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Antioxidant effect of *Chondrus crispus* and *Lentinula edodes* on different margarines

Efecto antioxidante de *Chondrus crispus y Lentinula edodes* en diferentes margarinas

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ABSTRACT

Lipid oxidation is the reaction responsible for food degradation. To solve this problem the agri-food industry uses antioxidants, preservatives and chemical stabilizers. Currently, there is an increasingly strong demand for healthier eating. Because of this, the industry is increasingly interested in replacing chemical compounds with natural products of equal or greater effectiveness. This project studies the oxidative stability, both qualitative and quantitative, of margarines of different compositions when independently adding dehydrated material from a fungus (*Lentinula edodes*) and a seaweed (*Chondrus crispus*) that are known for their antioxidant activity. To achieve this, Rancimat equipment was used at different temperatures: 110, 120, 130 and 140 ± 1.6 ºC. comparing treated margarine samples to their respective controls. The results varied depending on the temperatures used, the composition of margarines and the natural antioxidants added. The results show that margarines with a lower proportion of unsaturated fatty acids, and especially polyunsaturated fatty acids, have greater oxidative stability. Oxidative stability was higher when the fungus was added compared to seaweed.

KEYWORDS: *oxidative stability, Rancimat, lipid oxidation.*

RESUMEN

La oxidación lipídica es la reacción responsable de la degradación de los alimentos, para solventar este problema la industria agroalimentaria utiliza antioxidantes, conservantes y estabilizantes químicos. Actualmente hay una demanda cada vez más fuerte de una alimentación más saludable; debido a esto, la industria está cada vez más interesada en sustituir los compuestos químicos por productos naturales de igual o mayor efectividad. En este proyecto se estudia la estabilidad oxidativa en margarinas de distinta composición, tanto cualitativa como cuantitativa, al adicionar de forma independiente material deshidratado procedente de un hongo (*Lentinula edodes*) y de un alga (*Chondrus crispus*), que se caracterizan por tener actividad antioxidante. Para ello, se utilizó un equipo Rancimat a diferentes temperaturas: 110 ºC, 120 ºC, 130 ºC y 140 ºC ± 1.6 ºC, comparando las muestras de margarinas tratadas con sus respectivos controles. Los resultados variaron según las temperaturas empleadas, la composición de las margarinas y los antioxidantes naturales añadidos. Los resultados muestran que la margarina con menor proporción en ácidos grasos insaturados, y especialmente de poliinsaturados, presenta mayor estabilidad oxidativa y que esta aumenta en mayor grado cuando se añade el hongo con respecto a la adición del alga.

PALABRAS CLAVE: *estabilidad oxidativa, Rancimat, oxidación lipídica.*

INTRODUCTION

Oxidative stability is the resistance possessed by a lipid matrix to oxidation. Oxidation is a chemical reaction that occurs when combining oil with oxygen, and the oxidation rate increases over time [1]. This results in the formation of free radicals and hydroperoxides that break down over time, due to high temperatures or certain enzymes in products with a lower molecular weight containing carboxyl groups [2].

Oxidative stability can also be defined as the period of time it takes to reach a critical point of oxidation. This serves as a parameter for studying lipid behavior and lifespan [3]. This parameter depends on multiple factors: temperature, concentration and type of oxygen, processing and storage conditions, the presence of antioxidants, the composition of the oil and so on [4]. The literature has shown that unsaturated fatty acids (UFA) are more susceptible to oxidation relative to saturated fatty acids (SFA) and that, the more the degree of unsaturation, the more likely they are to oxidation [5]. For this reason, within UFA, polyunsaturated (PUFA) are more unstable and therefore more prone to oxidation in relation to monounsaturated fatty acids (MUFA).

To increase oxidative stability, the industry has traditionally used synthetic antioxidants. Synthetic antioxidants are aromatic compounds, with at least one hydroxyl group, that can have phenolic structures of various degrees of alchemical substitution. They have the disadvantage of being volatile so they break down easily at high temperatures and the big drawback is that they can produce reactions if you consume excess food that contains them. Gallates E-310 to E-312 can cause hyperactivity, stomach aches, other digestive discomfort and skin symptoms, mainly in allergic people and could lead to kidney injuries. The most commonly used in margarines are gallates, BHA, BHT, BHQT or synthetic tocopherols, among others [6]. It has been shown that they can cause cholesterol alterations, vitamin D deficiency, skin alterations (cracking) and be carcinogenic (lung, gastric, liver cancer, etc.). For these reasons, they have been strongly questioned regarding their toxicity and food safety, which has increased interest in the use and development of natural antioxidants. Chemical compounds showing antioxidant activity, such as flavonoids, polyphenols, vitamin C and vitamin E, have been seen to be present in aromatic, medicinal or other plant products.

Natural antioxidants can be phenolic compounds (tocopherols, flavonoids and acidic phenols), nitrogenous compounds (alkaloids, chlorophyll derivatives, amino acids and amines) or carotenoids [7]. Natural antioxidants also increase the quality and nutritional value of food [8]. Aromatic plants such as sage, thyme, mint, green tea or rosemary have been studied for their great antioxidant activity and polyphenolic composition. There is a positive linear correlation between phenolic content and the antioxidant capacity of these plant species. Rosemary is one of the plants with the greatest antioxidant activity, so several studies on the effectiveness of this plant, obtaining very favourable results, have been carried out [9].

Algae also have a very important antioxidant capacity. This can be explained by the presence of various apolar chemical compounds, such as chlorophyll, terpenoid and carotenoid derivatives. They also contain amino acids, capable of absorbing large amounts of ultraviolet (UV) radiation. The content of vitamins such as vitamin E (fat-soluble), or vitamin C (water-soluble) can contribute to its antioxidant properties [10].

Fig. 1. *Chondrus crispus,* whose common name is Ireland moss. (A) Dehydrated seaweed used in experimental trials. (B) Appearance of the seaweed in its natural habitat. *Source*: own elaboration.

This study investigates the antioxidant activity of *Chondrus crispus* seaweed (figure 1), a seaweed of commercial interest due to its high PUFA content with 20 carbon atoms [11]. It is an essential component in feeding several mariculture species and is suggested to reduce the risk of thrombosis, atherosclerosis and heart diseases in humans [12]. Red algae contain sulfated polysaccharides, such as galactans, carrageenans and agar. Of these sulfated polysaccharides, galactans are the ones with the greatest functional activity. They possess pharmacological activities including antiviral, antitumor, antiangiogenic, antithrombotic, anti-inflammatory, anticoagulant and immunomodulatory [13].

Fungi also have a very important antioxidant ability, which allows them to neutralize free radicals that can damage cells. Cell damage caused by free radicals appears to be a major contributor to aging and degenerative diseases. For this reason, it was decided to study the antioxidant activity of the fungus *Lentinula edodes* (figure 2), which is commonly named Shiitake. It is very popular in Asia for its flavor, medicinal properties and high nutritional value [14, 15]. Shiitake fungi owe their important medicinal, nutritional and functional properties to their special polysaccharides of type β – glucans such as Lentinan [16]. Recent studies claim that it has a high content of protein and fiber, and highlight the role of Lentinan and Eritadenin, its secondary metabolites, as biologically active agents in the prevention of certain types of cancer and cardiovascular diseases.

Fig. 2. The Shiitake fungus (*Lentinula edodes*). (A) Dehydrated fungus used in experimental trials. (B) Appearance of the fungus in its natural habitat. *Source*: own elaboration.

To study stability, the most commonly used process is the use of high temperatures to speed up oxidation. It has been observed in various studies that storing food at higher temperatures accelerates deterioration reactions and as a result decreases the shelf life. In this way, the shelf life of food may be obtained under normal conditions, if the data obtained in accelerated oxidation are extrapolated [17].

METHODS

Three margarines that differ in fat composition were selected for this study: Flora, Consum Light Margarine (MLC) and Lightweight Herd Margarine (MLH). All of the nutritional information is listed below in Table 1.

Table 1. Datasheet for margarines used in the study, quoting the company that markets them, their batch number, expiration date and the nutritional information of each margarine

Margarines	Flora		Consum Light Margarine		Hacendado Light Margarine	
Company	Upfield Spain S.L.U.		Doccas Food S.L		Vandemoortele Izegem NV	
Lot number	L024512088		L2020 01J/01		L0348	
Expiration date	16.01.2021		30.03.2021		19.02.2021	
Nutritional information (per 100g)	Energy	2588 kJ/ 618 kcal	Energy	1494 kJ/ 363 kcal	Energy	1521 kJ/ 370 kcal
	Fat	70 g	Fat	40 g	Fat	40 g
	Saturated	18 g	Saturated	12 g	Saturated	12 g
	Unsaturated	51 g	Unsaturated	28 g	Unsaturated	28.2 g
	Monounsaturated	19 g	Monounsaturated	11.5 g	Monounsaturated	20 g
	Polyunsaturated	32 g	Polyunsaturated	16.5 g	Polyunsaturated	8.2 g
	Carbohydrates	<0.5 g	Carbohydrates	<0.5 g	Carbohydrates	2.2 g
	Sugars	<0.5 g	Sugars	<0.5 g	Sugars	<0.5 g
	Proteins	<0.5 g	Proteins	<0.5 g	Proteins	<0.5 g
	Will	0.2 g	Will	0.4 g	Will	0.4 g
	Vitamin D	75 µg	Vitamin D	7.5 µg	Vitamin D	6.8 µg
	Vitamin E	18 mg	Vitamin E	22 mg	Vitamin E	12 mg

The variation in oxidative stability of these 3 types of margarines was studied. A sample of 3 grams of each margarine was used and 0.0155 g ± 0.00021 g dried fungus were added. Similarly, 0.0154 g ± 0.00025 g of freeze-dried seaweed extract were added to each margarine and the results were compared with their respective controls. Tables 2 and 3 illustrate the datasheet for natural additives and nutritional information. The tests were carried out at the following temperatures: 110, 120, 130 and 140 ± 1.6 ºC.

Table 2. Datasheet of natural additives used in the study

Natural ingredients	Company	Lot number
C. crispus	Port-Muiños SL	MM/200919
L. edodes	Mushroom Ship (The Socuello)	S/LE 0120/S

Table 3. Nutritional composition of the two antioxidants with which margarines are treated

Nutritional information (100 g)	C. crispus	L. edodes
Energy	636 kJ /155 kcal	130 kJ / 31 kcal
Fats of which	< 0.5 g	0.3 g
Saturated	< 0.1 g	0.07 g
Monounsaturated	-	-
Polyunsaturated	-	-
Carbohydrates	2.7 g	4 g
Of which sugars	0 g	<0.5 g
Food fiber	43 g	-
Proteins	14 g	1.8 g
Salt	6 g	0.013 g
Vitamin A (Retinol)	138.69 µg	-

A Rancimat 892 apparatus was used to study the variation in oxidative stability (figure 3). It consists of an analysis system for the simple and safe determination of stability in the oxidation of natural fats and oils where the sample undergoes an accelerated oxidation process through the introduction of continuous airflow and exposure to high temperatures. Heating the sample produces volatile metabolites that generate conductivity changes during the analysis process, which are recorded by the sensor. The time that elapses until the secondary products of the reaction are formed is called "an induction period" or "the oxidative stability index" (IP or OSI).

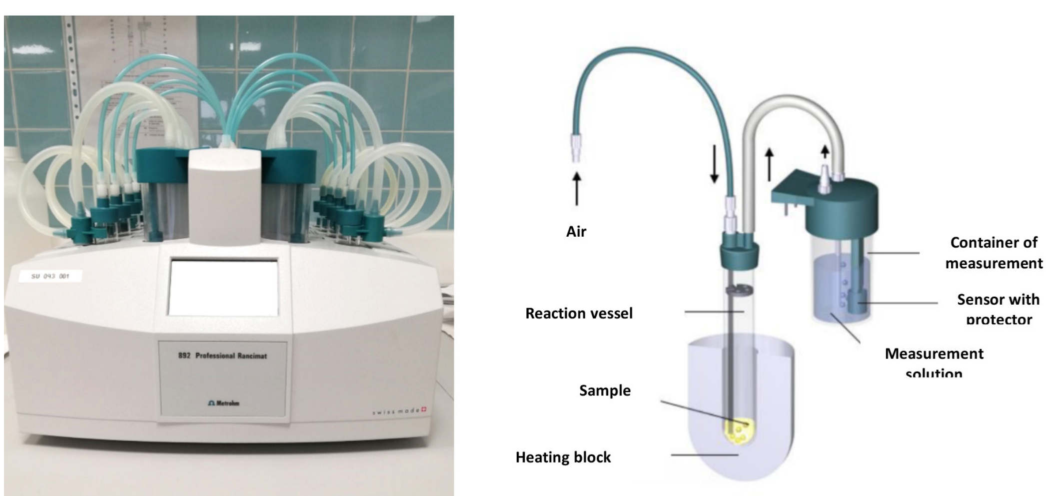

Fig. 3. A) Rancimat device. B) Detailed components of Rancimat during the measurement process. *Source*: own elaboration.

This value is indicative of the sample's resistance to oxidation, so a longer induction time implies that the sample is more stable. Conductivity-time curves are displayed live, for each measurement point in the database. The induction time was determined automatically from the second derivative of the measuring curve and the manufacturer's performance protocol for solid vegetable fats [18] was followed.

RESULTS AND DISCUSSION

Natural antioxidants have shown great importance in numerous studies related to the effectiveness of their antioxidant activity and the benefits to human health [19-26]. These articles are based on the use of natural antioxidants for application to improve the oxidative stability of foods including emulsions. Research articles on emulsions are generally scarce since it is such a complex water/oil system. Although margarine research has studied components that are famous for their antioxidant activity, research in this field remains rare.

Figures 5, 6, 7 depict the induction periods (IP) of untreated control margarine and margarine treated with *Chondrus crispus* (seaweed or Ireland moss) or *Lentinula edodes* (fungus) at 110, 120, 130 and 140 ± 1.6 ºC, with a suppression of 1.5 h and a continuous airflow of 20 L/h. For each test, 3.03 g ± 0.03 g of margarine were used and 0.0155 ± 0.00021 g of dried fungus and 0.0154 ± 0.00025 g of seaweed were added. For each sample, a total of 8 repetitions were performed.

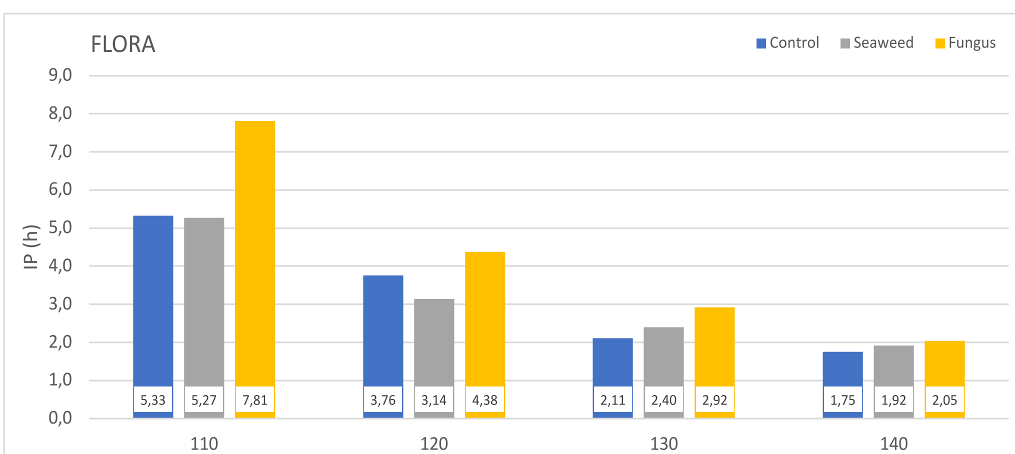

Fig. 4. Variation of IP by addition of natural antioxidants in Flora margarine. The results are presented as ± SD (standard deviation). *Source*: own elaboration.

At a temperature of 110 ± 1.6 ºC, an improvement in IP of 2.48 ± 0.44 h can be observed in the Flora margarine treated with the fungus (7.81 ± 0.48 h), compared to the control sample (5.33 ± 0.42 h). However, in the treatment with dehydrated seaweed (5.27 ± 0.28 h), IP decreased to slightly lower values than the control sample. When studying IP at 120 ± 1.6 ºC the same results were obtained, which means that the addition of fungus delayed the degradation of margarine at temperatures of 110 and 120 ± 1.6 ºC.

At temperatures from 130 ± 1.6 ºC, the results did not follow a pattern in terms of the difference between seaweed and fungus. Treatment with seaweed (2.40 ± 0.35 h) showed a variation of 0.29 ± 0.375 h versus the control sample (2.11 ± 0.025 h). Treatment with fungus (2.92 ± 0.14 h) showed better results at higher temperatures than seaweed, at 140 ºC ± 1.6 ºC, treatment with the fungus (2.05 ± 0.35 h) showed a difference of 0.3 ± 0.04 h with respect to the control (1.75 ± 0.17 h) which could be favorable considering the small IP values obtained at 140 ºC. At high temperatures, there was almost no difference between seaweed and fungus treatments, but in both cases the degradation of margarine was reduced.

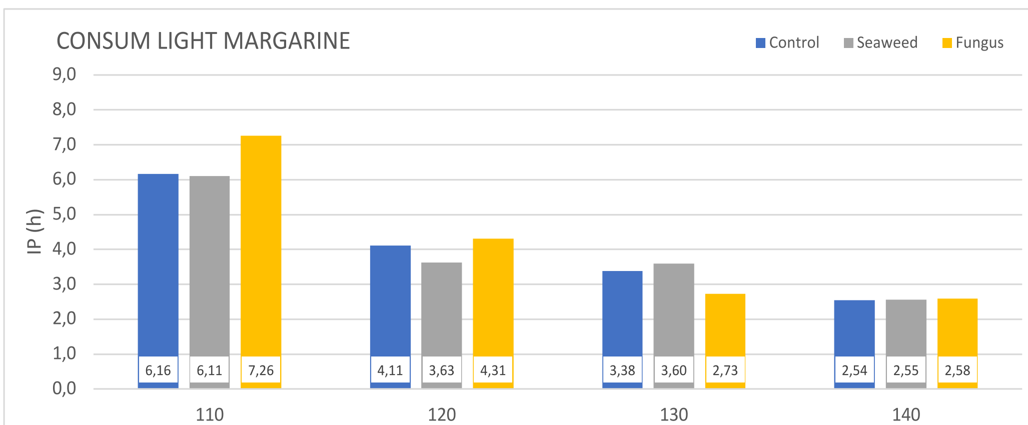

Fig. 5. IP variation by addition of natural antioxidants in CLM. The results are presented as an average ± SD. *Source*: own elaboration.

In the graph of figure 5, the results obtained at the temperature of 110 ± 1.6 ºC with the fungus (7.26 ± 0.26 h) showed an improvement compared to the IP of the control sample (6.16 ± 0.27 h). However, this is not the case when seaweed was added (6.11 ± 0.10 h). At other temperatures, no conclusive changes were seen. It was observed that treatment with seaweed showed slightly different results with respect to the control samples at 130 ± 1.6 ºC.

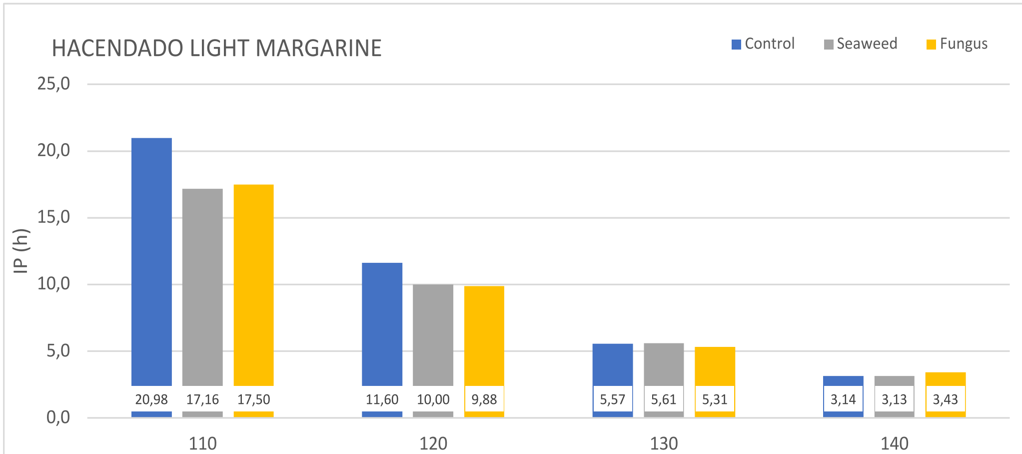

Fig. 6. IP variation by addition of natural antioxidants in HLM. The results are presented as an average ± SD. *Source*: own elaboration.

At 110 ± 1.6 ºC figure 6 shows an unexpected result in HLM control samples (20.98 ± 0.47 h). When all of the results are compared at that temperature no improvement in IP was seen with either treatment, because the IP value of the control sample (20.98 ± 0.47 h) was much higher than the values of the treated samples. From 130 ºC a linearity is seen in the results of the treatments; the values decreased as the temperature increased and no significant improvements were observed with the addition of the seaweed and fungus.

Table 4. Collection of total fat values in the three margarines. The amounts of polyunsaturated fats (PUFA), monounsaturated fats (MUFA) and the SFA/UFA ratio are shown

	Content in 100 g of product					Percentage on quantities in g			
	Total fat	SFA	UFA	PUFA	MUFA	SFA	UFA	PUFA	MUFA
Flora	70 g	19 g	51.0 g	32.0 g	19 g	27 %	73 %	63 %	37 %
CLM	40 g	12 g	28.0 g	16.5 g	12 g	30 %	70 %	59 %	43 %
HLM	40.2 g	12 g	28.2g	8.2 g	20 g	30 %	70 %	29 %	71 %

Variations in results are relative depending on the type of margarine used. The difference was attributed to its SFA and UFA ratio (Table 4) where the margarine with the greatest amount in UFA was less stable and therefore had a lower IP, which explains the behavior of Flora margarine (figure 7).

In the case of HLM and CLM they have the same proportion of SFA/UFA, but differ in their monounsaturated fats. HLM had the lowest proportion of PUFA (29 %) and the highest proportion of MUFA (71 %), which could explain the results obtained at 110 ± 1.6 ºC since a high percentage in monounsaturated fats confers greater stability on the sample (figure 8).

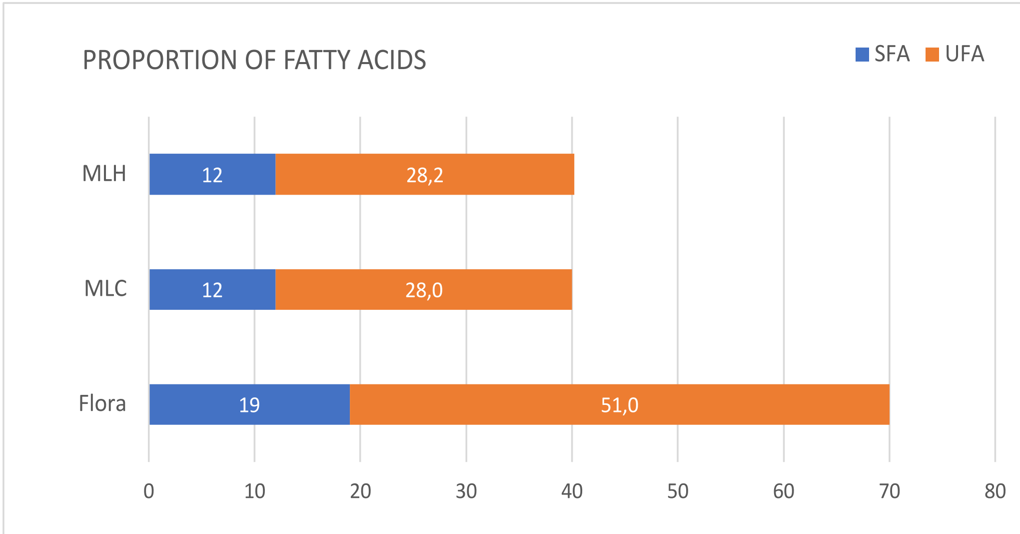

Fig. 7. Proportion of fatty acids in 100 g margarine. *Source*: own elaboration.

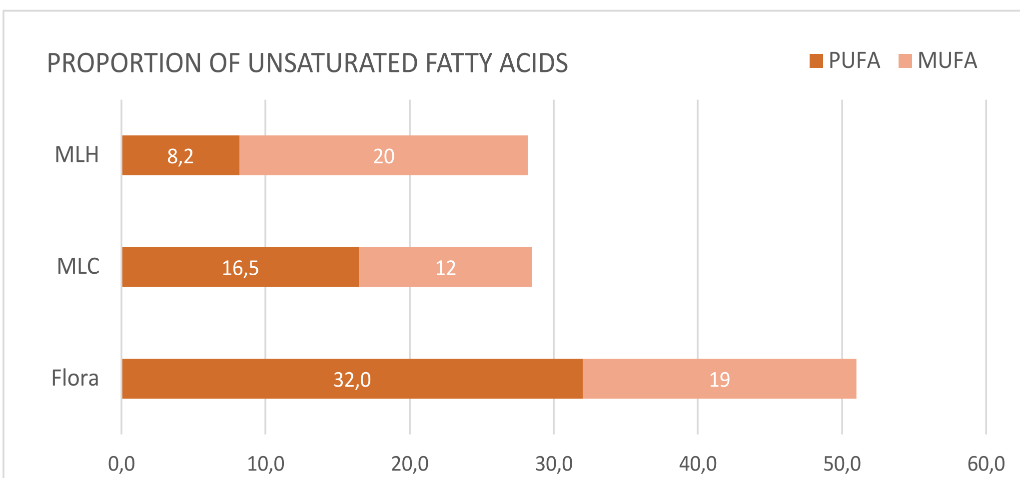

Fig. 8. Proportion of each type of UFA of each margarine. Source: own elaboration.

Studies show that there is a direct correlation between the number of total phenolic compounds and their antioxidant activity [27]. One of the components of Shiitake that is in greater proportion is linoleic acid [28]. As a result, it interacts better with margarine that possesses more polyunsaturated fats. Therefore, better results were obtained with Flora and CLM margarines. Most polyphenols have an apolar character as they are more related to fats, so an increase in IP is expected as shown in the figures above.

Comparing results from studies on the polyphenolic content of the extracts used in this project [29, 30, 31] the extract with the highest polyphenolic content was the fungus followed by seaweed. It can be confirmed that the concentration of polyphenols is decisive for the improvement of IP at temperatures below 140 ºC. This study determined that, for a margarine such as Flora (63% PUFA and 37% MUFA) or CLM (59% PUFA and 41% MUFA), the best treatment is the addition of the dehydrated fungus, discarding the treatment with seaweed in both cases. In order to obtain conclusive and measurable results in the IP study, it is not necessary to exceed 130 ± 1.6 ºC.

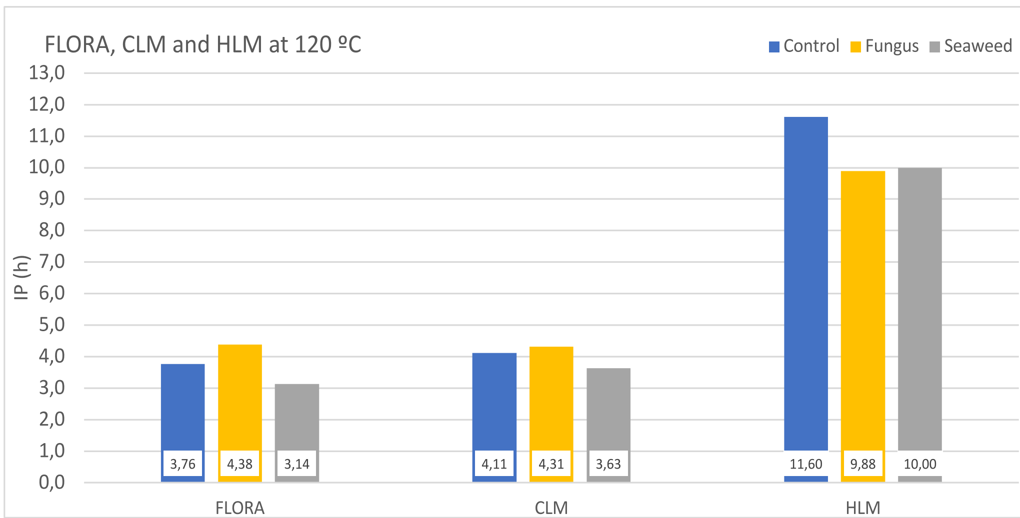

Fig. 9. Values of the IPs obtained from Flora, CLM and HLM margarines at 120 ºC. *Source*: own elaboration.

Figure 9 brings together the results obtained with the three margarines assayed at 120 ºC of controls and treatments with seaweed and fungus, to enable an overview of the variation in IP.

CONCLUSIONS

- The difference between HLM and CLM results is directly related to the proportions of MUFA and PUFA. This shows that not only the amount of UFA but also the proportion of polyunsaturated fatty acids present in the sample affected the IP. The study carried out with Flora margarine (19 % SFA and 51 % UFA), Hacendado Light Margarine (12 % SFA and 28.2 % UFA) and Consum Light Margarine (12 % SFA and 28 % UFA) demonstrated, that at the same test conditions (120 ± 1.6 ºC and 20 L/h), HLM margarine had a significant increase of 2.665 h in IP compared to the other two samples.
- Based on the results obtained we can determine that the same amount of SFA, but different amounts of UFA directly influenced oxidative stability. IP values in margarines with equal SFA content but lower UFA content have been shown to be significantly higher than margarines with the same percentage of SFA and a higher UFA percentage.

- The study of the variation in oxidative stability gave better results when working with margarines that have a lower % of unsaturated fats, especially lower polyunsaturated content, which makes them more efficient when working at high temperatures.
- *Chondrus crispus* caused a slight increase in IP, i.e. an increase in oxidative stability to 130 ºC in margarines with a higher percentage of PUFA.
- *Lentinula edodes* can be used as a natural antioxidant and it is clearly stated that 0.5 w/v of Shiitake powder at very high temperatures can act effectively as an antioxidant in margarines with a high % PUFA.
- The results showed that the optimal temperature to study the IP of margarines with the Rancimat test is 120 ºC. At ≥ 140 ºC the results of margarines treated with natural products are inconclusive and not reproducible. It is advisable to study at temperatures ≤ 130 ºC as it is inferred that at temperatures higher than 130 ºC, the amount of UFA and SFA does not affect the variation of oxidative stability in margarines.

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