Evaluation of the covering oil quality in canned sardines in olive oil (Sardina pilchardus) produced in Morocco

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Abstract
This study consists of evaluating the quality of the covering oil of canned sardines in olive oil compared to that using soybean and sunflower oils after sterilization at 122°C/28 min and after storage for 10 days. The effect of adding rosemary and thyme dried leaves as an ingredient on the covering oil stability was also studied. The type of oil increased significantly the acidity levels of the covering oils during storage of canned sardines. However, it influenced the peroxide value variation after both sterilization and storage, where the minimum increase in this value was obtained in canned sardines in olive oil. By flavoring canned sardines with thyme and especially rosemary dried leaves, the lowest alteration levels were showed for flavored samples after sterilization (0.36 to 0.66% (g of oleic acid/100 g of oil) - 0.07 to 0.57 meq O₂/Kg for flavored sample vs. 0.42 to 0.94% (g of oleic acid/100 g of oil) - 0.67 to 0.73 meq O₂/Kg for control sample) and after storage (0.07 to 0.24% (g of oleic acid/100 g of oil) - 0.07 to 0.50 meq O₂/Kg for flavored sample vs. 0.18 to 0.38% (g of oleic acid/100 g of oil) - 0.35 to 0.70 meq O₂/Kg for control sample). Results confirm that using olive oil in canned sardines and adding thyme and rosemary lead to a decrease in the covering oil’s level of oxidation.

Keywords: conserve, sardine, sterilization, storage, oil stability, rosemary, thyme.
Évaluation de la qualité de l'huile de couverture des conserves de sardines (*Sardina pilchardus*) à l'huile d'olive produites au Maroc

Résultats
Cette étude consiste à évaluer la qualité de l'huile de couverture des sardines en conserve à l'huile d'olive par rapport à celles utilisant des huiles de soja et de tournesol après stérilisation à 122 °C/28 min et après un stockage de 10 jours. L'effet de l'ajout de feuilles séchées de romarin et de thym en tant qu'ingrédient sur la stabilité de l'huile de couverture a également été étudié. Le facteur type d'huile a significativement augmenté les niveaux d'acidité des huiles de couverture pendant le stockage des conserves de sardines. Cependant, il a influencé la variation de la production des peroxydes après la stérilisation et le stockage, où la plus faible augmentation de cette valeur a été obtenue dans les conserves à l'huile d'olive. En aromatisant les sardines en conserve avec des feuilles séchées de thym et de romarin, les niveaux les plus faibles d'altération ont été obtenus dans les échantillons aromatisés après la stérilisation (0.36 à 0.66% (g d'acide oléique/100 g d'huile) - 0.07 à 0.57 meq O_2/Kg vs. 0.42 à 0.94% (g d'acide oléique/100 g d'huile) - 0.67 à 0.73 meq O_2/Kg enregistrés par les témoins) et le stockage (0.07 à 0.24% (g d'acide oléique/100 g d'huile) - 0.07 à 0.50 meq O_2/Kg vs. 0.18 to 0.38% (g d'acide oléique/100 g d'huile) - 0.35 to 0.70 meq O_2/Kg enregistrés par les témoins). Les résultats confirment que l'utilisation de l'huile d'olive dans les sardines en conserve et l'ajout de thym et de romarin permettent de réduire le niveau d'oxydation de l'huile de couverture.

**Mots clés :** conserve, sardine, stérilisation, stockage, stabilité de l'huile, romarin, thym.
تقييم جودة زيت تغطية السردين المعلب بزيت الزيتون المنتج بالمغرب.

مونيه الأكراتي، ملك الإدريسي الراجي، هند لقدم.

ملخص

تهدف هذه الدراسة إلى تقييم جودة زيت التغطية لسردين معلب في زيت الزيتون مقارنةً بالزيوت المستخدمة كزيت الصويا وزيت عباد الشمس بعد التعقيم عند 122 درجة مئوية / 28 دقيقة وبعد تخزين لمدة 10 أيام. كما تم دراسة تأثير إضافة أوراق الإكليل والزعتر الجافة على جودة زيت التغطية أثناء تخزين السردين المعلب. كما تأثرت قيمة مؤشر البيروكسيد بالتغيير بعد التعقيم والتخزين، حيث تم الحصول على أدنى زيادة في هذه القيمة في سرد من معلب في زيت الزيتون. بالإضافة أوراق الزعتر وخاصة أوراق الإكليل الجافة في السردين المعلب، أظهرت العينات المعنية بهذه الإضافة أقل مستويات التغيير بعد التعقيم (0.36 إلى 0.66٪ غ من حمض الأوليك/100 غ من الزيت) - 0.07 إلى 0.57 مللي إكسجين / كغم للعينات التي تحتوي على الأعشاب مقابل 0.42 إلى 0.94٪ غ من حمض الأوليك/100 غ من الزيت - 0.67 إلى 0.73 مللي إكسجين / كغم للعينات الشاهدة) وبعد تخزين (0.07 إلى 0.24٪ غ من حمض الأوليك/100 غ من الزيت) - 0.07 إلى 0.50 مللي إكسجين / كغم للفلتين المزينة مقابل 0.18 إلى 0.38٪ غ من حمض الأوليك/100 غ من الزيت) - 0.35 إلى 0.70 مللي إكسجين / كغم للعينات الشاهدة). تؤكد النتائج أن استخدام زيت الزيتون في سردين معلب وإضافة الزعتر والإكليل يؤدي إلى انخفاض مستوى أكسدة زيت التغطية.

الكلمات المفتاحية: السردين المعلب، التعقيم، التخزين، جودة الزيت، الإكليل، الزعتر.
Introduction

Canned sardines in olive oil are considered as products with high commercial and nutritional value because sardines contain large amounts of omega-3 (DHA, EPA and ALA) (Bouriga et al., 2022; Mesías et al., 2015), and olive oil is rich in omega-9 (oleic acid) and vitamin-E (tocopherol) (Boskou & American Oil Chemists’ Society, 2006; Lucci et al., 2020). The omega-3 are polyunsaturated fatty acids (PUFA) that have an important role in the prevention against cardiovascular and cerebrovascular diseases, hypertension, arthritis as well as other inflammatory, cancerous and autoimmune disorders (Dyall & Michael-Titus, 2008; Kamal-Eldin & Yanishlieva, 2002; Swanson et al., 2012; Vonschacky & Harris, 2007). Also, omega-9 that are monounsaturated fatty acids (MUFA) have several health benefits, including anti-inflammatory and anti-cancer characteristics (Farag & Gad, 2022).

Deheaded sardines are eviscerated, washed before putting them in cans. They are then cooked and the covering juice is added before sealing. After this step, the airtight containers are sterilized by a heat treatment to destroy pathogenic microorganisms and to guarantee a sanitary safety of the product. However, this sterilization as well as storage at high temperatures causes a degradation of nutritional compounds including lipids. These thermal sensitive molecules deteriorate due to the oxidation and hydrolysis reactions which affects the organoleptic quality of the product (Cuvelier & Maillard, 2012; Domiszewski & Mierzejewska, 2021; Gómez-Limia et al., 2021).

Many studies described the methods to evaluate the lipids alteration, in particular those of oils (Ben Tekaya & Hassouna, 2005; Cuvelier & Maillard, 2012). The most used ones to assess the quality of oils are the acidity and peroxide value indices. These two analyses provide important information regarding the freshness and oxidative stability of the oil. They aim to identify levels of primary oxidation products (the peroxide value PV) and secondary oxidation and hydrolysis products (the free acidity value) (Symoniuk et al., 2022; Wang et al., 2011).

This study aims to assess the quality of the oil used as a covering in canned sardines. It specifically focuses on sardines produced in Morocco, providing valuable insights into the characteristics of the olive oil used in this particular context. The findings of this research contribute to a better characterization of oil quality in canned sardines and can serve as a reference for further studies and improvements in the production process. This work has two goals, the first consists of evaluating the effect of sterilization and storage on degradation level of the covering oil for canned sardines in olive oil compared to those of sardine cans made with other vegetable oils (as soybean and sunflower oils). The second goal is testing the effect of using dried plants leaves (rosemary and thyme) on the quality of this studied oil.
Materials and methods

Sampling

Canned sardines (125g) were prepared on an industrial scale at a fish cannery in Agadir. The experiments were performed using three lots of sardines (Sardina pilchardus) caught off the Dakhla coast.

For the first experiment, each can is composed of 70% fish, 29% covering oil (one of these refined vegetal oil: soybean oil, sunflower oil and olive oil and 1% of salt. Concerning the second experiment, the canned sardines had the same composition except using just refined olive oil (imported from Spain) as covering oil. In addition, rosemary (Salvia rosmarinus) and thyme (Thymus ciliates) dried leaves were added to the sardine’s cans in this second experiment before sealing in three formulas, namely: 2% rosemary; 2% Thyme; 1% rosemary + 1% thyme.

In both experiments, cans were sterilized by heat treatment (HT) at 122°C for 28 minutes. This specific temperature and time couple is the standard practice followed by the fish cannery to achieve sterilization. The produced cans were stored for 10 days at 3 different temperatures (55 °C, 37 °C and room temperature evaluated between 25 and 28 °C).

Physico-chemical analysis

The alteration level of covering oils was monitored by analyzing the acidity and peroxide value (PV) according to ISO 660:2020 and ISO 3960:2017, respectively.

Statistical treatment

All measurements were carried out in triplicate (n=3), and the obtained results were tested by analysis of variance tests ANOVA completed by Duncan’s Multiple Range test at $P = 5\%$ significance level using IBM SPSS Statistics software version 26.0. Data are expressed as mean ± standard deviation.
Results and discussion

Experiment 1
Effect of sterilization on the quality of covering oils

Tables 1 and 2 summarize acidity and PV variation in the covering oils before and after sterilization of the three types of sardines cans. Results showed that type of oil doesn’t significantly affect the acidity level neither the peroxide value at a confidence level of 95% after sterilization. This increase in the alteration parameters after the heat treatment can be due to the presence of fish oil in it, which has undergone an oxidation in the processing chain in addition to the degradation of vegetable oil during the heat treatment.

The effect of the batch factor is not significant, because the three batches of sardines came from the same fishing spot, were transported under the same conditions and have incurred the same treatment.

| Table 1. Acidity variation of the covering oils in% before and after sterilization |
|----------------------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
|                                  | Sunflower oil    | Soybean oil      | Olive oil        |
|                                  | Before HT        | After HT         | Variation        | Before HT        | After HT         | Variation        | Before HT        | After HT         | Variation        |
| Lot 1                            | 0.18 ± 0.06      | 0.80 ± 0.12      | 0.62 ± 0.06      | 0.20 ± 0.12      | 0.70 ± 0.04      | 0.50 ± 0.06      | 0.14 ± 0.04      | 0.58 ± 0.01      | 0.44 ± 0.01      |
| Lot 2                            | 0.39 ± 0.12      | 0.96 ± 0.15      | 0.57 ± 0.08      | 0.32 ± 0.15      | 0.90 ± 0.04      | 0.58 ± 0.01      | 0.32 ± 0.04      | 0.74 ± 0.01      | 0.42 ± 0.01      |
| Lot 3                            | 0.76 ± 0.14      | 0.80 ± 0.10      | 0.04 ± 0.04      | 0.72 ± 0.10      | 0.87 ± 0.04      | 0.15 ± 0.04      | 0.70 ± 0.04      | 0.75 ± 0.18      | 0.05 ± 0.01      |

| HT: heat treatment (122°C / 28 min) |
| Means followed by the same letters as superscripts are not significantly different according to Duncan’s test. |

| Table 2. Peroxide value variation (meq O₂/kg) of the covering oils before and after sterilization |
|----------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                                  | Sunflower oil   | Soybean oil     | Olive oil       |
|                                  | Before HT       | After HT        | Variation       | Before HT       | After HT        | Variation       | Before HT       | After HT        | Variation       |
| Lot 1                            | 1.60 ± 0.57     | 2.30 ± 0.62     | 0.70 ± 0.06     | 1.00 ± 0.14     | 2.00 ± 0.01     | 1.00 ± 0.06     | 1.40 ± 0.01     | 2.20 ± 0.01     | 0.80 ± 0.01     |
| Lot 2                            | 2.20 ± 0.01     | 2.40 ± 0.02     | 0.20 ± 0.04     | 1.05 ± 0.07     | 1.80 ± 0.02     | 0.75 ± 0.04     | 1.60 ± 0.04     | 1.90 ± 0.01     | 0.59 ± 0.01     |
| Lot 3                            | 1.65 ± 0.49     | 2.45 ± 0.57     | 0.80 ± 0.08     | 1.60 ± 0.57     | 1.85 ± 0.21     | 0.25 ± 0.02     | 1.40 ± 0.28     | 1.85 ± 0.07     | 0.45 ± 0.01     |

| HT: heat treatment (122°C / 28 min) |
| Means followed by the same letters as superscripts are not significantly different according to Duncan’s test. |

Effect of storage on the quality of covering oils

The analysis of results shown in Fig. 1 indicates that the factors "Incubation temperature" and "Type of oil" have significant effects on the free acidity and PV of the studied oils during the storage. These results were subjected to a two-way analysis of variance completed by Duncan’s test. It is observed that the maximum degradation, in terms of acidity and peroxide production, was recorded after storage at 55°C. Therefore, the storage temperatures influenced significantly the lipid degradation since the increase in temperature is a factor which favors the
oxidation of fish and vegetable oils (Ben Tekaya & Hassouna, 2005; Cuvelier & Maillard, 2012; Shewfelt, 1981).

We also observe that the least amount of increase in both alteration indices was recorded by olive oil cans (Fig. 1), since the olive oil has a low PUFA content compared to other oils as it is rich in antioxidants such as tocopherols and phenolic compounds (Cuvelier & Maillard, 2012; Gimeno et al., 2002; Essiari et al., 2014). So, the oxidation process is influenced by the type of oil due to variations in its composition of polyunsaturated fatty acids (PUFAs) and the presence of compounds that can either promote oxidation (pro-oxidants) or inhibit oxidation (antioxidants).

The relatively low level of unsaturation found in olive oil in comparison to other oil types is noteworthy due to the high susceptibility to oxidation of PUFA to oxidation (Cuvelier & Maillard, 2012; Frankel, 2007; Graille, 2003). Indeed, the fatty acids composition of vegetable oils (SFA, MUFA and PUFA respectively), the olive oil contains 9-26%, 56-87%, 4-22%; the soybean oil is composed of 11-21%; 17-27%; 54-72%; and the sunflower oil consists of 10-16%; 15-26%; 62-70% (Morin & Pagès-Xatart-Parès, 2012). Moreover, sardines caught off the Dakhla coast present a fatty acid profile rich in PUFA (36.4 - 41.6 % of PUFA, 32.8 - 38.9 % of SFA and 24.0 - 28.1 % of MUFA) (Mkadem & Kaanane, 2020). These polyunsaturated fatty acids are mixed and analyzed with the covering juice.

This effect of the composition of the oil on oil quality was not proven after sterilization because the sterilization time was probably insufficient to detect the evolution of the studied quality parameters unlike the recorded variations after storage. Thus, the change in acidity even after heat treatment is due to the chemical hydrolysis of glycerides under the effect of heat and humidity. Enzymatic lipolysis probably does not occur since the lipases are deactivated during sterilization (Barros et al., 2010).

![Figure 1](attachment:image.png)

**Figure 1.** Acidity and peroxide value variations of the covering oils after storage


Columns annotated with identical letters are considered not statistically different according to Duncan's test.
Experiment 2
Effect of rosemary and thyme leaves after sterilization

After sterilization, the effect of using rosemary and thyme leaves and their combination is significant to reduce PV and free acidity level (Table 3 and Table 4). Where the lowest degradation values were recorded by flavored olive oil. This result is consistent with other studies previously reported (Ayadi et al., 2009; Caponio et al., 2003; Malheiro et al., 2009).

Table 3. Acidity variation (%) of flavored covering oils in olive oil cans before and after sterilization

<table>
<thead>
<tr>
<th></th>
<th>Before St. After HT</th>
<th>Rosemary Variation</th>
<th>Thyme After HT</th>
<th>Rosemary &amp; Thyme Variation</th>
<th>Control After HT</th>
<th>Variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lot 1</td>
<td>0.17 ± 0.00</td>
<td>0.37 b</td>
<td>0.60 ± 0.23</td>
<td>0.58 ± 0.05</td>
<td>1.11 ± 0.08</td>
<td>0.94 a</td>
</tr>
<tr>
<td></td>
<td>0.00</td>
<td>0.02</td>
<td>0.43 b</td>
<td>0.41 b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lot 2</td>
<td>0.17 ± 0.00</td>
<td>0.36 b</td>
<td>0.66 ± 0.09</td>
<td>0.55 ± 0.04</td>
<td>0.59 ± 0.01</td>
<td>0.42 a</td>
</tr>
<tr>
<td></td>
<td>0.00</td>
<td>0.16</td>
<td>0.49 b</td>
<td>0.38 b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lot 3</td>
<td>0.17 ± 0.00</td>
<td>0.41 b</td>
<td>0.42 ± 0.12</td>
<td>0.83 ± 0.17</td>
<td>0.99 ± 0.17</td>
<td>0.82 a</td>
</tr>
<tr>
<td></td>
<td>0.00</td>
<td>0.09</td>
<td>0.45 b</td>
<td>0.66 b</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

HT: heat treatment (122°C / 28 min)
Means followed by the same letters as superscripts are not significantly different according to Duncan’s test.

Table 4. Peroxide value variation (meq O₂/kg) of flavored covering oils in olive oil cans before and after sterilization

<table>
<thead>
<tr>
<th></th>
<th>Before St. After HT</th>
<th>Rosemary Variation</th>
<th>Thyme After HT</th>
<th>Rosemary &amp; Thyme Variation</th>
<th>Control After HT</th>
<th>Variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lot 1</td>
<td>1.50 ± 0.00</td>
<td>0.13 b</td>
<td>1.93 ± 0.12</td>
<td>1.70 ± 0.10</td>
<td>2.20 ± 0.09</td>
<td>0.70 a</td>
</tr>
<tr>
<td></td>
<td>0.00</td>
<td>1.63 ± 0.36</td>
<td>0.43 b</td>
<td>0.20 b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lot 2</td>
<td>1.50 ± 0.00</td>
<td>0.07 b</td>
<td>1.93 ± 0.25</td>
<td>1.90 ± 0.40</td>
<td>2.23 ± 0.12</td>
<td>0.73 a</td>
</tr>
<tr>
<td></td>
<td>0.00</td>
<td>1.57 ± 0.06</td>
<td>0.43 b</td>
<td>0.46 b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lot 3</td>
<td>1.50 ± 0.00</td>
<td>0.57 b</td>
<td>2.03 ± 0.25</td>
<td>2.17 ± 0.02</td>
<td>2.17 ± 0.06</td>
<td>0.67 a</td>
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<tr>
<td></td>
<td>0.00</td>
<td>2.07 ± 0.53</td>
<td>0.53 b</td>
<td>0.35 b</td>
<td></td>
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</tr>
</tbody>
</table>

HT: heat treatment (122°C / 28 min)
Means followed by the same letters as superscripts are not significantly different according to Duncan’s test.

As for the influence of the used plants after storage, as shown by the analysis of the represented results (Fig. 2), the both factors "storage temperature" and "formula in plants" have significant effects just on oil oxidation. So, the effect of the used plants seems more interesting in reducing oxidation than in decreasing acidity during storage. The best formulations consist of oil flavored with rosemary at 37°C and the combination of rosemary and thyme flavors at both room temperature and 37°C. The maximum deterioration for all formulations, including the control sample, is generally recorded at 55°C.
As we notice that the decrease of the PV of oils flavored, in particular with rosemary, are always lower than those unflavoured for all incubation temperatures. This showed that the richness of the used plants, mainly rosemary, by the polyphenols, makes it possible to improve the oxidative stability of the covering oil as proved by other studies (Servili & Montedoro, 2002), because they act as free radical scavengers and metal chelators, thus reducing lipid oxidation (Lacroix et al., 1997; Tomaino et al., 2005; Nozaki, 1989).

**Conclusion**

Olive oil is considered as a food product rich in unsaturated fats. It is used as a covering oil to produce high value canned sardines. These fatty compounds deteriorate due to heat during the process, particularly in sterilization, and during the storage.

When compared to other tested oils, olive oil showed minimal deterioration at different temperatures of storage (acidity: 0.18% at room temperature, 0.17% at 37 °C, 0.38% at 55 °C ; PV: 0.08 meq O₂/kg at room temperature, 0.35 meq O₂/kg at 37 °C, 0.49 meq O₂/kg at 55 °C). In addition, to preserve the quality of olive oil in cans of sardines, rosemary and thyme leaves can be used. It has been found that the oil PV values were 0.08 meq O2/kg using rosemary and thyme at room temperature; 0.07 meq O2/kg using rosemary at 37 °C; and 0.30 meq O2/kg using rosemary at 55 °C, compared respectively to 0.70 meq O2/kg; 0.35 meq O2/kg; 0.49 meq O2/kg for the control. The results have shown that the oxidation level of all studied flavored olive oils are generally lower than those of unflavored olive oil during sterilization and even after the 10 days of incubation.

In general, since rosemary and thyme are effective antioxidants sources for improving the stability of sardines canning in olive oil during process and storage, their combination can lead to an improvement in oxidative stability.
organoleptic quality can also be improved by this formulation, but it remains to be proven in further studies.

**Author contribution**

Mounia Lekrati: Conception, Methodology, Interpretation, Writing original draft. Malak El Idrissi Raji: Data acquisition, Formal analysis. Hind Mkadem: Data acquisition, Review. All authors have provided final approval of the version to publish.

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**Conflict of interest**

We have no conflict of interest to disclose. We declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
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