased the oxidative stability when compared to control samples with 0.05% α -tocopherol (Hailemariam & Emire, 2013).

The aims of this study were to: 1) analyse and select margarines of different composition; 2) investigate their oxidative stability; 3) evaluate the antioxidant capacity of different plant species: *Curcuma longa*, *Rosmarinus officinalis*, *Taraxacum officinale*, *Illicium verum*, *Thymus piperella* and *Thymus vulgaris* on margarines; 4) study and compare the effect of the antioxidant power of different plant species; 5) establish a relationship between oxidative stability and total phenolic compounds of plant species.

2. Materials and methods

2.1. Margarines

Twenty margarines were selected from different commercial brands and stored at 4 °C. The nutritional composition of each of the margarines was extracted from the manufacturers' packaging.

2.2. Plant material

The following six plant species were selected for their high content of phenolic compounds, flavonoids, tannins and diterpenes: *Curcuma longa*, *Rosmarinus officinalis*, *Taraxacum officinale*, *Illicium verum*, *Thymus piperella* and *Thymus vulgaris*.

The plant species were used as dehydrated material and were kept at room temperature (20 °C) and under dark conditions. Table 1 describes the manufacturer's information (name and batch number) of the plant species used in the assays.

2.3. Study of oxidative stability

Oxidative stability of the eight margarines individually and of the margarines with the five plant species (*Curcuma longa*, *Rosmarinus officinalis*, *Taraxacum officinale*, *Illicium verum*, *Thymus piperella* and *Thymus vulgaris*) was determined by evaluating the induction period (IP) with a Rancimat 892 system (Shakeri et al., 2010). The IP value is indicative of the resistance of the sample to oxidation, so that the longer the induction time, the more stable the analyzed sample is. Conductivity time curves are displayed live for each measurement point in the database. The induction time in hours (h) was determined automatically from the second derivative of the measuring curve, and the manufacturer's performance protocol for solid vegetable fats was followed.

For the tests a temperature of 120 ± 1.6 °C and an air flow of 20 L/h were used. Each analysis was carried out in quadruplicate. 3 g each of the margarines were used for each test. The plant species were supplied in powder form and homogenized together with the 3 g of each margarine using a percentage of 0.5% (w/w), as established by the European Commission and with previously conducted studies by Redondo-Cuevas et al. (European Commission, 2011; Redondo-Cuevas et al., 2017; Redondo-Cuevas et al., 2018). The variation of the oxidative stability of each of the different margarines with added vegetable extracts was studied and compared with the data obtained from their respective controls (margarine samples without added vegetable extracts) (Fig. 1).

2.4. Ferric reducing ability of power (FRAP) assays in plants

Extraction and testing sample preparations were adapted from Wojdyło et al. with some modifications. In short, 1 g of each ground aromatic plant was weighed in a test tube (Wojdyło, Oszmiański, & Czemerys, 2007). A total of 10 mL of 80% aqueous methanol was added, and the suspension was gently mixed. Tubes were sonicated twice for 15 min and then centrifuged for 10 min at 1500 rpm using a CompactStar CS4 centrifuge (VWR International Ltd, West Sussex, UK). Supernatants were collected and stored at 4 °C prior to their use within 24 h.

The total antioxidant potential of the five samples of plants was determined, using the FRAP assay adapted from Benzie and Strain, as a measure of antioxidant power (Benzie & Strain, 1996). The FRAP reagent was freshly prepared by mixing 25 mL of acetate buffer (300 mmol/L, pH 3.6), 2.5 mL of a solution of 10 mmol/L TPTZ in 40 mmol/L HCl, and 2.5 mL of 20 mmol/L FeCl_3 , and once prepared, it was kept in a

Table 1
Manufacturer information of the plant species used in the assays.

Plant species	Batch number	Manufacturer name/Location
<i>Curcuma longa</i>	F12747	Celeplame S.L./Barbate (Cádiz)
<i>Illicium verum</i>	3489	Celeplame S.L./Barbate (Cádiz)
<i>Rosmarinus officinalis</i>	F12449	Celeplame S.L./Barbate (Cádiz)
<i>Taraxacum officinale</i>	F12598	Celeplame S.L./Barbate (Cádiz)
<i>Thymus piperella</i>	5049	Celeplame S.L./Barbate (Cádiz)
<i>Thymus vulgaris</i>	11110.11110	Jesús Navarro S.A./Monforte del Cid (Alicante)

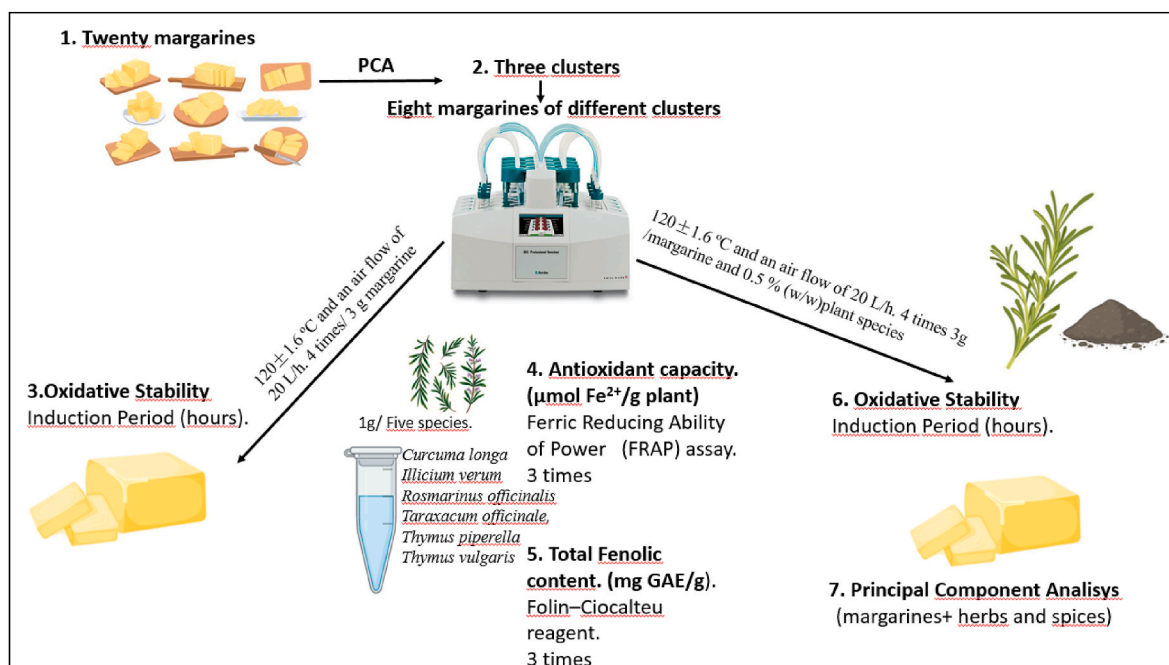


Fig. 1. Graphical summary of the different tests carried out in the study.

water bath at 37 °C. The following were added directly to each cuvette in a Pye Unicam UV-4 UV–VIS scanning spectrophotometer (Spectronic Camspec Ltd., Leeds, UK) thermostated at 37 °C: 90 µL H₂O, 900 µL FRAP reagent and 30 µL of sample (diluted 25 times), with standard or H₂O as a target. Absorbance readings were taken at 593 nm after 4 min at 37 °C. A solution of 1 mmol/L FeSO₄ was used as standard. All determinations were performed in triplicate. The results were corrected for dilution and expressed in µmol Fe²⁺ per gram of plant (Fig. 1).

2.5. Total phenolic content (TPC) of plants

Extraction and testing sample preparations were performed as follows: 1 g of each plant was weighed in a test tube, and 20 mL of methanol were added. The test tube was vortexed, homogenized for 2 min at 8000 rpm, left at a tube roller mixer for 1 h, and then centrifuged at 1100 rpm for 15 min. The supernatant was collected, and the procedure was repeated with another 20 mL of methanol. The two methanol extracts were combined, and the final volume was brought up to 40 mL with methanol. The total phenolic contents of the samples were determined using the Folin–Ciocalteu reagent as described by Parry et al. (Parry et al., 2005). In short, the reaction mixture contained 50 µL of testing sample solutions, 250 µL of the Folin–Ciocalteu reagent, 0.75 mL of 20% sodium carbonate, and 3 mL of pure water. After 2 h of reaction at room temperature, the absorbance at 765 nm was measured by a Pye Unicam UV-4 UV–vis scanning spectrophotometer (Spectronic Camspec Ltd., Leeds, UK) and was used to calculate the phenolic contents of oils using gallic acid as standard. Measurements were taken in triplicate. The results were expressed as mg gallic acid equivalents per gram of powder (mg GAE/g) (Fig. 1).

2.6. Statistical analysis

For the categorization and selection of the margarines in the study according to their nutritional composition, a multivariate Principal Component Analysis (PCA) was used. Total fatty acids (TFA), saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), vitamin A (Vit A) and vitamin E (Vit E) were used as variables for statistical analysis.

A descriptive analysis was performed using mean values, and

standard deviations of phenolic content, antioxidant capacity of plant species and Induction Periods of margarines. The normality of the variable distributions were verified by the Kolmogorov–Smirnov test.

A regression test (Pearson's *r*) was used to establish the possible correlation between the phenolic content (PC) and the antioxidant capacity of the herbs.

The analysis of variance (ANOVA) and Fisher LSD intervals with significance values of *P* < 0.05 were used to compare the Induction Periods of each margarine, and Induction periods of these without and with the herbs added. Statgraphics Centurion 19 software (Stat Point, Inc., Herndon, Virginia, USA) was used for this analysis.

To determine the influence of variables related to the nutritional composition of margarines (PUFA, SFA, MUFA/PUFA ratio), and antioxidant capacity of the herbs on the fat Induction Period obtained by adding the herbs, a multiple regression analysis was performed.

The multiple regression models and PCA for researching a possible relationship between the induction period and their fatty acid composition were performed using SPSS (vs. 21.0, IBM Corp., Chicago, IL, USA), and Knowledge Miner and Microsoft Excel for Office (2020) for Windows 10 OS.

3. Results

3.1. Antioxidant capacity of the plant species

The antioxidant capacity according to the FRAP assay and the total phenolic content of the plant species used in the tests are shown in Table 2.

Rosmarinus officinalis showed the higher phenolic content and the higher antioxidant capacity. The herbs of genus *Thymus* were the next in antioxidant capacity. *Taraxacum officinale* and *Illicium Verum* recorded the lowest values. A significant correlation between phenolic and antioxidant capacity of plant species was registered (*r* = 0.8449; *P*-value = 0.0342 < 0.05).

3.2. Preliminary selection of margarines

Table 3 shows the nutritional composition of the 20 margarines. Fats that were selected according to PCA were marked with an asterisk.

Table 2Phenol content and antioxidant capacity of the plant species used in the tests^a.

Plant species	Phenolic content (mg GAE/g)	Antioxidant capacity (μmol Fe ²⁺ /g)
<i>Curcuma longa</i>	240 ± 30	166.0 ± 0.7
<i>Rosmarinus officinalis</i>	410 ± 22	570 ± 70
<i>Taraxacum officinale</i>	33.90 ± 0.18	131.5 ± 0.9
<i>Illicium verum</i>	96.3 ± 0.5	88.9 ± 0.5
<i>Thymus piperella</i>	57.4 ± 0.4	213.5 ± 1.4
<i>Thymus vulgaris</i>	172.6 ± 1.4	242.3 ± 1.9

Data are presented as mean ± SD (n = 3).

^a g: grams. mg: milligrams. GAE: gallic acid equivalents.

Fig. 2 represents all the variables taken into consideration for the first two PCs (Plot of Components 1 and 2). The variance explained by PC1 and PC2 was 73.87%. PC1 (51.56% of the total variance) showed a positive loading with all variables. PC2, which explained 22.31% of the total variance and was positively correlated with Vit A and Vit E, and negatively correlated with SFA, TFA, MUFA and PUFA. Properties more remote from the 0.0 point were more important in describing the PCs.

The two-dimensional representation of margarines is shown in Fig. 3. The multivariate statistical analysis distributed the 20 margarines analyzed in three clusters based on their nutritional composition: Cluster 1 (F1>0, F2> 0), Cluster 2 (F1<0, F2>0), and Cluster 3 (F2<0). Cluster 1 (F1>0, F2> 0) exclusively included samples characterized by high TFA, SFA and Vit. A contents. Cluster 2 (F1<0, F2>0), included samples characterized by a low content in TFA, SFA and PUFA. Finally, the last group, Cluster 3 (F2<0), included samples with high content in MUFA, ω-3 and ω-6 fatty acids (Table 3).

Considering the results obtained in the statistical analysis, eight margarines were selected: Flora pro-activ colesterol original, Ligeresa, Flora oliva and Consum margarina ligera were grouped together. Consum margarina vegetal ¼, Tulipan original and Consum mantequilla were placed in a second group. A margarine was also chosen that was not placed into the two previous main groups, but separately in a third group: Flora original.

The labelling of these margarines as such did not show antioxidants

but there were substances used for other purposes that could have this action. Consum mantequilla (acidulant: citric acid, colorants: carotenes, vitamins: A, D and E); Consum margarina ligera (acidulant: lactic acid, colorants: carotenes, vitamins: A, D and E); Consum margarina vegetal ¼ (acidulant: lactic acid, vitamins: A, B1 and D); Flora oliva and Flora original (colorants: carotenes, vitamins: B1 and D); Flora pro-activ colesterol original (vitamins: A and D); Ligeresa (acidulant: lactic acid, vitamins: A, B1 and D); Tulipan original (acidulant: lactic acid, vitamins: A, D and E).

3.3. Induction period and fatty acid composition

Table 4 shows the fatty acid composition (in grams per 100 g of margarine) of each one of the selected margarines and the IP values obtained experimentally with the Rancimat equipment.

Analysing the clusters obtained from the statistical analysis and the composition determined by the Rancimat and that supplied by the manufacturers, it was found that Cluster 1 (F1>0, F2> 0) exclusively included samples characterized by high TFA, SFA and Vit A contents. Cluster 2 (F1<0, F2>0) contained samples characterized by a low content in TFA, SFA and PUFA. Finally, the last group, Cluster 3 (F2<0) comprised samples with high content in MUFA, omega-3 (ω-3) and omega-6 (ω-6) fatty acids (Tables 3 and 4, Fig. 3).

Fig. 4 shows the IP of the margarines that were selected and the trend line of the margarines belonging to the different clusters. The ordinate axis shows the different margarines used in the experiment and the abscissa axis shows the induction time in hours for the different margarines used.

It was observed that the IP increased in the direction: Cluster 1>Cluster 2>Cluster 3, which meant that cluster 1 margarines in general had higher oxidative stability and cluster 3 margarines had lower oxidative stability. In general, the margarines with the highest TFA content had the highest IP values (Tulipan original and Consum margarina vegetal ¼).

Something similar happened with the saturated fatty acid content. When comparing the margarines of cluster 1, namely Tulipan original and Consum margarina vegetal ¼, it was observed that the margarine with the higher SFA value (34 g for the former versus 15 g for the latter

Table 3Composition of the 20 margarines used in the Principal Component Analysis (PCA)^a.

Margarine Commercial brand	TFA (g)	SFA (g)	MUFA (g)	PUFA (g)	UFA (g)	MUFA/PUFA	Carbo-hydrates (g)	Proteins (g)	Vit. A (μg)	Vit. D (μg)	Vit. E (μg)	Salt (g)
Tulipan original ^b	80	34	23	23	46	0.50	0.5	0.5	800	7.5	17000	0.31
Tulipan con sal	60	18	15	26	41	0.63	0.5	0.5	800	7.5	16000	0.91
Tulipan simplemente vegano	50	12	20	17	37	0.46	0.5	0.5	0	7.5	10000	0.71
Flora original ^b	70	18	19	32	51	0.63	0.5	0	800	7.5	25000	0.20
Flora pro-activ colesterol original ^b	35	8	11	16	27	0.59	0.5	0.5	120	7.5	10000	0.3
Flora pro-activ colesterol oliva	35	8.1	12	15	27	0.56	0.5	0.5	800	7.5	10000	0.33
Flora pro-activ colesterol sabor mantequilla	70	16	23	31	54	0.57	0.5	0.5	120	7.5	18000	0.5
Flora Esencia	70	15	33	22	55	0.40	0.5	0.5	120	7.5	13000	0.4
Flora oliva ^b	40	11	13	17	30	0.57	0.5	0.5	800	7.5	12000	0.34
Flora Ligera	39	12	12	15	27	0.56	0.5	0.5	800	7.5	9700	0.37
Flora Delice	70	18	18	33	27	1.22	0.5	0.5	804	7.5	24700	0.21
Consum margarina vegetal ¼ ^b	60	15	17	27	51	0.25	0.5	0.5	650	5.4	24000	0.46
Consum margarina ligera ^b	40	12	17	11	28	0.39	0.5	0.5	853	7.5	22000	0.4
Margarina Hacendado	60	17	17	26	43	0.60	0.5	0.5	700	5.9	23000	0.3
Margarina con sal Hacendado	60	17	17	26	43	0.60	0.5	0.5	760	5.9	23000	0.9
Margarina ligera Hacendado	40	12	20	8.2	28	0.29	0.5	0.5	800	6.8	12000	0.4
Margarina de girasol Vitareform	80	23	20	37	57	0.65	0	0	900	2.5	40000	0.2
Margarina de girasol Vitareform baja en sal con calcio	60	13	23	24	47	0.51	0.5	0.5	800	7.5	27000	0.2
Ligeresa ^b	40	11	20	9	29	0.31	0.1	0.1	800	7.5	0	0.76
Consum mantequilla ^b	60	25	24	11	35	0.31	1	1	800	7.5	14000	0.4

^a g: grams. μg: micrograms. mg: milligrams. TFA: total fatty acids; SFA: saturated fats; MUFA: monounsaturated fats; PUFA: polyunsaturated fats; UFA: unsaturated fats; Vit.: vitamin.

^b Margarines then selected to add herbs or spices.

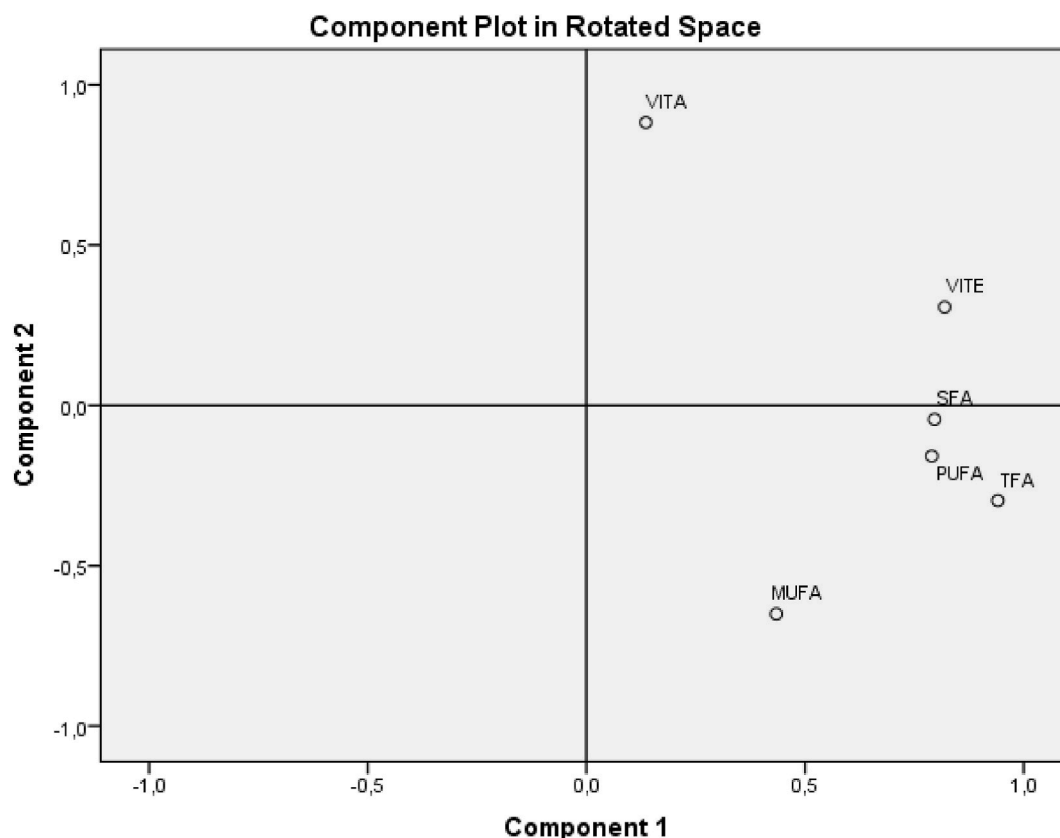


Fig. 2. Loadings plot for margarine samples. TFA: total fatty acids; SFA: saturated fats; MUFA: monounsaturated fats; PUFA: polyunsaturated fats; Vit.: vitamin.

margarine according to Table 4) presented a higher IP value (10.8 ± 0.3 h versus 5.88 ± 0.21 h) (Fig. 4).

In regard to cluster 2, the four margarines registered the lowest AGS values, and similar IP values were obtained (between 4.0 ± 0.3 h and 3.46 ± 0.17 h). Two margarines of this group (Flora oliva and Ligeresa) recorded the same content of total (TFA = 40g) and saturated (SFA = 11g) fatty acids and very similar values of unsaturated fatty acids (MUFA = 30 and 29 g, respectively) but different MUFA/PUFA ratios (0.76 vs. 2.22 respectively).

As for the only margarine from cluster 3 (Flora original), due to its high content of TFA (70 g) and SFA (18 g), and its low MUFA/PUFA ratio (0.59), a higher IP value would be expected. However, despite its fatty acid composition, its IP value was similar to those margarines of group 2 with high MUFA/PUFA ratios. This result could be due to the fact that this margarine (Flora original) was characterized by having the highest composition in grams of unsaturated fatty acids ω -3 and ω -6 (3.60 g and 29 g, respectively) (Table 4).

Although the margarines analyzed contained compounds with potential antioxidant activity, the Induction Periods obtained indicate that the presence of these compounds did not necessarily translate into greater oxidative stability. In fact, the margarines with the highest amount of antioxidant substances were not those that showed the longest induction times.

Fisher LSD intervals showed significant differences between the Induction Period of Tulipan original and Consum Margarina Ligera. Furthermore, the IP of these two margarines showed significant differences with the rest of the fats (data not shown).

3.4. Influence of added plants on the oxidation of margarines

Table 5 and Fig. 5 show the results of Induction Period averages and standard deviations in hours obtained with Rancimat, for eight margarines from different clusters, and these margarines with the six different

plant species added.

Of the six plant species used in the tests, four of them: *Rosmarinus officinalis*, *Curcuma longa*, *Thymus piperella* and *Thymus vulgaris* increased Induction Period times with respect to control margarines, which implies that their addition to margarines represented a protective measure to prevent their oxidation. However, the addition of *Illicium verum* and *Taraxacum officinale* produced a negative effect on the oxidative stability of the eight tested margarines (Table 5).

ANOVA and Fisher LSD intervals recorded that the improvement of the Induction Period was statistically significant when *Rosmarinus officinalis* was added to all margarines except Tulipan original. The increase produced by *Curcuma longa* was significant in Consum mantequilla and Consum margarina. *Thymus piperella* in Consum mantequilla and *Thymus vulgaris* in Consum mantequilla, Flora pro-activ colesterol original and Flora original.

The four plant species that improved the oxidative stability of all margarines did so to the greatest degree on the Consum mantequilla, which was characterized by the highest MUFA content (24 g) (Table 4). Therefore, this result indicated that the oxidative protective effect of the herbs used had a preferential effect on the MUFA content. *Rosmarinus officinalis* exerted its greatest protective effect against oxidation in the following margarines: Consum mantequilla (131.25%), Consum margarina ligera (94.48%), and Ligeresa (75.60%). *Curcuma longa* did so on Consum mantequilla (37.32%), Ligeresa (20.56%) and Consum margarina vegetal $\frac{3}{4}$ (15.15%). In the case of the two species of the genus *Thymus*, both improved the oxidative stability of two specific margarines: Consum mantequilla (*T. vulgaris* 104.67%; *T. piperella* 55.41% and Flora pro-activ original colesterol (*T. vulgaris* 44.07%; *T. piperella* 17.97%).

According to the results obtained in Table 5 and Fig. 5 on the IP values obtained in the different margarines after the individual addition of the four different herbs increasing the induction times, it was observed that Consum mantequilla was the margarine in which the

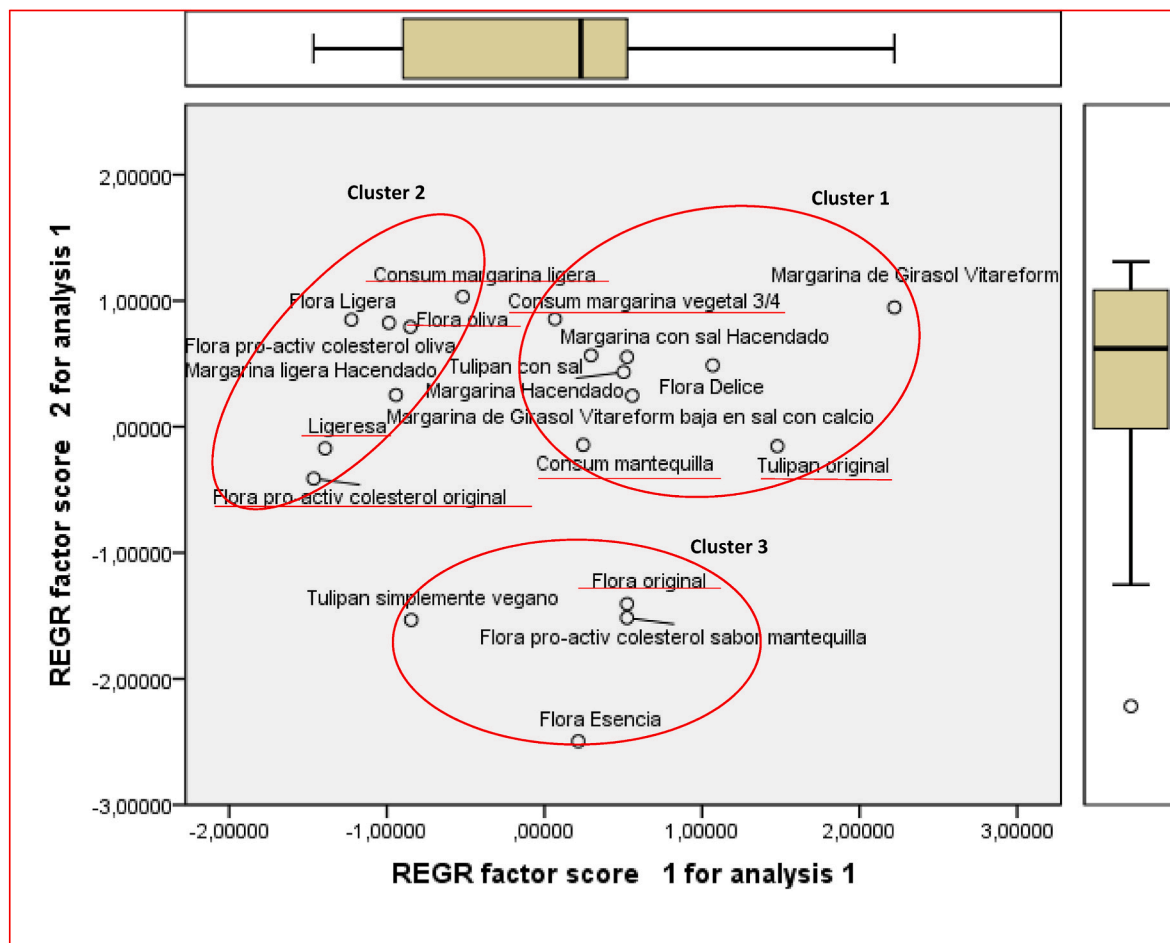


Fig. 3. Loadings plot for margarine samples.

Table 4

Fatty acid composition and Induction Period (IP) from the eight selected margarines in three clusters^a.

Cluster number	Margarine Brand	TFA (g)	SFA (g)	MUFA (g)	PUFA (g)	UFA (g)	MUFA/PUFA	UFA/SFA	ω-3 (g)	ω-6 (g)	IP value (h)
1	Tulipan original	80	34	23	23	46	1.00	1.35	0	0	10.8 ± 0.3
	Consum margarina vegetal 3/4	60	15	17	27	51	0.63	3.40	2.90	10	5.88 ± 0.21
	Consum mantequilla	60	25	24	11	35	2.18	1.40	0.00	11	2.65 ± 0.24
2	Flora pro-activ colesterol original	35	8	11	16	27	0.69	3.38	3.20	12	3.79 ± 0.09
	Flora oliva	40	11	13	17	30	0.76	2.73	1.90	15	3.74 ± 0.19
	Ligeresa	40	11	20	9	29	2.22	2.64	0	0	4.0 ± 0.3
	Consum margarina ligera	40	12	17	11	28	1.55	2.33	0	11	3.46 ± 0.17
											3.6 ± 0.3
3	Flora original	70	18	19	32	51	0.59	2.83	3.60	29	

Data are presented as mean ± SD (n = 4).

^a g: grams. h: hours. TFA: total fatty acids; SFA: saturated fats; MUFA: monounsaturated fats; PUFA: polyunsaturated fats; UFA: unsaturated fats; ω-3: omega-3; ω-6: omega-6.

effect of the addition of the four herbs was most noticeable. This margarine had one of the highest MUFA/PUFA ratios (2.18) and the highest MUFA content (24 g) (Table 4).

The plant species that provides greater oxidative stability to all tested margarines was *Rosmarinus officinalis* (in a range from 131.25% to 24.97%) followed by the two species of *Thymus* (*vulgaris* and *piperella*) and *Curcuma longa*. These results were consistent with their antioxidant capabilities (*Rosmarinus officinalis*: 570 μmol Fe²⁺/g; *Thymus vulgaris*: 242.3 μmol Fe²⁺/g; *Thymus piperella*: 213.5 μmol Fe²⁺/g and *Curcuma*

longa: 166.0 μmol Fe²⁺/g) (Table 2). Although the two *Thymus* species had similar antioxidant activity, *T. vulgaris* provided greater oxidative stability, probably because it had a higher phenol content than *T. piperella* (172.6 vs. 57.4 mg GAE/g, respectively) (Table 2).

In the case of the addition of rosemary, the improvement in the oxidative stability of the margarines was obtained in the following order from highest to lowest: Consum mantequilla, Consum margarina ligera, Ligeresa, Flora pro-activ colesterol original, Flora oliva, Consum margarina vegetal 3/4, Flora original, and lastly Tulipan original. The first

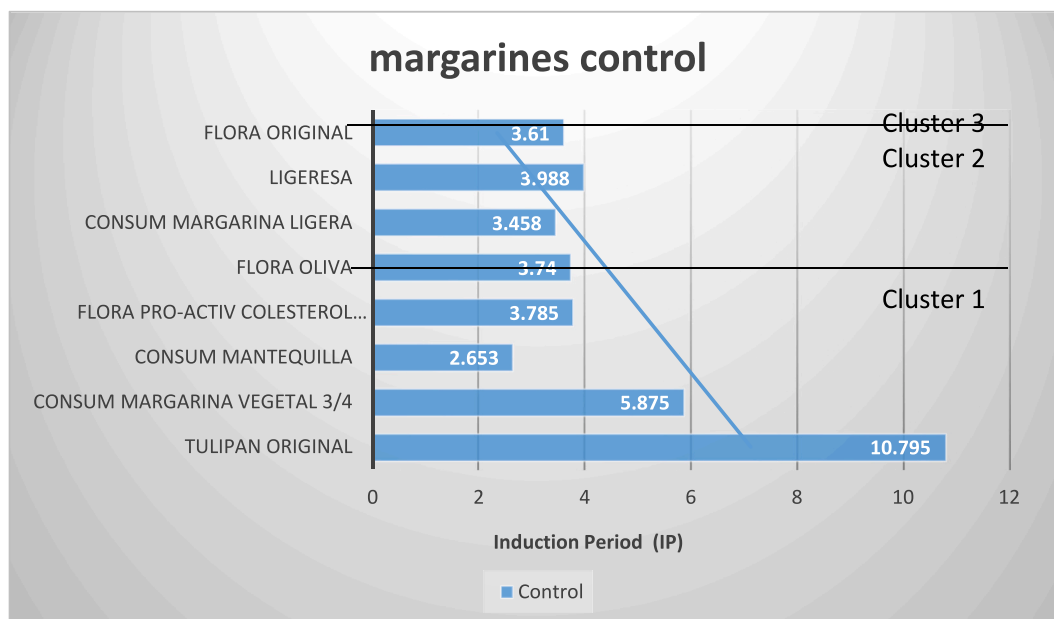


Fig. 4. Induction Period (IP) values in hours of eight margarines selected.

Table 5

Induction period (IP) in hours of eight selected margarines in three clusters after and before the addition of the six plant species (percentage improvement). The controls correspond to the margarine samples without the addition of any plant species.

MARGARINE BRAND	CLUSTER No.	ADDED HERBS						
		Control	<i>Rosmarinus officinalis</i>	<i>Curcuma longa</i>	<i>Illicium verum</i>	<i>Taraxacum officinale</i>	<i>Thymus piperella</i>	<i>Thymus vulgaris</i>
Tulipan original	1	10.8 ± 0.3	13.49 ± 0.23 (+24.97%)	11.87 ± 0.13 (+9.94%)	5.9 ± 0.5 (−45.21%)	6.41 ± 0.22 (−40.62%)	11.8 ± 0.8 (+9.03%)	11.87 ± 0.17 (+9.96%)
Consum margarina vegetal 3/4		5.88 ± 0.21	8.64 ± 0.24 ^a (+47.06%)	6.77 ± 0.18 ^a (+15.15%)	3.7 ± 0.8 (−36.89%)	4.20 ± 0.18 (−28.55%)	6.2 ± 0.6 (+6.04%)	6.6 ± 0.3 (+12.73%)
Consum mantequilla	2	2.65 ± 0.24	6.1 ± 0.6 ^a (+131.25%)	3.6 ± 0.6 ^a (+37.32%)	1.9 ± 0.3 (−28.94%)	2.31 ± 0.16 (−12.81%)	4.1 ± 0.5 ^a (+55.41%)	5.4 ± 0.5 ^a (+104.67%)
Flora pro-activ		3.79 ± 0.09	6.43 ± 0.07 ^a (+69.83%)	4.2 ± 0.03 (+10.91%)	3.1 ± 0.4 (−17.44%)	3.8 ± 0.3 (−0.92%)	4.47 ± 0.12 (+17.97%)	5.5 ± 0.3 ^a (+44.07%)
colesterol original		3.74 ± 0.19	6.32 ± 0.17 ^a (+68.98%)	3.81 ± 0.14 (+1.87%)	3.4 ± 0.3 (−8.82%)	3.6 ± 0.4 (−2.67%)	3.9 ± 0.4 (+4.01%)	3.94 ± 0.17 (+5.35%)
Flora oliva		3.46 ± 0.17	6.7 ± 0.5 ^a (+94.48%)	3.77 ± 0.13 (+9.02%)	2.2 ± 0.7 (−35.57%)	2.75 ± 0.10 (−20.39%)	3.76 ± 0.16 (+8.59%)	4.79 ± 0.10 (+38.43%)
Consum margarina ligera		4.0 ± 0.3	7.0 ± 0.9 ^a (+75.60%)	4.8 ± 0.3 (+20.56%)	2.37 ± 0.18 (−40.70%)	3.5 ± 0.3 (−12.78%)	4.0 ± 0.5 (+1.18%)	4.2 ± 0.5 (+4.19%)
Ligeresa		3.6 ± 0.3	5.28 ± 0.13 ^a (+46.26%)	3.84 ± 0.18 (+6.37%)	3.0 ± 0.4 (−17.17%)	3.57 ± 0.09 (−1.11%)	4.07 ± 0.19 (+12.74%)	4.4 ± 0.6 ^a (+21.05%)
Flora original		3						

h.

Data are presented as mean ± SD (n = 4).

^a Denotes statistically significant difference compared to control Induction Period values ($\alpha = 0.05$).

three margarines in which the greatest rises in the IP value were obtained after the addition of the mentioned herb (Consum mantequilla, Consum margarina ligera, and Ligeresa) were those that presented the highest values of the MUFA/PUFA ratio, which was greater than 1.55. The last three margarines with the lowest increases in IP value (Tulipan original, Flora original and Consum vegetable margarine 3/4) were the margarines with the highest UFA content (Table 4).

These same results were obtained when *Curcuma longa* was added: for two of the first three margarines (Consum mantequilla and Ligeresa) the IP value raised the most, and in one of the last three margarines (Flora original) it increased the least. Although the addition of *Curcuma longa* provided a lower oxidative stability improvement than *Rosmarinus officinalis*, this coincidence in the effect it produces on margarines could be due to its high phenol content (240 ± 30 mg GAE/g) (Table 2).

When the two species of the genus *Thymus* were added to the margarines, which had lower phenol content (172.6 ± 1.4 and 57.4 ± 0.4

mg GAE/g) than the tested species of *Rosmarinus* and *Curcuma*, the previous result was not obtained (Table 2).

When considering the average of the IP values obtained individually in each margarine, it was obtained that *Rosmarinus officinalis* increased by 69.80%, *Thymus vulgaris* by 30.87%, *Thymus piperella* by 14.37% and *Curcuma longa* by 13.89%. *Taraxacum officinale* and *Illicium verum* decreased by 14.98% and 28.84% respectively (Table 5).

3.5. Principal Component Analysis of the margarines together with the plant species

Table 6 indicates the total number of combinations of each margarine with one unique herb. The following 13 variables were used for statistical assessment: Induction Period obtained from the combinations of the eight margarines with the six added herbs (Table 5); total fatty acids, saturated fatty acids, monounsaturated fatty acids,

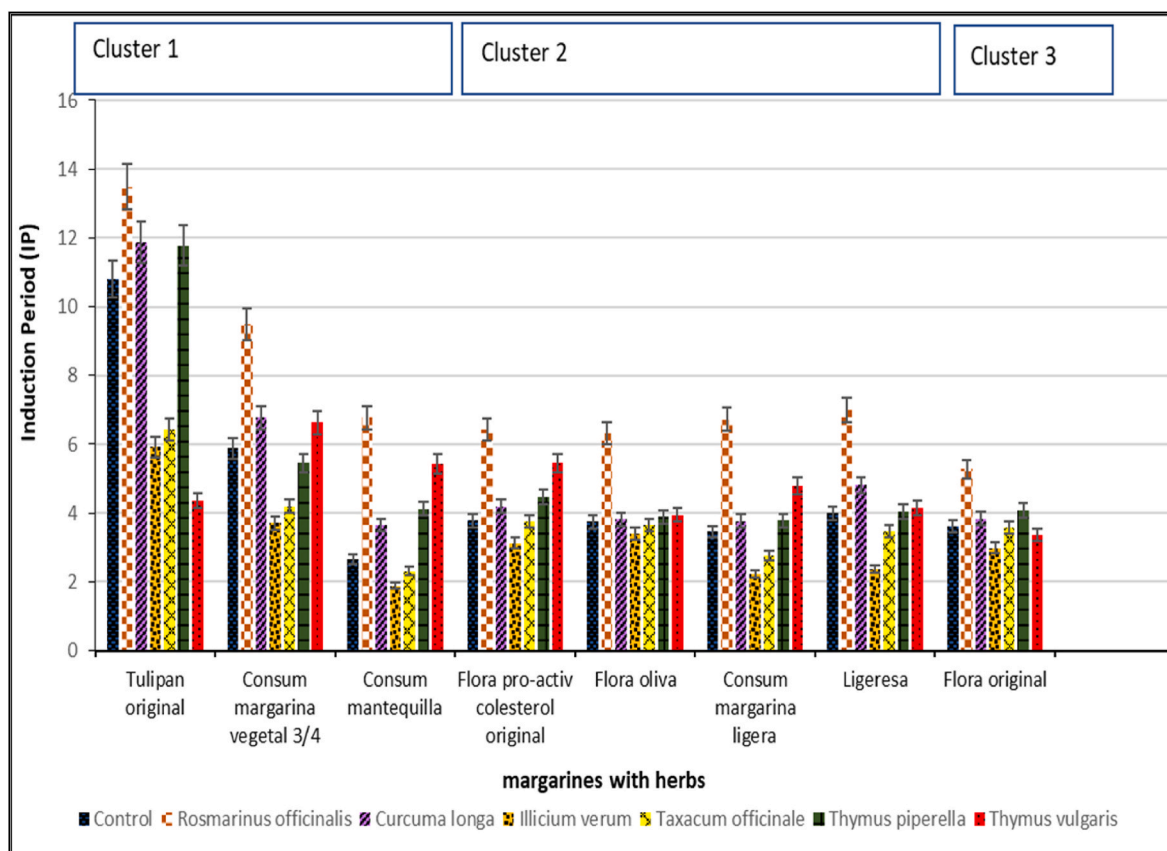


Fig. 5. Barchart representing the means and standard deviations of the Induction Periods of different treatments.

polyunsaturated fatty acids, unsaturated fatty acids, MUFA/PUFA ratio, vitamin A, vitamin E, ω -3, ω -6 from the margarine samples (Table 3); and phenolic content and antioxidant activity (AA) of the herbs (Table 2).

Fig. 6A presents the scores plot tri-dimensional representation of variables taken into consideration for the first three PCs. The total variance explained was 77.48% where: PC1 (37.084%), PC2 (23.94%), PC3 (16.45% of total). Fig. 6A shows that PC1 was positively correlated with all variables except PUFA; PC2 was positively correlated with TFA, SFA, MUFA, PUFA, UFA, Vit. A, Vit. E, IP, and MUFA/PUFA, and negatively correlated with ω -3, ω -6, PC, and AA; PC3 was positively correlated with the variables TFA, SFA, MUFA, PUFA, UFA, Vit. E, IP, PC, and AA, and negatively correlated with Vit. A, ω -3, ω -6, and MUFA/PUFA.

Loadings plot of margarines PC1 vs. PC2 shows eight groups of each margarine with all species as expected. Fig. 6B presents PC1 vs. PC3, where margarines were distributed into two major clusters: Cluster A solely included all margarines with *Rosmarinus officinalis* with highest IP values (1–8), Cluster B, all the rest and is divided into four subgroups: Subgroup 1 (11, 19, 27, 35, 43) formed by Flora pro-activ colesterol original with the rest of herbs. Subgroup 2 (16, 24, 32, 40, 48), Consum mantequilla with the rest of herbs with the lowest IP. Subgroup 3 (9, 17, 25, 33, 41), Tulipan original with the rest of herbs with the longest IP of the subgroups. Subgroup 4 included the rest of them.

The analysis highlighted the strong influence of *Rosmarinus officinalis* on the oxidative stability of the margarines as this herb generated an independent group, while the other groups depended only on each margarine with the other herbs.

3.6. Multiple regression analysis

After considering the different variables, the regression equation

obtained using the SPSS software was:

$$\text{Log IP} = (0.399 \pm 0.093) + (0.952 \pm 0.150) \omega\text{-3} - (0.010 \pm 0.003) \text{PUFA} + (0.0010 \pm 0.0001) \text{AA} + (0.021 \pm 0.002) \text{SFA} - (0.366 \pm 0.051) \text{MUFA/PUFA} \quad (1)$$

$$R^2 = 0.815; F = 36.910; \text{Standard Error of the estimate} = 0.872124841; \text{Durbin-Watson} = 1.2255.$$

The equation obtained by Knowledge Miner and Microsoft Excel for Office matched the parameters characterising the IP:

$$\text{Log IP} = 0.314647 + 0.942967 \omega\text{-3} - 0.006232 \text{PUFA} + 0.000691 \text{AA} + 0.019548 \text{SFA} - 0.330934 \text{MUFA/PUFA} \quad (2)$$

$$R^2 = 0.8083; \text{Prediction Error Sum of Squares} = 0.2090; \text{Mean Absolute Percentage Error} = 9.87\%; \text{Approximation Error Variance} = 0.1917; \text{Approximation Standard Deviation} = 0.4378.$$

Multiple linear regression correlation analysis (equations (1) and (2)) showed that ω -3, SFA; PUFA; MUFA/PUFA ratio and antioxidant activity AA were the main contributors to oxidative stability (OS, measured by IP) after adding plant species. SFA and AA were positively correlated with IP.

A positive dependence between OS and SFAs has been widely documented and the influence of herbal AA on oxidative stability has been confirmed. In this regard, original Tulipan with *Rosmarinus officinalis* was the herbal margarine with the highest oxidative stability (IP 13.49 h) because this margarine had the highest SFA content (34 g), and *Rosmarinus officinalis* was the herbal margarine with the highest AA.

PUFA and the MUFA/PUFA ratio were negatively correlated with the IP. Therefore, Consum mantequilla with high SFA values (25 g) and MUFA/PUFA ratio 2.18 was the margarine with the lowest oxidative stability (IP 2.65 ± 0.24 h) (equations (1) and (2)). When the two species of the genus *Thymus* were added to the margarines, which had lower phenol content (172.6 ± 1.4 and 57.4 ± 0.4 mg GAE/g) than the tested

Table 6
Combinations of the eight selected margarines with added herbs.

1. Tulipan original + <i>Rosmarinus officinalis</i>	17. Tulipan original + <i>Illicium verum</i>	33. Tulipan original + <i>Thymus piperella</i>
2. Flora original + <i>Rosmarinus officinalis</i>	18. Flora original + <i>Illicium verum</i>	34. Flora original + <i>Thymus piperella</i>
3. Flora pro-activ colesterol original + <i>Rosmarinus officinalis</i>	19. Flora pro-activ colesterol original + <i>Illicium verum</i>	35. Flora pro-activ colesterol original + <i>Thymus piperella</i>
4. Flora oliva + <i>Rosmarinus officinalis</i>	20. Flora oliva + <i>Illicium</i> <i>verum</i>	36. Flora oliva + <i>Thymus</i> <i>piperella</i>
5. Consum margarina vegetal ¼ + <i>Rosmarinus</i> <i>officinalis</i>	21. Consum margarina vegetal ¼ + <i>Illicium verum</i>	37. Consum margarina vegetal ¼ + <i>Thymus</i> <i>piperella</i>
6. Consum margarina ligera + <i>Rosmarinus</i> <i>officinalis</i>	22. Consum margarina ligera + <i>Illicium verum</i>	38. Consum margarina ligera + <i>Thymus piperella</i>
7. Ligeresa + <i>Rosmarinus</i> <i>officinalis</i>	23. Ligeresa + <i>Illicium</i> <i>verum</i>	39. Ligeresa + <i>Thymus</i> <i>piperella</i>
8. Consum mantequilla + <i>Rosmarinus officinalis</i>	24. Consum mantequilla + <i>Illicium verum</i>	40. Consum mantequilla + <i>Thymus piperella</i>
9. Tulipan original + <i>Curcuma longa</i>	25. Tulipan original + <i>Taraxacum officinale</i>	41. Tulipan original + <i>Thymus vulgaris</i>
10. Flora original + <i>Curcuma longa</i>	26. Flora original + <i>Taraxacum officinale</i>	42. Flora original + <i>Thymus vulgaris</i>
11. Flora pro-activ colesterol original + <i>Curcuma longa</i>	27. Flora pro-activ colesterol original + <i>Taraxacum officinale</i>	43. Flora pro-activ colesterol original + <i>Thymus vulgaris</i>
12. Flora oliva + <i>Curcuma</i> <i>longa</i>	28. Flora oliva + <i>Taraxacum officinale</i>	44. Flora oliva + <i>Thymus</i> <i>vulgaris</i>
13. Consum margarina vegetal ¼ + <i>Curcuma</i> <i>longa</i>	29. Consum margarina vegetal ¼ + <i>Taraxacum</i> <i>officinale</i>	45. Consum margarina vegetal ¼ + <i>Thymus</i> <i>vulgaris</i>
14. Consum margarina ligera + <i>Curcuma longa</i>	30. Consum margarina ligera + <i>Taraxacum</i> <i>officinale</i>	46. Consum margarina ligera + <i>Thymus vulgaris</i>
15. Ligeresa + <i>Curcuma</i> <i>longa</i>	31. Ligeresa + <i>Taraxacum</i> <i>officinale</i>	47. Ligeresa + <i>Thymus</i> <i>vulgaris</i>
16. Consum mantequilla + <i>Curcuma longa</i>	32. Consum mantequilla + <i>Taraxacum officinale</i>	48. Consum mantequilla + <i>Thymus vulgaris</i>

species of *Rosmarinus* and *Curcuma*, the previous result was not obtained (Table 4).

It may be striking that despite being acids ω-3 a mixture of PUFA compounds, they greatly favour oxidative stability while PUFA decrease it. This is demonstrated in both equations. The explanation is clear, ω-3 acids have the particularity of being α,β-unsaturated compounds that always have great stability by having double bonds alternated with single bonds, so they are a particular case, with a behaviour qualitatively different from the rest of PUFA (equations (1) and (2)).

4. Discussion

This manuscript investigated the antioxidant effect of *Curcuma longa*, *Rosmarinus officinalis*, *Taraxacum officinale*, *Illicium verum*, *Thymus piperella* and *Thymus vulgaris* after addition to eight margarines with different nutritional composition and oxidative stability.

Several publications have previously researched the antioxidant effect of the addition of different plant species to oils to delay the effect of their degradation (Rege, Momin, Wadekar, & Bhowmick, 2013; Ungureanu et al., 2020), but little data is available in the current scientific evidence on the effect of different plant species in margarines.

Fruehwirth et al. reported green tea extract as a promising antioxidant in margarine (Fruehwirth, Egger, Kurzbach, et al., 2021). In another study, a nanoemulsion loaded with sesamol and retinol improved the oxidative stability of this vegetable fat (Yousefi, Nateghi, & Rashidi, 2024). The methanolic extract of *Moringa oleifera* showed a higher antioxidant capacity compared to margarines without antioxidants or with vitamin E (Ouahrani, Casal, Bachir-bey, & Zaidi, 2022). Moreover, it was concluded that *Lentinula edodes* extract could be used to act effectively as an antioxidant in margarines with a high PUFA

percentage (Carvache Meneses, Mura, Serra Bisbal, Sarrión Sos, & Fagoaga García, 2021).

As was seen in this trial, herbs and spices such as *Rosmarinus officinalis*, *Curcuma longa* and *Thymus* spp. increased the induction times, and their addition was a protective measure to prevent oxidation of the different margarines. Of these four species, three of them belong to the taxonomic family *Laminaceae* (rosemary and thyme) whose different extracts have been reported in several studies for their antioxidant activity (Nickavar & Esbati, 2012).

Rosmarinus officinalis was the plant species with the highest antioxidant capacity of all the margarines tested belonging to the three groups, generally increasing their average IP values by up to 69.80%. In this regard, several studies on rosemary have identified the following compounds with antioxidant activity: carnosol, 12-O-methylcarnosic, rosmannol, caffeic acid, rosmarinic acid, caffeoyl derivatives, phenolic diterpenes (carnosic acid), carnosol, epirosmannol and flavonoids (Embuscado, 2015). This may be the cause of the high antioxidant protection obtained in the margarines tested.

After rosemary, plant species belonging to the genus *Thymus* showed the greatest antioxidant activity. Of the 500 species catalogued in this genus, *Thymus vulgaris* and *Thymus piperella* are very common in southeastern Spain and important bioactive compounds have been described (Nieto, 2020). This species contains phenolic acids (gallic acid, caffeic acid, rosmarinic acid), thymol, phenolic diterpenes and flavonoids as molecules that act to increase oxidative stability (Embuscado, 2015).

The fourth best plant species that improved average IP value by 13.98 % over different margarines was *Curcuma longa*, Turmeric belonging to the Zingiberaceae family, a yellow spice of high economic importance due to its extensive medicinal values. Antioxidant capacity of some species and varieties of turmeric has been reported previously (Kim, Yang, Lee, & Kang, 2011; Tanvir et al., 2017), and many compounds have been isolated from turmeric for a number of biological effects. Much work has been carried out on the antioxidant activities of compounds derived from turmeric rhizomes. The curcuminoids [i.e., curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione), demethoxycurcumin, and bisdemeth-oxycurcumin] are major antioxidative compounds described. In addition to the curcuminoids, other compounds possessing these capabilities include: γ-terpinene, ascorbic acid, β-carotene, β-sitosterol, caffeic acid, campestrol, camphene, dehydrocurdione, eugenol, *p*-coumaric acid, proto-catechuic acid, stigmaterol, syringic acid, turmerin, turmeronola, turmeronol-B and vanillic acid (Gonzalez-Albadalejo et al., 2015).

Regarding the other two plant species used in the trials (*Taraxacum officinale* L. and *Illicium verum*); despite having antioxidant agents in their composition, their addition to margarines did not improve their oxidative stability. Unlike the previous species, these accelerated the degradation of margarines showing a decay in the IP values. *Taraxacum officinale* decreased, on average, the IP value by 14.98% and *Illicium verum*, by 28.84%.

With respect to *Taraxacum officinale* and *Illicium verum*, their seeds contain unsaturated fatty acids (linoleic and oleic acids), as well as other antioxidant agents (Cheng-Hong et al., 2012; Ivanov, 2014). However, most of the phenolic compounds of both these species are not soluble in non-polar media (such as oils and fats); justifying those, they did not have an antioxidant effect in the (non-polar) margarines tested. Moreover, many of the phenolic compounds such as trans-anethole (in the case of *I. verum*) and citric acid (in *T. officinale*) are unsaturated compounds that increase the total UFA content, and this could be the reason why these two plant species decreased the IP value and, therefore, the oxidative stability of the margarines. It could also be due to thermal sensitivity of the compounds losing their activity.

The results obtained in this study suggest that in the formulation of commercial margarines, natural additives extracted from the herbs and spices investigated in this study, mainly from *Rosmarinus officinalis*, *Thymus vulgaris*, *Thymus piperella* and *Curcuma longa* extracts, are an

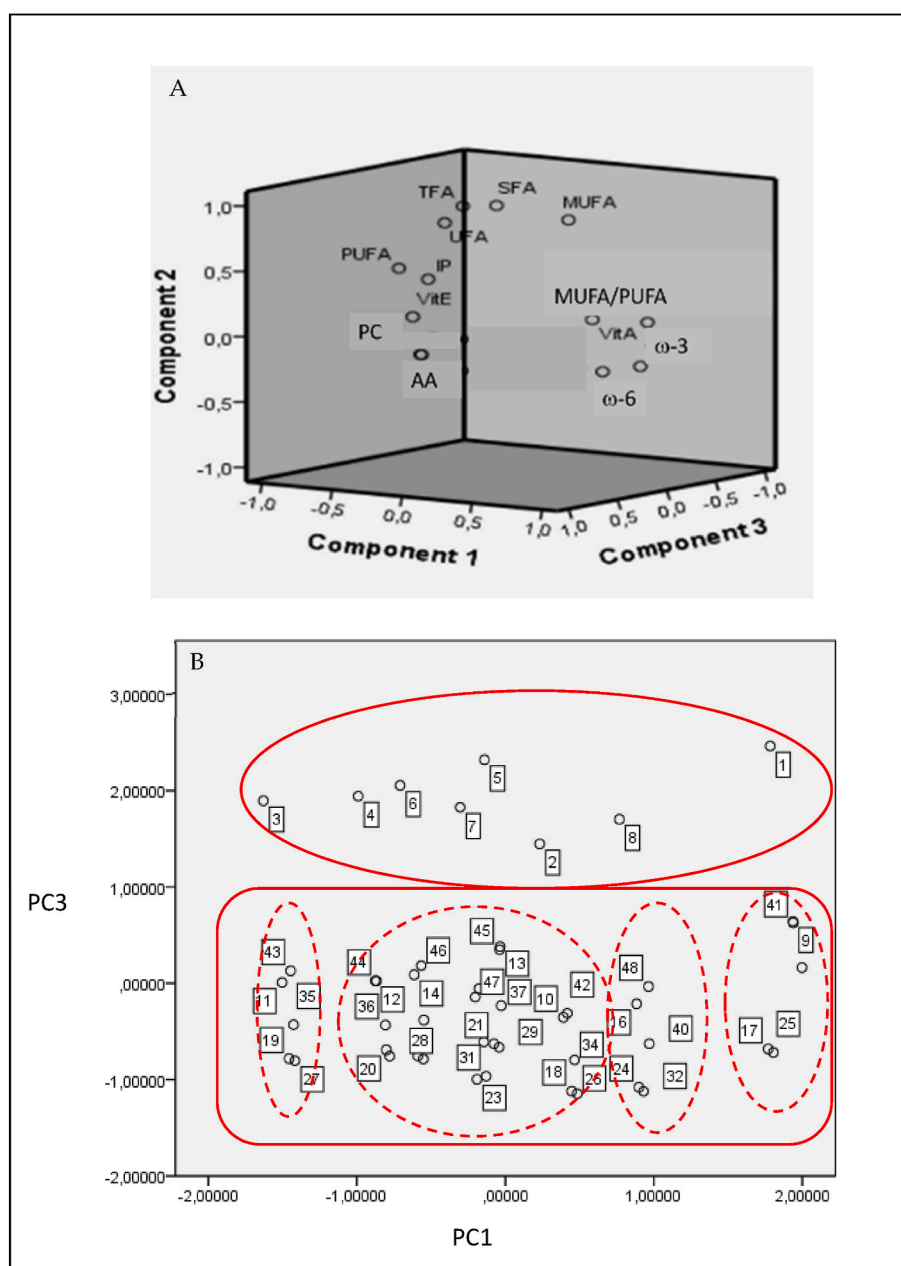


Fig. 6. A. Score plot of variables. B. Loading plot PC1 vs PC3 of margarines with herbs added grouped in clusters A (up) and B (down).

alternative to the synthetic compounds traditionally used. The natural antioxidants presented in the species studied can enhance the activity of endogenous antioxidant systems providing additional protection against oxidative stress and prevent certain diseases resulting from the consumption of artificially obtained compounds. In this way, functional foods are generated that can provide better health conditions to the consumer, in line with the growing demand for healthy products. Their low cost and high availability, the preservation of the organoleptic qualities of the fats due to the small amounts used and their effectiveness in maintaining the quality of margarine by preventing or delaying the adverse effects of oxidation on texture, flavour and nutritional value, prolonging its shelf life make further studies necessary.

5. Conclusions

Water/oil emulsions have been poorly studied and characterisation of the long-term stabilisation of margarine remains a challenging task in

food science, which limits their potential application in the food industry. It is essential to understand which factors affect its oxidative stability and in the case of margarine, this is difficult to determine as various additives such as emulsifiers, metal ions, NaCl, citric acid or carotenoids and other factors whose mechanism is still unknown could influence it.

In the future, synergistic effects between the different herbs and spices studied in margarines could be studied and the results compared with those obtained in the present study. Which compounds are involved in antioxidant activity and the effect of temperature on this activity could be researched. Their efficacy in laboratory-produced margarines could be tested. Their effect in other food matrices, such as different types of oils, butters, etc. could be analyzed.

CRediT authorship contribution statement

Juan José Serra: Investigation. Carmen Fagoaga: Writing –

original draft, Validation, Methodology, Investigation, Formal analysis. **Jessica Mura:** Investigation. **Francisca Sempere-Ferre:** Writing – review & editing, Writing – original draft, Validation, Software, Methodology, Formal analysis. **Gloria Castellano:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

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Declaration of competing interest

The authors declare that they have no competing interests.

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Data availability

Data will be made available on request.

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