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# Effect of drying and gamma irradiation on some physical and microbial attributes of round sardinella muscles and by-product powders


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**Abstract -** The aim of this study is to investigate the effect of combined drying and gamma irradiation processes on the final quality of muscles and byproduct powders of round sardinella *Sardinella aurita*. Sardinella muscles with fishbone were dried at 75°C while byproducts (heads and viscera) were cooked at 90°C, pressed and dried at 75°C. Such process allowed decreasing moisture content of the muscles (from 73.38 ±1.01% to 3.295±1.05% -wet basis) and of the byproduct (from 70.00±0.85% to 6.40±0.90% -wet basis). Dried muscles and byproduct powders undergo gamma irradiation at 5 and 10 kGy doses in a Co-60 d-irradiator. Microbial analysis (coliform bacteria, *Escherichia coli*, mesophilic bacteria, pseudomonas, and sulfite-reducing bacteria, yeasts and molds) were performed to investigate products stability at room temperature. The pH values of muscle and byproduct powders were 6.15±0.14 and 5.86±0.06 respectively. Such values were maintained constant after 42 days of storage at room temperature for irradiated byproduct powders (5.80±0.03 for 5kGy and 5.90±0.02 for 10 kGy); whereas, the pH of the 5kGy-irradiated muscles powder decreased to a value of 5.99±0.03. The global color difference of irradiated muscles and byproduct powders showed little variations during storage at room temperature. The microbial analysis showed that the non-irradiated byproduct powder was more salubrious that non-irradiated muscles powder and this could be attributed to cooking operation of the byproduct before drying. The irradiation of both products at 5 and 10 kGy doses provided products with acceptable color and microbial quality

**Key words:** Sardinella, Muscles, Byproduct, Drying, Irradiation, Quality

## 1. Introduction

Round Sardinella (*Sardinella aurita*), is a small pelagic fish belonging to the Clupeidae family. This species is characterized by a low economic value, despite its high nutritional value (protein ~19.09 ± 0.29 g / 100 g fat: 2.42 ± 0.12 g / 100g) (Djendoubi et al., 2011) and abundance throughout the world including the Mediterranean Sea. Moreover the marketing of such species as fresh product pose problem because of its high perishability, especially during hot season and the possibility of microbial and biochemical alterations (Mbarki, Sadok and Barkallah, 2009; Hocaoğlu et al., 2012). Microbial contamination is the most common cause of fish alteration. Thus, large numbers of microorganisms are present on the fish surface which causes odor and flavor changes (Adam, Paul et Ehlermann, 1982). For pelagic fish high initial charge of microflora (mesophilic bacteria: 2×104 CFU/g, psychrophils 30 CFU/g; 5×103 CFU/g of total coliforms) were found (Mbarki et al., 2009). This bacterial flora changes depending on some factors such as the season, environmental conditions and water quality. On the other hand, the chemical alterations of these sea products are explained by their high levels of fat and unsaturated fatty acids (~ 30% of total fatty acids) which undergo changes inducing alteration of smells and tastes, development of rancidity and color changes (Adam, Paul and Ehlermann, 1982). Freezing, salting and drying are the most common methods of fish preservation.

Thus, the main processed products of sardine are canned, salted and / or unsalted dried products. These processes generate huge amounts of waste, nowadays considered as byproduct (about 40% of the raw material). Several papers in literature reported drying kinetics of whole sardine or sardine muscles and described the effect of different dying parameters (salting and drying method, temperature, salt concentration…) on the water and salt transfers occurring during drying (Bellagha et al., 2005; Bellagha et al., 2007; Boudhrioua et al., 2009). Worldwide, fish waste causes difficulties of transportation and storage and pollution problems (FAO, 2005). In fact, the degradation of the large amount of organic matter of fish byproduct generates a lot of gases into the atmosphere, requiring rapid evacuation (Lourhzal, Tahri et Faid, 2002). Several studies have been undertaken to limit these problems such as Bouallagui et al. (2009) and Eiora et al. (2012). Different processes are available for byproduct valorization such as fishmeal production, silage and bioactive compounds extraction (collagen, gelatin, hydroxipatite, protein hydrolysats, fat) (Ferrero et al, 2013). Fishmeal is widely used as a food supplement for all types of animals feeding offering a unique combination of nutrients. The control of nutritional aspects and microbiological quality of the fishmeal should be considered. Data dealing with muscles and byproduct treatment by dehydration are scarce in literature. Development of integrated process for transformation of both muscles and byproduct of fishes to other products such fishmeal is an interesting way for valorization of low unit value fishes.

Irradiation is one of the alternative treatments that could be used combined or not to other processes for food stabilization. The irradiation uses the advantages' effects of ionizing for agro-food preservation (Kumtau et Sreenivasaan, 1970) and is also considered, by the World Health Organization (WHO) and Organization of Food and Agriculture (FAO) as a safe and effective process in the fight against foodborne disease (Arvanitoyannis, Stratakos and Tsarouhas, 2009). Gamma irradiation is an effective process for inactivating food pathogens and reducing their microbial population (Arvanitoyannis, Stratakos and Mente, 2009). Food irradiation is performed by the exposition of the product to ionizing power source, usually Cobalt-60. Microorganisms' inactivation by ionizing radiation is mainly due to DNA damage which inhibits reproduction capabilities and other functions of the microbial cell. However, because of the absence of consumer confidence and aversion about the effectiveness of this method, it is highly restricted in most countries and permission must be requested before the marketing of irradiated foods. Through several studies over three decades, it was proved that irradiation is a cost-effective technology that can provide healthy food to consumers (Kamat and Thomas, 1998; Hwang and Xuetong, 2015). Several studies about irradiation application on fish focus on the effect of gamma irradiation on the chemical composition, microbial, sensory quality and shelf life extension (Mbarki et al., 2008; Hocaglu et al., 2012; Badr, 2012), the nucleotides degradation (Ozogul et al, 2010) or lipid oxidation (Maltar-Stremecki et al, 2013). The aim of this work is to suggest an integrated process for sardine processing and to investigate the effect of combined drying –irradiation processes on the final microbial quality, pH and the color of muscle and byproduct powders of sardinella.

## 2. Material and methods

### 2.1. Evisceration, morphological parameters and indices determination

Fresh sardinella (*sardinella aurita*) was bought from Kelibia fishing port (Nabeul, Tunisia) in 2014-2015 and transported in ice to INSTM laboratory (La Goulette, Tunis). On arrival at the laboratory, fish were washed and eviscerated.

Before dissection biometric measurements are taken using an ichthyometer and a balance (Exacta 6274, Germany) with a precision of $10^{-4}$ g. The measured parameters are: total length (l), standard length (L) and weight (W). After dissection and filleting, following measurements were taken: visceral weight (VW), liver weight (LW), fillets weight (FW) and byproduct weight (BW). The following table (Table 1) summarizes the morphological parameters and indices determined on fresh fish.

**Table 1**: Morphological parameters and indices of *sardinella aurita*

| Indices | Fillets rate | Byproduct rate | Visceralo-somatic index | Hepato-somatic index | Condition factor K |
|---|---|---|---|---|---|
| Formula | (FW/TW)×100 | (BW/TW)×100 | (VW/TW)×100 | LW/TW | (W/SL3)×100 |

FW: Fillet Weight, TW: Total Fish Weight, BW: Byproduct Weight, LW: Liver Weight, VW: Viscera Weight, W: Weight; SL: Standard Length

Muscles (with fishbone) and byproducts (heads and viscera) are collected separately and stored in freezer bags at -80°C until any further treatments.

**2.2. Processing**

Productions of muscles and byproduct powders are made according to Figure 1: Sardinella muscles with skeleton were dried at 75°C in a combined microwave-air oven (Whirpool®, France) until obtaining a constant mass. The dried product was ground using a coffee grinder to obtain a muscle powder (Moulinex®, France). Sardinella byproducts were cooked at 90°C for 20 minutes and after that they were pressed to get a cake and press juice. The juice has undergone successive centrifugations giving different phases; an oil phase, water release and the press cake. Cake and sludge were dried at 75°C in a combined microwave-air oven (Whirpool®, France) to a constant mass. The obtained product is subsequently ground to get byproduct flour.

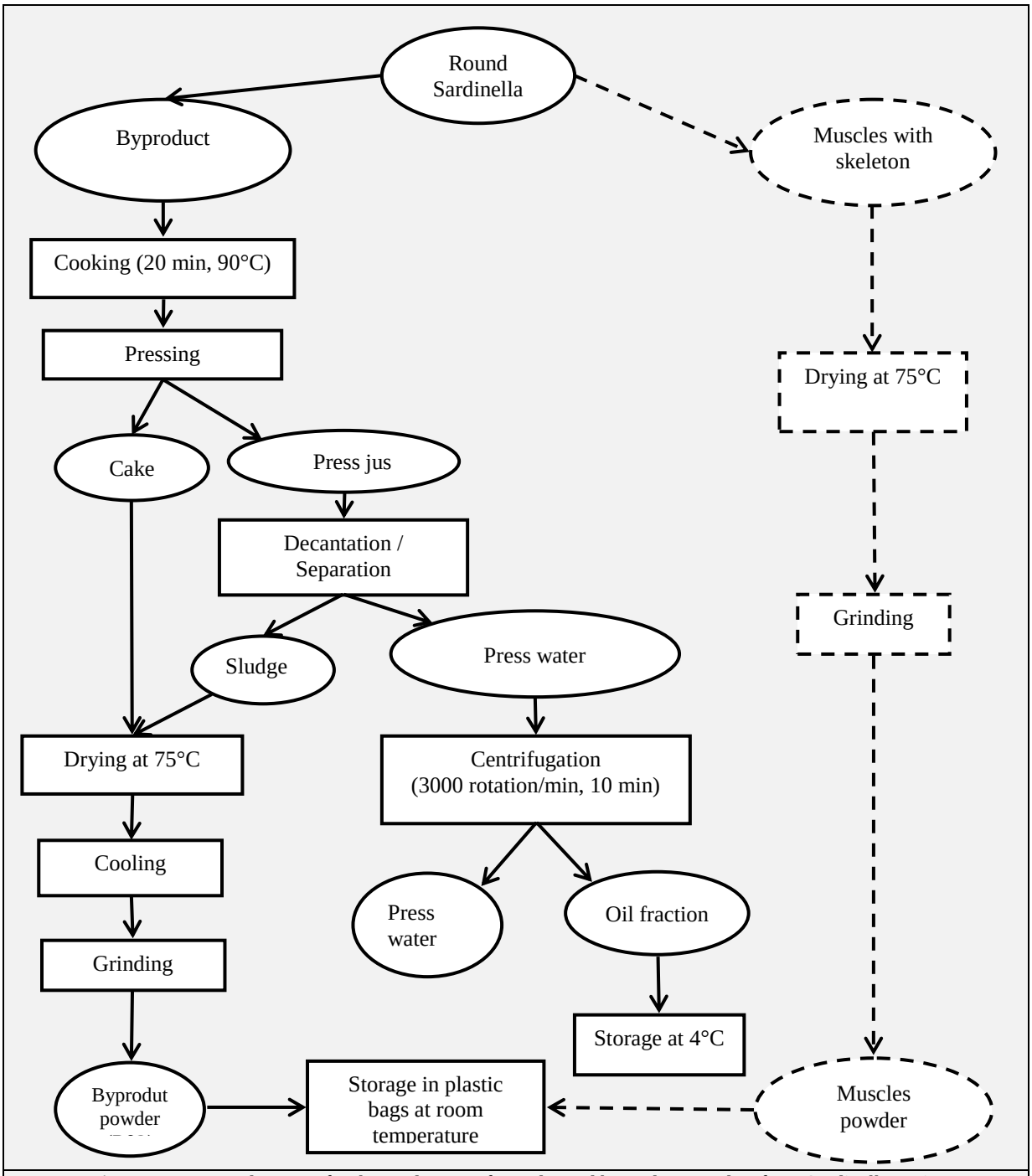


**Figure 1:** Integrated process for the production of muscles and byproduct powders from *Sardinella aurita*

Both products (muscle and byproduct powders) undergo separately gamma irradiation treatment. Irradiation treatments were performed at the Tunisian semi-industrial 60Co gamma-irradiator (National Center of Nuclear Sciences and Technologies, Tunis) at doses of 5 and 10 kGy, and at dose rate of 55.304 Gy/min using Harwell perspex polymethylmethacrylate (PMMA) dosimeter. The irradiation treatment was carried out at room temperature (20°C). Control samples were prepared in the same way but without exposition to irradiation. Non-irradiated and irradiated samples at 5 and 10 kGy were then stored at room temperature during 42 days.

### 2.3. Physicochemical analyzes

#### 2.3.1. Moisture determination

The determination of the moisture content was carried out by gravimetric method: dehydration of the sample was made until a constant weight (at 105°C for 24 hours). The moisture content is expressed either as dry basis or as wet basis (AOAC, 2005).

#### 2.3.2. pH determination

The pH measurement was performed on the control and the irradiated samples the day of byproduct and muscle powders production and at the end of storage period at room temperature.

#### 2.3.3. Colorimetric parameters

The colorimetric parameters of byproduct and muscle powders were evaluated by using a colorimeter (CPCE, TCR200, and Spain). The latter measures the spectrum of the reflected light and converts it into a color coordinate set the CIE Lab. L * value is the value of the brightness from 0 (black) to 100 (white); the a * value is from -100 (green) to 100 (redness) and b * ranges from -100 (blue) to +100 (yellow). The total color difference (ΔE) was determined by using the following equation (Eq. 1) where the subscript "0" in equation 1 refers to the color of irradiated and non-irradiated samples at day 0 of storage.

$$\Delta E = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \quad \text{(Eq.1)}$$

### 2.4. Microbial analyzes

Microbiological counts were made on irradiated and non-irradiated (control) muscle and byproduct powders samples. Germs and culture media used to detect the microbial flora present in the powders of muscles and byproduct are given in the following table (Table 2):

**Table 2.** Microorganisms and culture media used for microbial analysis

| **Bacteria** | Mesophilic | Yeast/Mold | Total coliforms | Sulphite-reducing micro-organismes | Pseudomnas | E.coli |
|---|---|---|---|---|---|---|
| **Culture media** | Plate count agar PCA | Sabouraud | VRBL | Tryptone sulphite neomycine TSN | Certrimide | Tryptone bile glucuronide agar TBX |
| **Incubation condition** | 30°C/48h | 30°C/48h | 30°C/48h | 46°C/48h | 37°C/48h | 37°C/48h |

### 2.5. Statistical analysis

All analyzes were repeated at least 3 times (n=3 for pH, moisture determination and color measurement and n=30 for morphological parameters and indexes determination); average and standard deviations were calculated. Statistical analysis was carried out using the software package IBM. SPSS 20.0 and the comparison of averages of each treatment were based on the analysis of variance (ANOVA) at significance level 5%. Values followed by the same letter are not statistically significant according to Duncan's multiple range test at significance level $p < 0.05$.

## 3. Results and discussion

### 3.1. Morphological parameters and indices

Results of different morphological parameters measured on fresh sardinella are illustrated in Table 3. The byproduct rate determined for June 2014 is about 30%. Fillets rate and byproduct rate vary significantly ($p<0.05$) between period of analysis. This variation can be explained by trophic changes and the life cycle of sardine. In fact, in some periods of the year, fish accumulated reserves in muscles

and in others periods of the year, the fish energy is reserved for spawning and fertilization. Consequently, the yields of the fillets and byproduct and nutritional value of the different parts of the fish show significant variation throughout the year. This data should be of great interest for industrial and professional of fish industry. The hepato-somatic index represents the liver weight expressed as a percentage of the total weight of the fish. This determination provides information on fish metabolism and the whole weight changes provide information on the mode of storage and use of fish reserves. Indeed, fat content, which is the most affected by season variation, is more important in summer (food abundance) than in winter. This observation was reported in several studies dealing with sardine (Zlatanos et Kostas; 2007) and other marine products (Goskse and al; 2004). The condition factor expresses the variations of the fish state; it reflects the recent food conditions and its changes according to the fish sexual maturity cycle. The value of the condition factor (K) is used to estimate the condition or well-being of species. In this study, values measured for sardinella at different periods did not show significant difference.

**Table 3**: Morphological parameters and indexes measured for sardinella

| Fishing period | Fillets rate | Byproduct rate | Hepato-somatic index | Visceralo-somatic index | Condition factor K |
|---|---|---|---|---|---|
| **June 2014** | 63.18±0.17$^{b}$ | 33.10±0.89$^{a}$ | 0.54±0.05$^{a}$ | 4.98±0.13$^{b}$ | 1.18±0.02$^{a}$ |
| **November 2014** | 68.87±2.41$^{a}$ | 30.56±1.18$^{b}$ | 0.39±0.03$^{b}$ | 6.15±0.59$^{b}$ | 1.20±0.02$^{a}$ |
| **April 2015** | 63.39±0.70$^{b}$ | 34.78±0.96$^{a}$ | 0.54±0.04$^{a}$ | 7.98±0.87$^{a}$ | 1.18±0.03$^{a}$ |

Results are presented as means ± S.D. (n=30).
Values with the same letter are not significantly different at p <0.05.

### 3.2. Drying kinetics

The drying kinetics of sardinella muscles and its byproduct are illustrated in **Figure 2**. Initial moisture content varies from 70±0.85% (for byproduct) to 73±1.01% (for muscles). These values are in agreement with those reported by Castrillon, Navarro and Alvarez-Pontes (1997); Kamat and Tomas (1998) and Lourhzal et al. (2003). Drying at 75°C allowed the decreases of the moisture content of sardinella muscles (with skeleton) and its byproduct from 73.38 ±1.01% to 3.295±1.05% (wet basis) and from 70.00±0.85% to 6.40±0.90% (wet basis) respectively. Drying curves showed a fast decrease of moisture content at the beginning of drying and then the drying process becomes slower. The lack of constant drying rate period is due to the progressively water activity reduction of the external cells. When a great number of cell layers in the tissue have lost a considerable amount of moisture, dried cell layers offer a much greater resistance to water diffusion through the interface and the drying rate slows down rapidly. These trends are in agreement with previous studies dealing with drying of fishes and other biological products (Djendoubi et al., 2011).

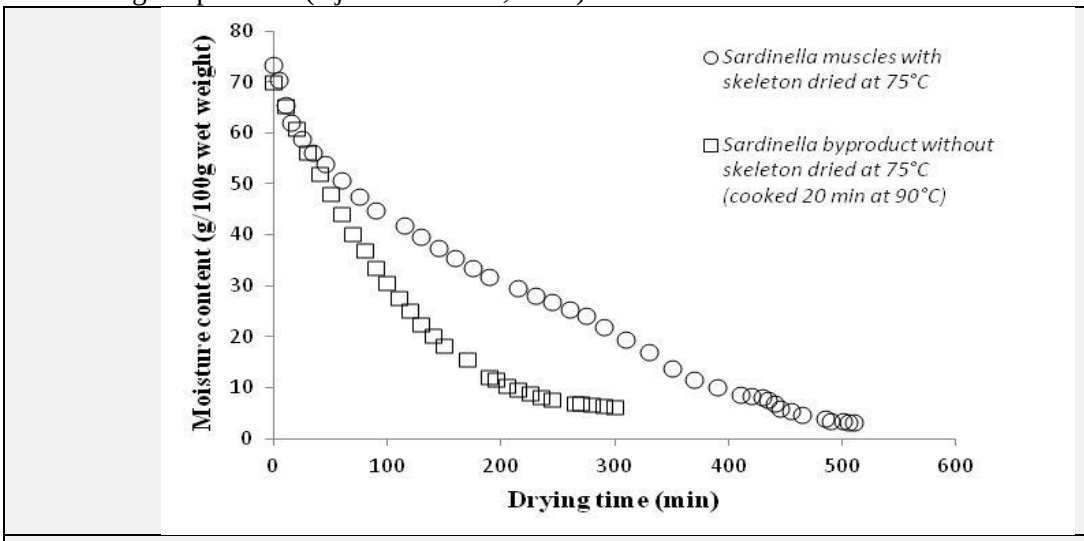


**Figure 2** Drying kinetics of sardinella muscles and byproducts

The stability of fish flour during storage is only ensured if it contains no more than 10% moisture. Beyond this level, the flour is very prone to spoiling. This is manifested by the appearance of mold, which destroyed the flour organoleptic characteristics. Whereas, too low moisture content will give very dusty flour and causes clogging and tamping (Guerrerio and Retiere, 1991).

### 3.3. Measurement of pH

Table 4 shows pH of controls and irradiated samples at 5 and 10 kGy. All samples (powder of the muscle and byproduct flour) are acid. The values of pH of sardinella muscle and byproduct powders are in agreement with those reported by Kamat and Thomas (1998). The authors mentioned that pH of sardine flour and its co-product is around 5.90 ± 0.20. The pH values of the byproduct flour are inferior to those of muscles powder; this is favorable for a longer shelf life. This could be attributed to the viscera initially present in the byproduct. Irradiation at 5 kGy seems to induce fluctuations in the samples acidity.

**Table 4:** pH of muscle powder and byproduct powders of sardinella

| | Control samples | Irradiated samples at 5 kGy | Irradiated samples at 10kGy |
|---|---|---|---|
| **Muscles powder** | 6.15±0.14[a] | 5.99±0.03[a] | 6.12±0.09[a] |
| **Byproducts powder** | 5.86±0.06[ab] | 5.80±0.03[b] | 5.90±0.02[a] |

Results are presented as means ± S.D. for triplicate analysis. Values with the same letter are not significantly different at p <0.05.

### 3.4. Microbiological analyzes

For both sardinella muscles and its byproduct powders, microbial analyzes show the absence of sulphite-reducing microorganisms, yeast, molds and pseudomonas bacteria. However total coliforms and mesophilic bacteria are present in the control (non-irradiated) samples of muscles and byproduct powders. Table 5 and Table 6 show the microbial counts of irradiated and non-irradiated muscle powder and byproduct flour during the storage at room temperature.

**Table 5**: Change in microbial counts of irradiated and non-irradiated muscle powder of sardinella
(0) absent, (*) the analyzes were suspended

| | Dose | Day 0 | Day 7 | Day 14 | Day 21 | Day 35 | Day 42 |
|---|---|---|---|---|---|---|---|
| **Micro-organisms** | | | | CFU/ 10 g | | | |
| **E. Coli** | | | | | | | |
| | C | 0 | 0 | | 0 | 0 | 0 |
| | 5 | 0 | 0 | | 0 | 0 | 0 |
| | 10 | 0 | 0 | | | | |
| **Sulphite-reducing** | | | | | | | |
| | C | 0 | 0 | | 0 | 0 | 0 |
| | 5 | 0 | 0 | | 0 | 0 | 0 |
| | 10 | 0 | 0 | | 0 | 0 | 0 |
| **Total Coliforms** | | | | | | | |
| | C | 22700 | 108000 | | 96 | Bacterial mat | Bacterial mat |
| | 5 | <15 | 191000 | | <15 | <15 | <15 |
| | 10 | 0 | | | | | |
| **Pseudomonas** | | | | | | | |
| | C | 0 | 0 | | 0 | 0 | 0 |
| | 5 | 0 | 0 | | 0 | 0 | 0 |
| | 10 | 0 | 0 | | 0 | 0 | 0 |
| **Yeast and molds** | | | | | | | |
| | C | 0 | 0 | | 0 | 0 | 0 |
| | 5 | 0 | 0 | | 0 | 0 | 0 |
| | 10 | 0 | 0 | | 0 | 0 | 0 |
| **Mesophilic bacteria** | | | | | | | |
| | C | <15 | 175 | | 230 | Barterial mat | Bacterial mat |
| | 5 | <15 | 212 | | 76 | <15 | <15 |
| | 10 | <15 | * | | * | * | * |

**Table 6:** Change in microbial counts of irradiated and non-irradiated byproduct flour of sardinella
(0) absent, (*) the analyzes were suspended

| | Dose | Day0 | Day 7 | Day 14 | Day 21 | Day35 | Day 42 |
|---|---|---|---|---|---|---|---|
| **Micro-organisms** | | | | CFU/ 10 g | | | |
| **E. Coli** | | | | | | | |
| | C | 0 | 0 | | 0 | 0 | 0 |
| | 5 | 0 | 0 | | 0 | 0 | 0 |
| | 10 | 0 | 0 | | * | * | * |
| **Sulfite-reducing** | | | | | | | |
| | C | 0 | 0 | | 0 | 0 | 0 |
| | 5 | 0 | 0 | | 0 | 0 | 0 |
| | 10 | 0 | 0 | | 0 | 0 | 0 |
| **Total Coliforms** | | | | | | | |
| | C | 256 | <15 | | <15 | <15 | <15 |
| | 5 | <15 | <15 | | <15 | <15 | 0 |
| | 10 | * | * | | * | * | * |
| **Pseudomonas** | | | | | | | |
| | C | 0 | 0 | | 0 | 0 | 0 |
| | 5 | 0 | 0 | | 0 | 0 | 0 |
| | 10 | 0 | 0 | | 0 | 0 | 0 |
| **Molds and yeast** | | | | | | | |
| | C | 0 | 0 | | 0 | 0 | 0 |
| | 5 | 0 | 0 | | 0 | 0 | 0 |
| | 10 | 0 | 0 | | 0 | 0 | 0 |
| **Mesophilic bacteria** | | | | | | | |
| | C | 750 | <15 | | <15 | <15 | <15 |
| | 5 | 18 | 30 | | <15 | <15 | 0 |
| | 10 | * | * | | * | * | * |

The counts of total coliforms and total mesophilic in control samples increased during storage period. After irradiation the microbial charge decreased to remain steady during the storage period except for the 5kGy-lot, which shows total coliforms of 191000 cfu/10g at day 7. Samples irradiated at 10 kGy seem to be salubrious at the day of their production (day 0), the microbial analysis is thus suspended. Total coliforms is about 256 CFU/10g and total mesophilic is about 750 CFU/10g in non-irradiated byproduct powder (day 0). After irradiation the microbial charge in byproduct powder decreased to a steady state during the storage period. According to microbial analyzes of control samples, it seems that muscles powder is less salubrious than byproduct powder; The stability and safety of this latter is provided by the both heat treatments (cooking at 90°C and drying at 75°C) applied during powder production. Irradiated samples at 5kGy and at 10 KGy showed an acceptable microbiological quality of both products. Irradiation improves the microbial quality and inhibits the proliferation of microorganisms in fish as reported by different authors (Mbarki et al., 2009; Badr et al., 2012; Hocaoglu et al., 2012; Aly et al 2014; Prakash et al., 2014). In fact, Mbarki et al. (2012) reported that application of gamma irradiation (1 kgy and 2 kGy) before storage of Mediterranean horse mackeral reduced significantly the microbial charge (mesophilic, psycrophils, and total coliforms germs) and inactivated completely fecal coliforms, staphylococcus and E.coli during freezing. These results are in agreement with those reported for irradiated shrimp (at 3 and 5 kGy) stored at -18°C (Hocaoglu et al., 2012).

### 3.5. Color measurement

The colorimetric parameters of irradiated and non-irradiated samples of muscles and byproduct powders during 42 days of storage at room temperature are illustrated in Figure 2 and Figure 3.

The global color difference (ΔE) of non-irradiated and irradiated products (flour byproduct and muscle powder) shows significant variation ($p<0.05$) during storage. This variation is less outstanding respectively at 5kGy and at 10 kGy. The color changes observed for non-irradiated samples may be explained by spontaneous lipid oxidation and/or the microbial charge that causes lipids oxidation and browning of the product. This phenomenon is mentioned especially for fatty fishes (FAO, 1999).

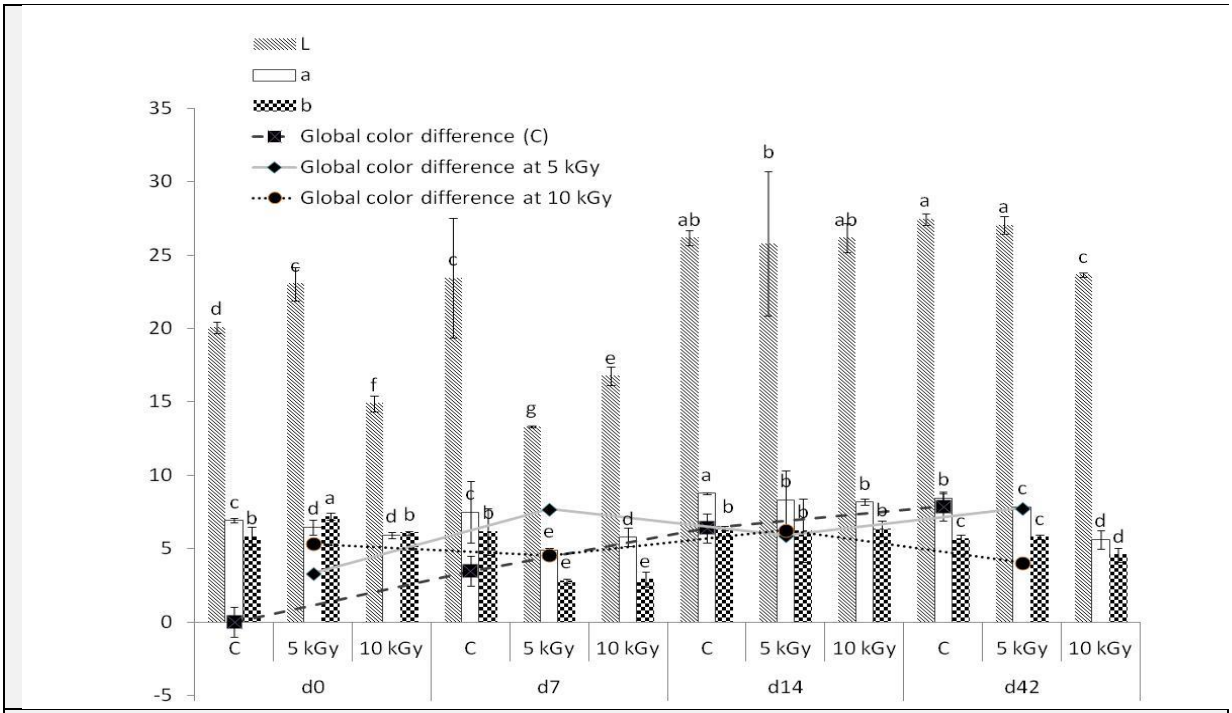


**Figure 2:** Colorimetric parameters of sardinella muscles powder (C: control, irradiated at 5 kGy and 10 kGy samples) for 42 days of storage at room temperature

Results are presented as means ± S.D. for triplicate analysis. Values with the same letter are not significantly different at $p<0.05$.

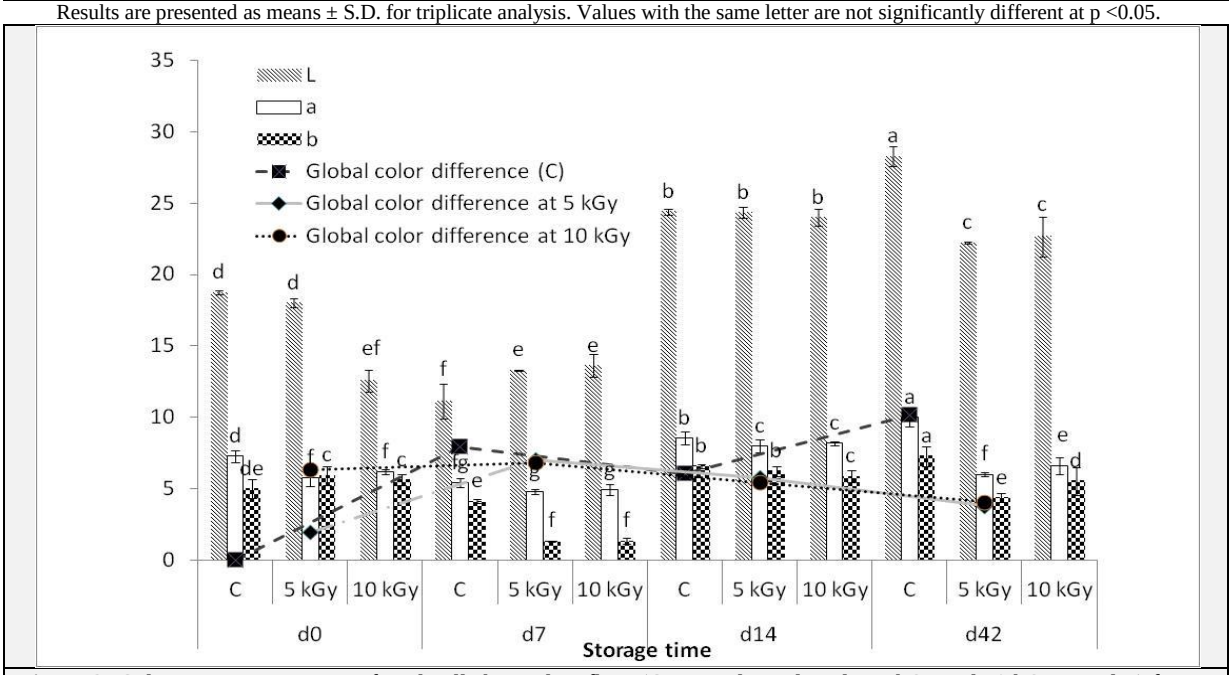


**Figure 3:** Colorimetric parameters of sardinella byproduct flour (C: control, irradiated at 5 kGy and 10 kGy samples) for 42 days of storage at room temperature.

Results are presented as means ± S.D. for triplicate analysis. Values with the same letter are not significantly different at $p<0.05$.

## 4. Conclusion

The combined drying-gamma irradiation processes seem to be an efficient way for sardinella muscle and byproduct powders stabilities. The microbial analysis showed that the non-irradiated byproduct

powder produced by cooking/drying/grinding was more salubrious that non-irradiated muscles powder. Drying allows reducing the moisture content of the muscle and byproduct powders to a level inferior to 6.40% (wet basis) and the irradiation (at 5 or at 10 kGy) following drying step ensures the inhibition of the microbial growth of both products. Microbiological quality and the color of both products were enhanced and maintained relatively constant after irradiation at 5 and 10 kGy during the 42 days of storage.

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