The effect of different marinating baths on sensory properties and shelf life parameters of cold marinated anchovies

(Engraulis encrasicolus, L.)

V. Šimat¹, T. Bogdanović², M. Bulić³

Summary
Synergistic effect of acid and salt content plays the most important role in the marinating process. Selection of suitable procedures for preparing raw fish material for marination, the marinating baths compositions and storage conditions, are carried out in order to achieve a longer shelf life, to maintain quality and achieve good sensory characteristics of marinated products. In this paper 22 samples of cold marinated anchovies containing marinating baths of different composition (different proportions of vine vinegar, alcoholic vinegar, salt and water) were investigated, in order to determine changes in sensory characteristics during maturation, as well as the shelf life parameters. For this purpose, pH values, water activity, NaCl content and sensory properties of marinated anchovy fillets, the content of volatile amines and the index of fat oxidation were determined. The fillets marinated in the marination bath prepared from 45% of water, 30% of vine vinegar, 25% of alcoholic vinegar and 7% of salt obtained the best sensory scores, a pH value of 3.09, 3.60% NaCl and water activity of 0.84. A weak accumulation of volatile amines during storage (<20mg TVB-N / 100g; <1mg TMA/100g) and statistically significant increase of the TBA index (6.41 to 7.36 malondialdehyde / kg) were observed during ripening, but the rancidity was not described as an intensive sensory property of the marinated fillets.

Key words: Engraulis encrasicolus, cold marinade, shelf-life, TVB-N, TBA

Introduction
Anchovy (Engraulis encrasicolus, L.) is a small pelagic fish and the only European representative of the Engraulidae family, thus an endemic species of the Mediterranean-Atlantic region. The anchovies are caught along the Mediterranean shores and in the Adriatic Sea all year round, but mainly within the spring-autumn period (Sinovčić, 2000). It represents a species of an important economic value in all parts of the Mediterranean. The average annual capture production of European anchovy from 2002 till 2008 in the Mediterranean and Black Sea was around 480000 tons (Anon., 2010). The Croatian catch of anchovies in the Adriatic Sea increased four times since 2003 and had reached 13200 tons in 2007 (Anon., 2009). Most of the catch is processed by salting, marinating or freezing and utilized for human consumption. Healthy living and Mediterranean kitchen encourage the consumption of fish and seafood products; especially lightly preserved products, such as cold marinades or carpaccio-like products.

The basic principle of cold marinating preservation is associated with the synergic activity of organic acids and salt from marinating bath to the changes in fish fillets. These products are made out of fresh, frozen or salted fish. Organic acids and salt are added to the fish to retard the microbiological and enzymatic activity, but also to change the taste and textural properties of the fish, resulting with a lightly preserved product with limited shelf life (Poligne and Collin-gan, 2000; Pons-Sanchez-Cascado et al., 2005; Yeannes and Casales, 2008; Olgunoğlu et al., 2009, Šimat, 2010). The microbiological activity of cold marinades is restricted by low pH values, low water activity and high NaCl content. However, in order to insure the continuity in production process and best sensory properties of the product acid type and concentration, and NaCl content must be carefully chosen. Numerous studies were conducted in search of right procedures and preparation of raw fish for marinating, compositions of marinating baths and cover brines of marinated products, adequate storage for these products, all with purpose to achieve a longer shelf life and preservation of

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**Materials and methods**

**Marinating process**

Anchovies (*Engraulis encrasicolus*) caught in January 2010 in Central Adriatic fishing region GSA 17 (GMU 37.2.1) were used for product preparation. The fish were placed in self-draining polystyrene boxes, packed in flake ice and delivered to the local factory where they were frozen using combined brine/air IQF freezing process (Tacore, Spain) and stored at -24(±1) °C for 6 months. Approximately 100 kg of anchovies were thawed and used in cold marinating process (Figure 1.). The size category of the fish was 42 individuals per kilogram. The process consisted of thawing and filleting of the anchovies, followed by rinsing of the fillets in rinsing tank filled with mild salt solution, and the preparation of the products. Fillets were placed in the plastic containers, marination baths made out of different types of vinegar and salt contents (Table 1.). The ratio between fish and marination bath was 1:1.5. During the entire production process as well as storage, fish fillets were completely immersed in the marination bath. Products numbered 18 through 22 from the table were brined in 24% salt solution before marination, so no additional salt was added into the marination bath. After 48 hours 11 products with best sensory properties were selected, and placed in the second marinating bath, consisted of the same ratio of acid and salt as the first one. These 11 products were stored at 4(±2) °C for 25 days.

**Sensory assessment**

Sensory assessment of the marinated anchovy fillets was carried out by a sensory panel of six trained and
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Proximate analysis

For proximate composition analysis 20 individual fish of both raw and thawed anchovies were sampled, gutted, filleted and homogenised using a laboratory blender (KINEMATICA Mikrotron MB 550, Switzerland). The proximate composition was determined from homogenates as water content (drying the samples at 105°C to constant weight), crude protein (Kjeldhal method, N × 6.25), crude fat (acid hydrolysis method) and crude ash (calcinations at temperatures ≤ 500°C) (AOAC, 2000). Proximate composition of marinated fillets was determined for the three products with best sensory score after 25 days of storage. Fillets were drained and homogenised for analysis. All analyses were done in triplicate and presented as percentage wet weight of anchovy fillet.

pH, NaCl and water activity analysis

The pH, NaCl and water activity were determined for raw material (fresh, thawed, rinsed, brined anchovies) and 11 marinated anchovy products after 2 and 25 days of storage. The pH was measured using digital pH meter (702 SET/MET Titrino, Metrohm), equipped with glass electrode, calibrated at 4 and 7. The electrode was dipped into the mixture of fish and distilled water (1:1), at ambient temperature (Kyrana and Loug-ovious 2002).

A method by Morh was used to determine salt content in the fillets.

Water activity was determined on sample homogenate using a meter Rotonic AG, HygroPalm AW1-set40, equipped with a calibrated digital probe with measurement accuracy ±0.005.

Determination of thiobarbituric acid index and volatile amine content

Thiobarbituric acid index and volatile amine content were determined for raw material (fresh, thawed, rinsed and brined) and marinated anchovy fillets from three products with best sensory scores at the end of marination process.

Thiobarbituric acid index (TBAi) was determined as previously described by Vyncke (1970) and Lemon (1975), using spectrophotometer (PRIM Advanced, Secomam, France). After extraction with trichloroacetic acid (100 g/L), antioxidant propyl gallate (1 g/L) and chelating agent ethylenediaminetetraacetic acid (1 g/L) were added into solution to lower the possibility of erroneously formed malondialdehyde or other TBA reactive substances during blending and filtering of the sample. Calibration curves were done using 1,1,3,3-tetraethoxy-propane (TEP). Results were expressed as mg malondialdehyde/kg muscle (mg MA/kg).

Total volatile base nitrogen (TVB-N) and trimethylamine nitrogen (TMA-N) were determined by direct distillation of fish extracts as previously described in Šimat et al. (2009) and expressed as mg TVB-N/ TMA-N per 100g of fish muscle.

Statistical analysis

Graphic figures and statistical evaluations (analysis of variance, least significance difference, correlations) of obtained data were done using Microsoft Office Excel 2007 package and software Statgraphics® Plus v. 5.1 Professional (Manugistics, Inc., Rockville, MD, USA).

Results and discussion

The limited shelf-life of the cold marinades and other lightly preserved products is changeable due to high post mortal pH of fish muscle, high content of free amino acids, the presence of trimethylamine oxide (TMAO), the presence of specific spoilage bacteria and the differences in chemical composition and condition of the specimens of wild fish populations (Dalgaard, 2000). The
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Table 2. Water activity (a_w), pH values and NaCl content of raw anchovy fillet used for the preparation of the products.

<table>
<thead>
<tr>
<th>Analyzed anchovy sample</th>
<th>Parameters determined</th>
<th>Proximate analysis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Određeni parametri</td>
<td>Kemijski sastav (%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Water content</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Udio vode</td>
</tr>
<tr>
<td>Fresh Svježi</td>
<td>a_w 0.994±(±0.001)</td>
<td>pH 6.16±(±0.01)</td>
</tr>
<tr>
<td>Thawed Odmrznuti</td>
<td>a_w 0.987±(±0.002)</td>
<td>pH 6.30±(±0.02)</td>
</tr>
<tr>
<td>Rinsed Ispirani</td>
<td>a_w 0.84±(±0.001)</td>
<td>pH 5.86±(±0.01)</td>
</tr>
<tr>
<td>Brined Salamureni</td>
<td>a_w 0.766±(±0.002)</td>
<td>pH 5.77±(±0.01)</td>
</tr>
</tbody>
</table>

* Values in the same column labelled with different letters are significantly different (p < 0.05).
* Vrijednosti u istoj koloni označene različitim slovom se statistički značajno razlikuju (p < 0.05).
ent marinating baths as described in Table 1. For products numbered from 1-16 rinsed anchovy fillets were used, while products from 17 to 22 were made with brined fillets. Two days after marination, the first sensory assessment was carried out and products with best sensory properties were selected for further investigation. Different composition of the marinating baths significantly affected the sensory properties of the products. Among the 22 products, 11 were selected for best sensory properties. Eight products (2, 3, 7, 9, 11, 13, 14, 16) made with rinsed anchovies and three products (17, 18, and 20) from brined anchovy fillets were chosen. All were put in a new marinating bath and stored at 4(±2) °C for 25 days. After 2 and 25 days of marinating remains around 3.85. Varlik et al. (2000) found that the pH value of the marinated products should range between 4.1 and 4.5. However, lower pH values of marinated fish were recorded in scientific literature. In the study by Poligne and Collignan (2000) the pH value of the marinated anchovies increased from 3.9 to 4.2 with storage time, but after 20 days of storage it remains constant until the end of storage. According to Dokuzlu (1997), shelf-life is ensured if the pH value of anchovy fillets after 2 and 25 days of storage.

Table 3. Water activity ($a_w$), pH value and NaCl content of selected marinated anchovy fillets after 2 and 25 days of storage.

<table>
<thead>
<tr>
<th>Sample number*</th>
<th>Determined parameters</th>
<th>Number of days in marinating bath</th>
<th>Broj dana u kupelji za mariniranje</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$a_w$</td>
<td>pH</td>
<td>NaCl (%)</td>
</tr>
<tr>
<td>2</td>
<td>0.853</td>
<td>0.805</td>
<td>3.53</td>
</tr>
<tr>
<td>3</td>
<td>0.854</td>
<td>0.803</td>
<td>3.60</td>
</tr>
<tr>
<td>7</td>
<td>0.856</td>
<td>0.806</td>
<td>3.57</td>
</tr>
<tr>
<td>9</td>
<td>0.828</td>
<td>0.809</td>
<td>3.44</td>
</tr>
<tr>
<td>11</td>
<td>0.801</td>
<td>0.811</td>
<td>3.49</td>
</tr>
<tr>
<td>13</td>
<td>0.846</td>
<td>0.840</td>
<td>3.47</td>
</tr>
<tr>
<td>14</td>
<td>0.811</td>
<td>0.843</td>
<td>3.49</td>
</tr>
<tr>
<td>16</td>
<td>0.802</td>
<td>0.847</td>
<td>3.88</td>
</tr>
<tr>
<td>17</td>
<td>0.812</td>
<td>0.856</td>
<td>3.45</td>
</tr>
<tr>
<td>18</td>
<td>0.851</td>
<td>0.849</td>
<td>3.48</td>
</tr>
<tr>
<td>20</td>
<td>0.818</td>
<td>0.848</td>
<td>3.45</td>
</tr>
</tbody>
</table>

* Samples descriptions are given in Table 1.
* Uzorci su opisani u Tablici 1.

The lowest water activity was recorded in samples with the highest NaCl content (11 and 14). Only these two samples had 10% of salt in the marinating baths. The lowest salt content was found in samples prepared with brined fillets (17, 18, 20) that had no additional salt in the marinating baths. It decreased even further with storage time. Generally, fillets in the products with a higher proportion of vinegar (3 and 9) absorbed more salt after marinating process. However, the NaCl content in all products decreased with storage, with the exception of the product 16 (55% of vinegar and 7% of NaCl). Using marinating baths without the addition of NaCl is not suitable for marination, regardless of pre-treatment of the raw material. Salt content of 4% is sufficient to insure marinated fish from spoiling (Fuselli et al. 1994). According to this criterion, the product 17, with salt content <4% during storage, was not satisfactory (Table 3.).

Although the pH value of all marinating baths used was 3.4-3.5, the effect of different sources of acid acted differently on the pH of fillets. The effectiveness of organic acids to achieve an antimicrobial effect in food varies depending on the degree of acid dissociation (Ray and Bhunia, 2008). With respect to the achieved pH of the fillets in all products, both types of vinegar (vine and alcohol) have shown to be a good choice of acid for fish marinate. After 25 days of storage at 4(±2) °C, pH value decreased in all samples and ranged from 3.0 to 3.3 (Table 3.). Samples marinated with alcohol vinegar had lower pH than others, which affected the sensory evaluation, and the acidity of these products was rated as more intensive sensory characteristics (Figure 2.). Varlik et al. (2000) found that the value of pH in the marinated products ranged from 3.9 to 4.2 with storage time, but after 20 days of storage it remains constant until the end of storage. According to Dokuzlu (1997), shelf-life is ensured if the pH value of anchovy fillets after marinating remains around 3.85.

The addition of vinegar affected the sensory perception of salt content in the fillets. Although the salt content in the marinated fillets ranged from 3.7 to 7.2%, due to the acidity of certain products salinity was not described as an expressive organoleptic parameter. Such influence of acid in product suppressing the salinity was recorded in the literature, but also a reverse effect, depending on the concentration of salt and acid in the product (Poligne and Collignan, 2000; Aubour and Ugliano, 2002). Breslin (1996) concluded that regardless of NaCl concentration in the solu-
After 25 days of marination the best sensory score was given to samples 13 and 14. The marination baths of these two products contained 30% of vine vinegar, 25% of alcoholic vinegar, 45% of water and they differed in the NaCl content (13-7%, 14-10%). The maturity of the fish was identified by taste, consistency and colour, and by shiny, glassy appearance of meat. The odour was rated as very good to excellent in all the samples (a slight smell of vinegar was noted) and rancidity was not present in any of the products. Samples 18 and 20 received the lowest scores for juiciness (3, neither good nor bad), while products 11 and 14, whose marinated baths contained 10% of NaCl were described as good. The best sensory scores on the 2nd day of marinating were given to samples 2 (60% of vinegar, 40% of water, 7% of NaCl) and 13 (30% of vinegar, 25% of vinegar, 45% of water, 7% of NaCl).

After 25 days in marinating baths, the aftertaste (product 9 - 60% of vinegar, 40% of water and they differed in the NaCl content (13-7%, 14-10%). The maturity of the fish was identified by taste, consistency and colour, and by shiny, glassy appearance of meat. The odour was rated as very good to excellent in all the samples (a slight smell of vinegar was noted) and rancidity was not present in any of the products. Samples 18 and 20 received the lowest scores for juiciness (3, neither good nor bad), while products 11 and 14, whose marinated baths contained 10% of NaCl were described as good. The best sensory scores on the 2nd day of marinating were given to samples 2 (60% of vinegar, 40% of water, 7% of NaCl) and 13 (30% of vinegar, 25% of vinegar, 45% of water, 7% of NaCl).
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The ripening process has shown a great importance in cold marinade production. After 25 days of slow ripening at 4°C the sensory properties of marinated products were improved and the maturation was finalized. These semi-products were than packed in oil with spices, and stored at refrigeration temperatures (from 0 to 8°C) to provide high quality life of the marinades for several weeks (Shenderyuk and Bykowski, 1990).

Volatile amine content, TBA index and proximate analysis of three marinated samples with best sensory scores were determined after 25 days of marinination (Table 4). Nutritive quality of the anchovy fillets was preserved by cold marinating process. The total volatile accumulation was weak; however the increase in TVB-N content during storage indicates the existence of an enzymatic maturation of the fillets. Both TVB-N and TMA content were under the limit of acceptance for seafood of 25 - 35 mg TVB-N/100g and 5 - 10mg TMA/100g (Anon., 2008).

Statistically significant increase of TBA index was recorded during maturation, but not over the limit of acceptance (Table 4). Olgunoğlu et al. (2009) found the amount of 4.24 ± 0.01 mg malondialdehyde / kg in the anchovy fillets after 7 months of storage of marinated anchovies (4.5% of vinegar, 10% of salt and 0.2% of citric acid) at 1 (±1) °C. Kilinc and Cakli (2004) have not recorded a significant increase in the amount of MA during the 22 days of marinating fillets of sardine (7% of acetic acid and 10% of NaCl). However, further storage of marinated sardine fillets in a mild solution (2% of acetic acid and 4% of NaCl) through 6 months at +4 °C showed an increase from 4.33 to 9.25 mg MA/kg of pasteurized, and from 4.47 to 9.49 mg MA/ kg in non-pasteurized samples. Despite the increasing amount during storage, sensory evaluation of marinated anchovies did not indicate rancidity as a negative attribute.

Before packing in oil and spices, marinated fillets are centrifuged, thus some water from the fillet gets removed. This slight increase of the pH (3.86) and the salt content (4.52) were observed after centrifugation, but no changes in water activity of the fillets.

Conclusions

The quality of raw material directly determines the quality of the final product and the technological process of cold marinating of the highest quality raw materials needed, given that it is a process without thermal treatment, dependent on the ripening process. Salt and acid ratios have an important role in determining the shelf-life of the product, as well as in defining the final sensory properties. The products received better sensory scores after 25 days of marinination. Marinating time (maturation) has
also a key role in defining the sensory properties of cold marinated anchovies. The samples containing vine vinegar had better sensory scores. However, the best sensory scores were given to sample 13, marinated in a bath with 45% of water, 30% of vine vinegar, 25% of alcoholic vinegar and 7% of NaCl, which corresponds to the NaCl content of 3.60% in the fillet, water activity of 0.840 and a pH value of the fillet 3.09 after marination process.

References


Šimat, V. (2010): Promjene parametara kvalitete u filetu hladno mariniranog inçuna (Engraulis encrasicolus). Prehrambeno-
Učinak naljeva različitih koncentracija octa i soli na senzorska svojstva i parametre kvalitete hladno mariniranih inćuna (Engraulis encrasicolus L.)

Sažetak
Sinerijski učinak kiseline i soli ima ključnu ulogu u procesu mariniranja. Odabir pogodnih postupaka pripreme sirove ribe za mariniranje, sastava kupelji za mariniranje, sastava naljeva mariniranog proizvoda i uvjeta skladištenja, provode se u svrhu postizanja duže trajnosti, očuvanja kvalitete i postizanja dobrih senzorskih svojstava mariniranih proizvoda. U radu je provedeno istraživanje na 22 uzorka hladno mariniranih inćuna koji su sadržavali naljeve različitog sastava (različiti omjeri vinskog octa, alkoholnog octa, soli i vode), s kako bi se, tijekom zrenja, istražile promjene senzorskih svojstava i čimbenika koji određuju rok trajnosti. U tu svrhu određena je pH vrijednost, aktivitet vode, udio NaCl i senzorska svojstva mariniranih fileta inćuna, kao i sadržaj hlapljivih amina i indeks oksidacije masti. Dobiveni rezultati ukazali su da je za postizanje najboljih senzorskih svojstava najprikladnija kupelj pripravljena od 45% vode, 30% vinskog octa, 25% alkoholnog octa i 7% soli, čime se postiže pH vrijednost mesca od 3,09, udio NaCl od 3,60% i aktivitet vode od 0,84. Zabilježena je slaba akumulacija hlapljivih amina tijekom skladištenja mariniranih proizvoda (<20 mg TVB-N /100g; <1mgTMA/100g) i statistički značajan porast TBA indeksa (6,41-7,36 malondialdehida/kg) tijekom zrenja, ali užeglost fileta nije bila opisana kao intenzivna svojstvo.

Ključne riječi: Engraulis encrasicolus, hladna marinada, rok trajanja, TVB-N, TBAi


Wirkung des Aufgusses von Essig- und Salzkonzentration auf sensorische Eigenschaften und Qualitätsparameter kaltmarinierter Anschovis (Engraulis encrasicolus L.)

Zusammenfassung
Die synergetische Wirkung von Säure und Essig hat die Schlüsselrolle im Marinadeprozess. Die Auswahl der richtigen Verfahrensvergänge bei Vorbereitung des rohen Fisches für das Marinieren, die Zusammensetzung des Marinadebades, die Zusammensetzung des Aufgusses des marinierenden Erzeugnisses und die Lagerungsbedingungen werden durchgeführt, um eine verlängerte Dauerhaftigkeit, Qualitätsschutz und Erreichung der guten sensorischen Eigenschaften der Marinadeerzeugnisse zu erzielen. In der Arbeit wurde die Forschung auf 22 Mustern der kalt marinierter Anschovis durchgeführt, die Aufgusse verschiedener Zusammensetzung enthielten (verschiedene Verhältnisse von Weinessig, Alkoholesig, Salz und Wasser), um Veränderungen der sensorischen Eigenschaften und Faktoren, die die Dauerhaftigkeit bestimmen, während der Reifezeit zu untersuchen. Zu diesem Zweck wurden pH-Wert, Wasserkonzentration, Anteil von NaCl und sensorische Eigenschaften der marinierter Anschovisfilets, Inhalt der verdunstbaren Amine und Index von Oxydationsfetten bestimmt. Die bekommten Resultate haben gezeigt, dass für die Erzielung der besten sensorischen Eigenschaften das Bad zümmengesetzt aus 45 % Wasser, 30 % Weinessig, 25 % Alkoholesig und 7 % Salz am geeignetsten ist, wobei der pH Wert des Fischfilisches von 3,09, Anteil des NaCl von 3,60 % und Wasserkonzentration von 0,84 erreicht werden. Es wurde eine schwache Akkumulation von verdunstbaren Aminen während der Lagerungszeit der marinierenden Erzeugnisse beobachtet (<20 mg TVB-N/100g; <1mgTMA/100g) und der bedeutende Aufstieg des TBA Indexes (6,41-7,36 Malondialdehida/kg) während der Reifezeit, aber die Dauer der Filets wurde nicht als intensive Eigenschaft beschrieben.

Schlüsselwörter: Engraulis encrasicolus, kalte Marinade, Dauerhaftigkeit, TVB-N, TBAi

Effetto di aggiunta di differenti concentrazioni d’aceto e sale sulle caratteristiche e i parametri di qualità di acciuge marinate a freddo (Engraulis encrasicolus L.)

Sommario
L’effetto sinergico di acido e di sale fa un ruolo chiave nel processo di marinata. La scelta del procedimento di preparazione del pesce crude destinato alla marinata, la composizione di marinata e delle condizioni di immagazzinamento si esibiscono con lo scopo di conseguire una durata più lunga, una qualità mantenuta e delle buone caratteristiche sensoriche di prodotti marinati. Quest’articolo presenta anche una ricerca fatta su 22 campioni di acciuge marinate a freddo, le cui marinate avevano contenuti diversi (percentuali differenti di aceto di vino, aceto alcolico, sale e acqua), per esaminare i cambiamenti di caratteristiche sensoriche e dei fattori che determinano la data di scadenza, durante la maturazione. Perciò si dovevano determinare il valore pH, attività dell’acqua, la percentuale di NaCl e le caratteristiche sensoriche di filetti di acciuge marinate, e anche il contenuto delle amine evaporanti e l’indice d’ossidazioni di grassi. I risultati ottenuti dichiarano che per ottenere le migliori caratteristiche sensoriche bisogna preparare la marinata che contiene il 45% d’acqua, il 30% di aceto di vino, il 25% di aceto alcolico e il 7% di sale. Così il valore della carne diventa il 3,09, la percentuale di NaCl il 3,60% e l’attività dell’acqua lo 0,84. Si nota una debole accumulazione delle amine evaporanti durante l’immagazzinamento dei prodotti marinati (< 20 mg TVB-N/100g; < 1 mg TMA/100g) e una statisticamente notevole aumento dell’indice TVB (6,41-7,36 Malondialdehida/kg) durante la maturazione, però i danno ossidativo di filetti non era descritto come una caratteristica intensa.

Parole chiave: Engraulis encrasicolus, marinata a freddo, data di scadenza, TVB-N, TBAi

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