Lipid hydrolysis and oxidation in farmed gilthead seabream (*Sparus aurata*) slaughtered and chilled under different icing conditions

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1. INTRODUCTION

Different mechanisms have been found responsible for fish deterioration during chilled storage (Whittle et al., 1990; Olafsdóttir et al., 1997). Among them, the lipid fraction has been reported to undergo hydrolysis and oxidation reactions that can lead to important losses in sensory and nutritional qualities with an important impact on its commercial value (Harris and Tall, 1994; Chaouqy et al., 2008).

In order to avoid fish damage development during chilled storage, recent research accounts for advanced strategies. One such technology is slurry ice (SI) which, when employed in the place of traditional flake ice (FI) has shown many advantages such as a lower storage temperature, faster cooling, lower physical damage to the product and better heat exchange power. As a result, the application of this chilling strategy has led to an important inhibition of autolysis development, microbiological activity and lipid oxidation in different kinds of marine products (Yamada et al., 2002; Medina et al., 2009).

For some decades now, fish technologists and the fish trade have been especially attracted by aquaculture development as a source of fish products. Among cultivated fish, gilthead seabream (*Sparus aurata*) deserves great attention because of its increasing production in Mediterranean countries such as Greece, Turkey and Spain (FAO, 2007). Previous research carried out during the chilled storage of this species accounts for changes in sensory (Kyrana et al., 1997; Erkan, 2007), microbial (López-Caballero et al., 2002; Cakli et al., 2006; Özogul et al., 2007), physical (Alasalvar et al., 2002; Lougvois et al., 2003) and chemical (Tejada et al., 2003; Caballero et al., 2009) properties. Concerning the lipid fraction, most research on gilthead seabream has been focused on the effect of the diet provided (Yildiz et al., 2008; Fountoulaki et al., 2009) and the harvesting season (Grigorakis et al., 2003; Senso et al., 2007) on the fatty acid profile of the fish product; however, research concerning lipid damage during the chilled storage of this species has been scarce and complementary to other kinds of quality analyses (Kyrana et al., 1997; Grigorakis et al., 2003; Cakli et al., 2006).
The present work focuses on the slaughtering and chilling steps related to the commercialization of farmed gilthead seabream as a fresh product. An SI system was applied and evaluated comparatively to traditional FI with the objective of extending shelf life. In this study, lipid hydrolysis and oxidation were analyzed, sensory acceptance was compared and trimethylamine formation (microbial activity) was undertaken.

2. MATERIALS AND METHODS

2.1. Icing systems

Two different icing systems (FI and SI) were employed for the fish slaughter and chilled storage. FI was prepared with an Icematic F100 Compact device (Castelmac SPA, Castelfranco, Italy). The temperature of the FI was \(-0.5^\circ\text{C}\), with the temperature of the fish in the range of \(0^\circ\text{C}\) to \(-0.5^\circ\text{C}\).

SI was prepared using a FLO-ICE prototype (Kinarca S.A.U., Vigo, Spain). The composition of the SI binary mixture was 40% ice/60% water, prepared from filtered seawater (salinity: 3.3%). The temperature of the SI mixture was \(-1.5^\circ\text{C}\), being the temperature of the fish in the range of \(-1.0^\circ\text{C}\) to \(-1.5^\circ\text{C}\).

2.2. Fish material, slaughter and chilled storage

Sixty specimens of farmed gilthead seabream (\textit{Sparus aurata}) (individual weight range: 0.42-0.50 kg; individual length range: 28-30 cm) were obtained (day 0) from an aquaculture facility (Cultipeix S. L., Valencia, Spain) and slaughtered at the farm by immersion either in FI (30 individuals) or SI (30 individuals). Individuals were kept under such conditions for 24 hours until arrival at the laboratory. At this time (day 1), six individuals belonging to each icing condition were separated and taken for sensory and chemical analyses as starting fish. Specimens from each icing condition were divided into three groups (two individuals in each group) which were studied separately (\(n = 3\)). The remaining fish were placed in an isothermal room at \(2^\circ\text{C}\) and were surrounded by either FI or SI at a 1:1 fish to ice ratio. Fish sampling was then continued on days 4, 7, 11 and 14 of icing treatment, according to the same sampling design (\(n = 3\)). Throughout the experiment, both the FI or SI were renewed when required.

2.3. Sensory analyses

Sensory analysis was conducted by a sensory panel consisting of five experienced judges, according to guidelines concerning fresh and refrigerated fish (DOCE, 1989). Sensory assessment of the fish included the examination of the following parameters: skin, eyes, external odor, gills, consistency and flesh odor (Table 1). Four categories were ranked: highest quality (E), good quality (A), fair quality (B) and unacceptable quality (C).

The panelists included in this study had been involved in sensory analysis of different fish species for ten years. Previously to the present experiment, the panelists were specially trained with chilled gilthead seabream. At each sampling time, the fish specimens were presented to panellists and were scored individually. The panel members shared samples tested.

2.4. Trimethylamine (TMA) formation

Trimethylamine-nitrogen (TMA-N) values were determined by means of the picrate method, this

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Highest quality (E)</th>
<th>Good quality (A)</th>
<th>Fair quality (B)</th>
<th>Unacceptable (C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin</td>
<td>Very intense pigmentation; transparent mucus</td>
<td>Milky mucus; insignificant pigmentation losses</td>
<td>Slightly greyish mucus; pigmentation without shine</td>
<td>Widely opaque mucus; important pigmentation losses</td>
</tr>
<tr>
<td>Eyes</td>
<td>Convex; transparent cornea; bright and black pupil</td>
<td>Convex and slightly sunken; slightly opalescent cornea; black and cloudy pupil</td>
<td>Flat; opalescent cornea; opaque pupil</td>
<td>Concave and milky cornea; internal organs blurred</td>
</tr>
<tr>
<td>External odor</td>
<td>Sharply seaweedy and shellfish smell</td>
<td>Weakly seaweedy and shellfish smell</td>
<td>Incipiently putrid or rancid</td>
<td>Putrid or rancid</td>
</tr>
<tr>
<td>Gill</td>
<td>Brightly red; without odor; lamina perfectly separated</td>
<td>Rose coloured; without odour; lamina adhered in groups</td>
<td>Slightly pale; incipient fishy odor; lamina adhered in groups</td>
<td>Grey-yellowish color; intense ammonia odor; lamina totally adhered</td>
</tr>
<tr>
<td>Consistency</td>
<td>Presence or partial disappearance of rigor mortis symptoms</td>
<td>Firm and elastic; pressure signs disappear immediately and completely</td>
<td>Presence of mechanical signs; elasticity notably reduced</td>
<td>Important shape changes due to mechanical factors</td>
</tr>
<tr>
<td>Flesh odour</td>
<td>Sharply seaweedy and shellfish smell</td>
<td>Weakly seaweedy and shellfish smell</td>
<td>Incipiently putrid or rancid</td>
<td>Putrid or rancid</td>
</tr>
</tbody>
</table>
including a spectrophotometric (410 nm) assessment (Beckman Coulter DU 640, London, UK) (Tozawa et al., 1971). This involves the preparation of a 5% (w/v) trichloroacetic acid extract of fish muscle. The results were expressed as mg TMA-N/100 g muscle.

2.5. Lipid hydrolysis

Lipids were extracted by the Bligh and Dyer (1959) method. Quantification results were expressed as g lipid/100 g muscle.

Free fatty acid (FFA) content was determined in the lipid extract of the fish muscle by the Lowry and Tinsley (1976) method based on complex formation with cupric acetate-pyridine followed by spectrophotometric (715 nm) assessment. Results were expressed as g FFA/100 g lipids.

2.6. Lipid oxidation

The peroxide value (PV) was determined in the lipid extract of the muscle according to the ferric thiocyanate method (Chapman and McKay, 1949). Results were expressed as meq active oxygen/kg lipids.

The thiobarbituric acid index (TBA-i) was determined in a 5% (w/v) trichloracetic acid extract of the fish muscle (Vyncke, 1970). Results were expressed as mg malondialdehyde/kg muscle.

Formation of fluorescent compounds was determined in the lipid extract of the muscle by measurements at 393/463 nm and 327/415 nm as previously described (Aubourg et al., 1998). The relative fluorescence (RF) was calculated as follows: RF = F/F_{st}, where F is the fluorescence measured at each excitation/emission maximum, and F_{st} is the fluorescence intensity of a quinine sulphate solution (1 µg/ml in 0.05 M H₂SO₄) at the corresponding wavelength. The fluorescence ratio (FR) was calculated as the ratio between the two RF values: FR = RF/393/463 nm/RF/327/415 nm.

Browning development was measured in the lipid extract by spectrophotometric (420 nm) assessment. Results shown were calculated according to the formula: B x V / w, where B is the absorbance lecture obtained, V is the volume (ml) of the organic extract and w is the weight (g) of the fish muscle employed (Smith et al., 1990).

2.7. Fatty acid analysis

Lipid extracts were converted into fatty acid methyl esters (FAME) by employing acetyl chloride and then analyzed by gas chromatography according to a previous procedure (Aubourg et al., 1996). FAME were analyzed by means of a Perkin-Elmer 8700 chromatograph employing a fused silica capillary column SP-2330 (0.25 mm i.d. x 30 m, Supelco Inc., Bellefonte, PA, USA). Peak areas were automatically integrated, 19:0 fatty acid being used as internal standard for quantitative analysis. The concentration of each fatty acid was calculated as g/100 g total FAME.

2.8. Statistical analyses

Data (n = 3) obtained from the different sensory and chemical analyses were subjected to the ANOVA method to explore differences in two different ways: icing condition (FI/SI comparison) and icing time (Statsoft Inc., Statistica, version 6.0, 2001). Comparison of means was performed using the least-square differences (LSD) test. Confidence interval at the 95% level (p < 0.05) was considered in all cases. Correlation analysis among parameters was also carried out. Linear fittings are expressed; otherwise, the kind of fitting is mentioned.

3. RESULTS AND DISCUSSION

3.1. Sensory evaluation and microbial activity assessment

As long as the storage time increased, a progressive sensory acceptance decrease could be observed in fish corresponding to both icing conditions (Table 2). However, some differences could be assessed between both fish groups. Thus, a shelf life time of 11 days was obtained for individuals kept under FI conditions, while their counterparts stored under SI system were still acceptable at the end of the experiment. External odor (putrid odor development) and gills (color and odor) were found to be limiting factors.

The absence of rancid odor development can be partially explained on the basis of the lipid content obtained (0.75-1.20 g/100g muscle), this being lower than that reported for the same farmed species in previous research (Kyrana et al., 1997; Alasalvar et al., 2002; Grigorakis et al., 2003; Özogul et al., 2007; Yildiz et al., 2008). Lipid content differences in muscle can be justified as a result of a different feed composition. Present results on lipid content are in agreement with those previously obtained for this species under wild conditions (Alasalvar et al., 2002; Yildiz et al., 2008).

Shelf life values obtained in this study for gilthead seabream kept under traditional flake ice can be considered in agreement with previous research. Thus, previous reports account for a shelf life time of 9-12 days (Cakli et al., 2006), 12 days (Erkan, 2007), 14 days (Kyrana et al., 1997) and 18 days (Özogul et al., 2007). Differences among experiments can be explained as a result of different factors such as fish-to-ice ratio, fish size, slaughtering condition, starting raw fish quality, etc.

Concerning the employment of the SI system, it is worth pointing out that this treatment has not
produced a cloudy appearance in the eyes in this study. This problem was previously detected (Huidobro et al., 2001) and could be attributed to the employment of a relatively low temperature (round –2.2 °C); however, quality benefits were found by Huidobro et al. (2001) from the physico-chemical point of view when SI was used as a slaughter method for gilthead seabream in replacement of the FI system.

In a recent research (Kilinc et al., 2007), SI conditions provided some advantages when applied to gilthead seabream in replacement of FI. Thus, both icing conditions were applied as a cooling pre-treatment for 2 h before a further refrigerated storage at 4 °C up to 15 days. As a result, individuals previously treated under SI conditions showed a higher shelf life time (15 days) than their counterparts kept under FI condition (13 days) during the refrigerated storage.

Microbial activity was measured in the present study by the TMA formation (Figure 1). In both kinds of fish, an increasing TMA formation could be depicted as long as the storage time increased ($r^2 = 0.93-0.94$, quadratic fitting); however, only fair correlation values ($r^2 = 0.80-0.90$) could be observed with the different sensory attributes. Differences between individuals from both icing conditions could only be observed at the end of the experiment (day 14), when higher ($p < 0.05$) TMA values were obtained for individuals corresponding to FI conditions.

Results obtained in fish kept under the FI system can be considered low when compared to previous related research concerning the same farmed species (Cakli et al., 2006; Erkan, 2007). Since TMA is produced during chilled storage from trimethylamine oxide (TMAO) by bacterial enzyme reduction, present results could be explained on the basis of a low TMAO value in the muscle. In this sense, the TMAO content in farmed fish muscle has shown a great dependence on feed composition (Kyrana et al., 1997) and differences among different edible parts of the fish body (Alvarez et al., 2009).

Previous research has also shown a lower TMA formation in gilthead seabream as a result of replacing FI with SI as cooling system (Kilinc et al., 2007); in this cooling pre-treatment experiment, refrigerated (4 °C) gilthead seabream individuals previously treated under FI conditions reflected a higher TMA formation than their counterparts corresponding to the SI system.

### 3.2. Lipid hydrolysis

No significant ($p > 0.05$) FFA formation could be assessed in either kinds of iced fish during the 1-7 day icing period (Figure 2). At day 11, an important FFA formation ($p < 0.05$) could be depicted in individuals corresponding to FI conditions; for SI-fish, a marked hydrolysis development ($p < 0.05$) could be observed only at the end of the experiment. Comparison between both kinds of fish led to a higher ($p < 0.05$) FFA formation in FI-individuals during the 11-14 day period.

FFA formation has been reported to be produced during the first stages of the chilling process (up to day 9, approximately) as a result of endogenous enzyme (namely lipases and phospholipases) activity (Pigott and Tucker, 1990; Whittle et al., 1990). Later on, microbial activity should gain importance, so that FFA formation is then mostly produced as a result of bacterial catabolic processes. According to this profile, present results on FFA formation in gilthead seabream show that endogenous enzyme activity has been negligible, while the microbial contribution has been significant during the 11-14 day period for FI-fish and at the

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**Table 2**

<table>
<thead>
<tr>
<th>Attribute</th>
<th>FI</th>
<th>SI</th>
<th>FI</th>
<th>SI</th>
<th>FI</th>
<th>SI</th>
<th>FI</th>
<th>SI</th>
<th>FI</th>
<th>SI</th>
<th>FI</th>
<th>SI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin</td>
<td>E</td>
<td>E</td>
<td>A</td>
<td>A</td>
<td>E</td>
<td>E</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eyes</td>
<td>E</td>
<td>E</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>B</td>
<td>B</td>
<td>B</td>
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<td>External odor</td>
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<td>E</td>
<td>A</td>
<td>B</td>
<td>A</td>
<td>B</td>
<td>A</td>
<td>C</td>
<td>B</td>
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<td>A</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Consistency</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>B</td>
<td>A</td>
<td>B</td>
<td>A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flesh Odor</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>B</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Freshness categories as expressed in Table 1.

---

**Figure 1**

Trimethylamine (TMA) formation in gilthead seabream after slaughtering and chilled storage in flake ice (FI) or slurry ice (SI), respectively. * Average values (mg TMA-N/100g muscle) of three (n = 3) independent determinations. Standard deviations are indicated by bars.
end of the experiment for SI-fish. According to this microbial activity on FFA formation, a good correlation value was obtained between FFA and TMA contents ($r^2 = 0.86-0.93$).

An important aspect to be taken into account related to the microbial activity, and accordingly, to FFA formation is the presence of salt in the SI system. Thus, previous research has shown an important inhibitory effect of salt on lipid hydrolysis development in stored fish (Aubourg and Ugliano, 2002; Chaouqy et al., 2008). Therefore, an important role of salt can be inferred on the different developments of lipid hydrolysis in both kinds of fish in the present study.

Regarding the sensory assessment, FFA accumulation has been associated in some extent to the lack of sensory acceptability, being strongly interrelated with off-odor development (Refsgaard et al., 2000). However, poor correlation values were obtained in the present experiment for the FFA content with the different sensory attributes ($r^2 = 0.68-0.87$) and with the external odor in particular ($r^2 = 0.75-0.82$). In this sense, it has to be taken into account that the external odor detected by the sensory panel was judged as putrid odor and not as rancid odor. Accordingly, a better correlation of external odor was obtained with the TMA ($r^2 = 0.80-0.90$, above mentioned) than with FFA formation.

In addition to the low lipid content, the absence of rancid odor development in the present study can also be explained on the basis that the final FFA values can be considered low when compared to FFA values obtained during storage under FI conditions for the same farmed species (Kyrana et al., 1997) or related farmed species such as blackspot seabream (Pagellus bogaraveo; Álvarez et al., 2009) and sea bass (Dicentrarchus labrax; Pena et al., 2009). While the formation of FFA itself does not lead to nutritional losses, its assessment is deemed important when considering the development of rancidity. Thus, a pro-oxidant effect of FFA on lipid matter has been proposed and explained on the basis of a catalytic effect of the carboxyl group on the formation of free radicals by the decomposition of hydroperoxides (Miyashita and Takagi, 1986; Aubourg, 2001). In addition, FFA are known to oxidize faster than higher-molecular weight lipid classes (namely, triglycerides and phospholipids) by providing a greater accessibility (lower steric hindrance) to oxygen and other pro-oxidant molecules (Labuza, 1971).

### 3.3. Lipid oxidation

Lipid oxidation was studied by means of different quality indexes corresponding to different steps included in the development of the lipid oxidation mechanism.

Peroxide formation (primary oxidation) was evident at day 7 in both kinds of fish samples, according to a marked content increase ($p < 0.05$) (Table 3). At the end of the experiment, an important peroxide content decrease is observed, being likely to be produced as a result of partial peroxide breakdown into other kinds of lipid oxidation compounds (Pokorný, 1981; Aubourg, 1999). Highest mean PV obtained (days 7 and 11 for individuals corresponding to SI and FI, respectively) remain below a 10 value and can not be considered especially high.

Concerning secondary lipid oxidation compound formation (Table 3), the assessment of thiobarbituric acid reactive substances (TBARS) showed a low and progressive formation in both kinds of fish with icing time ($r^2 = 0.88-0.90$, quadratic fitting); a significant increase was only observed at the 11-14 day period for individuals from FI and SI conditions. Throughout the whole experiment, no differences ($p > 0.05$) could be defined between the two kinds of fish.

Previous research concerning the chilled storage of gilthead seabream under FI conditions accounts for a low (Kyrana et al., 1997) and a marked (Grigorakis et al., 2003; Cakli et al., 2008) TBA-i increase with chilling time. In such cases, starting and 14-days-storage values were markedly higher than in the present study. Previous research has also shown a lower TBARS formation as a result of replacing FI by SI as cooling system (Klinic et al., 2007); in this cooling pre-treatment experiment, refrigerated gilthead seabream individuals previously treated under FI conditions reflected a higher secondary lipid oxidation compound formation.

Among the different chemical lipid oxidation parameters studied in the present experiment, secondary lipid oxidation compounds (TBA-i) are known to be the most closely related to the formation of rancid flavors (White, 1994). In the present experiment, the TBARS formation was low and can be considered partially responsible for the fact that no rancid development was detected during the sensory analysis. Accordingly, poor correlation values were obtained between the TBA-i and the external odor ($r^2 = 0.86-0.67$).

Formation of tertiary lipid oxidation compounds was measured according to the fluorescence and browning development in lipid extracts from
the gilthead seabream muscle (Pokorný, 1981; Aubourg, 1999) (Table 3). Similar conclusions were obtained for both fluorescence and browning property assessments. Thus, individuals corresponding to FI condition showed higher mean values than their counterparts from the SI system throughout the whole experiment; however, differences were not found significant. An increasing tendency with icing time could also be depicted for individuals from both icing conditions, although correlation values were not good ($r^2 = 0.79-0.88$); however, differences as a result of the icing times. In addition, no differences ($p > 0.05$) could be assessed as a result of the icing time for both chilling conditions. Previous research (Aubourg et al., 1998) has shown an important negative effect of lipid oxidation development on the PUFA content (PI decrease) during the chilled storage of a fatty fish species (sardine; Sardina pilchardus). However, in the present study, increases found for some lipid damage indexes have not produced a negative effect ($p > 0.05$) on the polyene index.

### 4. CONCLUSIONS

An important quality loss could be assessed in gilthead seabream during slaughtering and chilled storage, according to sensory analysis and TMA formation. However, lipid damage development was found low, according to the different lipid quality indexes considered (lipid hydrolysis; primary, secondary and tertiary lipid oxidation), these

<table>
<thead>
<tr>
<th>Icing time (days)</th>
<th>Peroxide value</th>
<th>Thiorbituric acid index</th>
<th>Fluorescence formation</th>
<th>Browning development</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FI</td>
<td>SI</td>
<td>FI</td>
<td>SI</td>
</tr>
<tr>
<td>1</td>
<td>2.95 a</td>
<td>2.84 a</td>
<td>0.12 ab</td>
<td>0.14 ab</td>
</tr>
<tr>
<td></td>
<td>(0.84)</td>
<td>(1.35)</td>
<td>(0.08)</td>
<td>(0.06)</td>
</tr>
<tr>
<td>4</td>
<td>2.80 a</td>
<td>2.82 a</td>
<td>0.06 a</td>
<td>0.09 a</td>
</tr>
<tr>
<td></td>
<td>(1.04)</td>
<td>(1.69)</td>
<td>(0.01)</td>
<td>(0.02)</td>
</tr>
<tr>
<td>7</td>
<td>6.46 b</td>
<td>8.13 c</td>
<td>0.08 a</td>
<td>0.12 a</td>
</tr>
<tr>
<td></td>
<td>(1.54)</td>
<td>(0.80)</td>
<td>(0.05)</td>
<td>(0.03)</td>
</tr>
<tr>
<td>11</td>
<td>6.69 b</td>
<td>4.37 ab</td>
<td>0.19 b</td>
<td>0.22 b</td>
</tr>
<tr>
<td></td>
<td>(1.41)</td>
<td>(0.97)</td>
<td>(0.06)</td>
<td>(0.03)</td>
</tr>
<tr>
<td>14</td>
<td>z 2.78 a</td>
<td>y 5.71 b</td>
<td>0.30 c</td>
<td>0.31 c</td>
</tr>
<tr>
<td></td>
<td>(0.92)</td>
<td>(1.02)</td>
<td>(0.02)</td>
<td>(0.03)</td>
</tr>
</tbody>
</table>

* Average values of three ($n = 3$) independent determinations. Standard deviations are indicated in brackets.

** For each quality index and for each icing time, mean values preceded by a different letter (y, z) denote significant differences ($p < 0.05$) between both icing conditions. For each parameter and for each icing condition, mean values followed by different letters (a, b, c) denote significant differences ($p < 0.05$) as a result of icing time.
including no effect on the rancid odor development and no loss of polyunsaturated fatty acids.

The employment of SI as a slaughtering and chilling strategy was found useful in order to partially inhibit quality losses in gilthead seabream. Thus, a shelf life increase and a partial TMA and FFA formation inhibition could be obtained.

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