E-ISSN: 2073-3720
https://doi.org/10.33997/j.afs.2025.38.2.006

SHORT COMMUNICATION

Influence of Fat Content on the Salt-Ripening Process of the Peruvian Anchovy (*Engraulis ringens*)

VERÓNICA VALVERDE-VERA[1], ESTRELLITA ROJAS DE-LOS-SANTOS[1], AARÓN MONDRAGÓN-MARTÍNEZ[2], JESSICA VELA[1], FABIOLA OLIVARES[3], MIGUEL ALBRECHT-RUIZ[4,*]

[1]CITE Pesquero Callao, Curados, Callao, Peru
[2]Natural Environment S.A.C. Lima, Lima, Peru
[3]Facultad de Pesquería, Universidad Agraria de la Molina, Lima, Peru
[4]Dirección de Investigación DIDITT, Instituto Tecnológico de la Producción, Callao, Peru

*E-mail: valbrecht@itp.gob.pe | Received: 06/05/2024; Accepted: 27/05/2025

Abstract

The study evaluated the influence of fat content on the salting ripening process of Peruvian anchovy (*Engraulis ringens*) at an industrial scale. Two batches of two tonnes each of *E. ringens* were processed, with heads and viscera removed before placing the fish in barrels with salt for ripening. The first batch contained 9.0 % fat, and the second 6.8 %. Sensory qualities were evaluated and the documented ripening times revealed no significant differences between batches; however, the higher-fat batch exhibited a slower decrease in water activity (aw) and reached sensory ripe slightly later than the batch with lower fat content. Physico-chemical analyses within each batch showed average increases in the proteolysis index (PI), total volatile basic nitrogen (TVB-N), and trimethylamine (TMA), but no significant differences between batches. The high variability inherent to industrial-scale processing limited the establishment of a direct correlation with fat content. Regarding amino acid composition, the initial histidine content in raw fillets was 2.1 g.100 g^{-1}, representing 81 % of free amino acids; however, during salting, most histidine diffused into the brine, leaving only 0.3 g.100 g^{-1} in the ripened product. Ripening led to an increase in free amino acids, particularly leucine, lysine, and glutamic acid, while the total amino acid content in the muscle decreased due to osmotic processes, salt-induced protein solubilisation, and proteolytic activity. Histamine levels in both batches remained below 50 mg.kg^{-1} at the end of ripening, complying with food safety standards.

Keywords: anchoveta ripening, amino acids, salted anchovy, proteolysis index, histamines, food safety

Introduction

The traditional salt-ripening process of European anchovies (*Engraulis encrasicolus*) in Mediterranean countries yields salt-ripened fillets known as "anchoa". However, overfishing and climate change have led to a decline in European anchovy catches, now representing only 6 % of the total anchovy catch. Consequently, other anchovy species in South America, such as Argentine anchoita (*E. anchoita*) and Peruvian anchovy or anchoveta (*E. ringens*), are being utilised in Europe (Ministerio de Agricultura y Pesca, Alimentación y Medio Ambiente – MAGRAMA, 2017). According to the Observatory of Economic Complexity (OEC), Peru exported salted/brined (not dried or smoked) anchoveta worth USD1.5 million in 2022, representing 1.74 % of the global total (https://oec.world/en/profile/hs/saltedbrined-anchovies-not-driedsmoked).

Anchoveta is the most harvested fish species globally, with an average annual yield of 4.249 million metric tonnes (Mood and Brooke, 2024). In 2022, Peru exported approximately 80,000 MT of salt-cured anchoveta for direct human consumption (Ministerio de la Producción - PRODUCE, 2022). It is marketed in various forms, including anchovies preserved in barrels with salt, salt-ripened fillets packaged in vacuum bags, and semi-preserved in glass or metal containers. (Instituto Nacional de Defensa de la Competencia y de la Protección de la Propiedad Intelectual- INDECOPI, 2013).

Variations in the preparation of salt-ripened anchovies, along with differences in proximate composition and raw material quality, significantly influence the physico-chemical and sensory characteristics (i.e. appearance, taste, smell) of the final product (Czerner, 2011). The salt-ripened anchoveta is distinguished by its darker colour and more intense flavour compared to other anchovies, possibly due to its high iron content in the dark muscle and a greater concentration of n-3 fatty acids present in its muscle (Albrecht-Ruiz and Salas, 2015).

During the preparation process, fresh anchovies are submerged in saturated brine. They are then partially gutted and arranged in barrels, alternating layers of fish with layers of salt until the barrel is filled. Finally, pressure is applied to the product, and saturated brine is added to expel any trapped air (De la Torre Boronat and Soler Segón, 1984). The barrels are subsequently stored for approximately 4 to 6 months, with saturated brine being added as necessary during this period.

The ripening of salted anchovies is a result of the prolonged action of muscular and digestive enzymes on the fish meat immersed in salt, potentially facilitated by the participation of halophilic microbes (Hernández-Herrero et al., 1999a; Hall, 2011; Czerner and Yeannes, 2014). This process involves complex physico-chemical and biochemical transformations that give rise to sensory characteristics typical of the product – such as flavour, odour, colour and texture. These transformations include myofibrillar protein hydrolysis (Hernández-Herrero et al., 2000; Czerner, 2011), continual increases in total volatile basic nitrogen (TVB-N) (Hernández-Herrero et al., 1999b), and total ester index (Filsinger et al., 1982), as well as a reduction in polyunsaturated fatty acids of the n-3 series (Czerner et al., 2015).

In Peru, anchoveta fishing for direct human consumption takes place throughout the year, with variations in crude fat content attributed to its spawning periods (Lam, 1968; Salas-Maldonado et al., 2002). Consequently, the ripening times of salted anchovies may be affected by their fat content under the skin, which can serve as a barrier to salt penetration (Czerner and Yeannes, 2013; Astruc et al., 2022). In this context, the objective of this study is to investigate the relationship between fat content and ripening time of anchovies, while also assessing the sensory characteristics and physico-chemical changes during the salt-ripening process.

Materials and Methods

Ethical approval

This study did not involve live animals or any form of experimental animal manipulation. The raw material used (anchoveta, *Engraulis ringens*) was sourced from a commercial purse seine fishery regulated by Peruvian authorities. As such, no animal ethics approval was required.

Raw material

Anchoveta were fished in FAO Fishing Area 87 (09°04'S, 78°36'W), off the coast of Chimbote port, Peru. The first catch, in March 2017, had a fat content of 9.0 % and was used to prepare batch 1, while the second catch, in May 2018, had a fat content of 6.8 % and was used to prepare batch 2. Each catch comprised approximately two tonnes of anchovies, which were kept on ice (2 ± 1 °C) until processing.

Fish processing

The anchovies were manually beheaded, and the ventral area was removed (headed and gutted), leaving the body clean and intact. Care was taken to retain part of the viscera to enhance the ripening process. (Czerner et al., 2011). Subsequently, the fish were immersed in saturated brine for approximately 16 to 24 h at room temperature. Following this, the fish were layered in barrels with salt at a ratio of 5:1 (w:w), with each barrel containing approximately 150–200 kg of fish. These barrels were then stored until the anchovies reached an over-ripened state, typically after 28 weeks. Throughout the study, the average room temperature was maintained at 22.9 ± 3.1 °C. For each batch, 4 barrels (1 ton) were processed, with two barrels undergoing ripening in different processing plants.

Sample preparation

Periodically, 3 kg of salted anchoveta were extracted from the barrels at each processing plant and sent for analysis. Upon arrival at the laboratory, they were immersed in saturated brine at 80 °C for 2 sec to facilitate skin removal. The backbone was then removed to obtain fillets, which were subsequently washed in cold saturated brine and dried on the outside using absorbent paper towels. These fillets were homogenised in a grinder to produce a paste suitable for physico-chemical tests.

Sensory quality evaluation

A scale previously reported for *E. anchoita* (Filsinger et al., 1982) was employed in this study to evaluate the following five sensory qualities: flavour (disregarding salt), flesh colour, odour, flesh consistency, flesh adherence to the backbone (Table 1). The final score was determined by summing the scores obtained for each evaluated attribute. Six trained panellists conducted the evaluations. The product was considered "ripened" when the score reached values of 30 ± 2.

Physico-chemical tests

Water activity (aw) was directly measured in the sample using an AQUALAB® PRE-Decagon water activity meter, employing the chilled-mirror dew point technique (Nordic Committee on Food Analysis – NMKL, 2001).

Table 1. Sensory characteristics assessed in *Engraulis ringens* fillets during the salt-ripening process. The scale employed was adapted from the one utilised by Filsinger et al. (1982) for *E. anchoita*.

Score	0	2	4	6	8
Flavour (disregarding salt)	Raw fish	Neutral	Slightly ham-like	Ham-like cured meat	Rancid off-flavours
Flesh colour	Natural fresh fish	Natural around borders, deep red in the middle, pink in between	Light pinkish meat, deep red or pink in the middle	Uniformity in the pink tone distribution	Dark red, black, red blots and/or black dots
Odour	Fresh fish	Neutral (smells like brine)	Smooth agreeable odour to volatile esters	Smells of agreeable volatile esters, "Characteristics anchovy odour"	Rancid, acid, ammoniacal or Sulphur us off-odours
Flesh consistency	High elasticity, damp	Less elasticity, less damp	Slight elasticity, more compact, does not feel damp.	No elasticity, firm and resistant to finger pressure	Flimsy
Flesh adherence to backbone	Very adherent, does not separate	Very adherent, does not separate easily	Adherent, it separates (incomplete filleting)	Very little adherence, it separates neatly (adequate filleting)	Flesh gets torn in the filleting process

Proximate composition: Moisture was determined by oven drying at 101 °C using a Memmert UE 400 (Germany) oven until constant weight (Bradley, 2010). Ash content was determined in a muffle furnace at 500 °C (48000 Barnstead/Thermolyne, USA) until constant weight (Marshall, 2010). Crude protein was determined by the Kjeldahl method using a Kjeldatherm/Turbosog/Vapodest system (C. Gerhardt GmbH & Co. KG, Germany) with a total nitrogen (TN) to protein conversion factor of 6.25 (Chang, 2010). Crude fat was obtained using hexane as an extraction solvent with a Soxtherm® equipment (C. Gerhardt GmbH & Co. KG, Germany) (Min and Ellefson, 2010).

Total volatile nitrogenous bases (TVB-N) were quantified by steam distillation of sample extracts obtained in 6 % perchloric acid, following the methodology recommended by the Official Journal of the European Communities (1995), utilising a Vapodest® system (Gerhardt GmbH & Co. KG, Germany). Trimethylamine (TMA) was measured using the microdiffusion method in a Conway unit (Clancy et al., 1995), and its values were expressed as mg N.100 g^{-1} of muscle.

Proteolysis index was calculated as the ratio of soluble nitrogen (SN) to total nitrogen (TN). To determine SN, 5 g of the sample was homogenised with 50 mL of 5 % trichloroacetic acid, and nitrogen content was quantified using the Kjeldahl method.

Amino acids were quantified in freeze-dried skinless fillets of fresh and salt-ripened anchoveta at 2, and 24 weeks of ripening, following standard methodologies (AOAC, 2006) for tryptophan (AOAC 988.15), amino acids (AOAC 982.30), cystine and methionine (AOAC 994.12), and free amino acid profile (AOAC 999.13).

To determine the total esterification index (TEI), 2 g of the sample were boiled in 10 mL of 0.5 N ethanolic KOH under reflux for 60 min. Afterward, the solution was titrated with 0.5 N HCl, using 100 µL of 1 % phenolphthalein as indicator. A blank was prepared using the same procedure. The results were expressed in grams of KOH.100 g^{-1} of dry sample (Filsinger et al., 1982).

Histamine was quantified via dansyl chloride derivatisation. A sample (~1 g) was homogenised with 10 mL of 5 % TCA, centrifuged, and filtered. The supernatant (100 µL) was mixed with 0.25 M sodium bicarbonate (400 µL), 0.5 % dansyl chloride in acetone (200 µL), and acetone (300 µL), incubated at 60 °C for 60 min, cooled, and filtered (0.45 µm). HPLC analysis used a C18 column, methanol:water (80:20) as the mobile phase, 30 °C, 1.0 mL m^{-1} in flow rate, and 254 nm detection (Norma Chilena Oficial NCh 2637.Of 2001).

Statistical analysis

Proximate composition, amino acid content, and histamine were analysed in duplicate, while the other physico-chemical assays were conducted in triplicate or quadruplicate. Differences in mean values between batches were evaluated using the U-Mann Whitney test, while the Friedman test of related measures was employed to assess the ripening process. Statistical analysis was conducted using SPSS 29.0.0 software.

Results and Discussion

Changes in sensory quality

Peruvian anchovy does indeed exhibit a darker hue and a stronger flavour profile that distinguishes it from other species within the *Engraulidae* family. However,

despite these sensory differences, its ripening process bears similarities to that of *E. anchoita*.

The ripened anchovy fillets are characterised by their ease of backbone detachment, a light pink colour with beige hues at the edges and a central red line. They are firm to the touch, emit volatile esters in aroma, and possess a taste reminiscent of cured-ripened meat (Filsinger, 1987). These attributes closely resemble those of *E. anchoita* (Table 1). In contrast, over-ripened anchoveta exhibits distinct characteristics, including the absence of ammoniac or sulphurous odours, a flimsy texture, slight moisture, lack of adherence, and a cracked and pasty consistency.

Throughout the study, dark spots were observed on the inner surface of the fillets, seemingly related to incomplete evisceration and cutting techniques. Additionally, batches with higher fat content were perceived to have more appealing sensory characteristics by the panellists, although this hedonic criterion was not quantitatively measured.

Ripening times did not exhibit significant differences between batches. However, batch 1, with the highest fat content, displayed a longer ripening time, reaching 30 points at week 16, compared to batch 2, which achieved the same value at approximately week 13 (Fig. 1). Despite the slight disparity in crude fat content

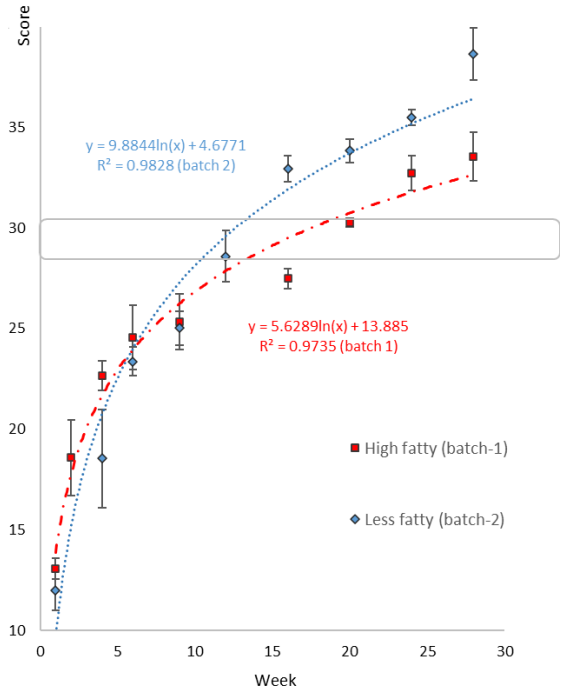

Fig. 1. Changes in the sensory evaluation score of Peruvian anchovy during salt ripening. The product was considered 'ripe' when the score reached 30 ± 2.

between the raw materials (6.8 % vs. 9.0 %), it remains uncertain whether this influenced the ripening times. Consideration of fat content is crucial in the ripening of salted anchoveta, as this species can have fat levels ranging from 0.8 % to 13.3 %, depending on age and spawning period (Ayala-Galdós et al., 2002). A study involving a larger number of batches with greater differences in fat content could provide a clearer understanding of the effects of fat on ripening time. This ripening time could be important for the production time for commercial orders.

Physico-chemical variations

During the initial stages of the salting process, osmotic dehydration rapidly reduced the water activity (aw) values in the fillets. There is a rapid decrease during the first days of salting until equilibrium is reached (Table 2).

The batch with the highest fat content reached osmotic equilibrium in week 5 (aw = 0.74), likely due to the fat content acting as a barrier for salt penetration. The slightly lower aw values in this batch may be attributed to the reduced availability of free water caused by the higher fat content, remaining consistent throughout the study. Conversely, the batch with the lowest fat content reached osmotic equilibrium in week 2 (aw = 0.76). From week 6, a slight increase in the aw values were observed (aw = 0.77), reaching values of 0.80 by week 28. This increase may indicate over ripeness, although additional data are required to confirm this. The initial stabilisation times differed from those reported for *E. encrasicolus*, where stabilisation occurred at week 10 (Ababouch and El Marrakchi, 2009). It is important to note that aw values at equilibrium should ideally be around 0.76, corresponding to saturated solutions of NaCl (Neves-Martins et al., 2023). Maintaining control of water activity (aw) is crucial during the ripening process, as values greater than 0.80 can lead to bacterial contamination, compromising the quality, safety, and sensory characteristics of the product.

Proximate composition analysis was conducted on both the raw material and the homogenised fillets (Table 2).

The analysed fillets exhibited a lower fat content compared to whole fresh anchovies, attributed to the removal of skin and subcutaneous fat. Minor variations in protein and fat content may be attributed to ripening-associated reactions that increase soluble compounds.

The proteolysis index (PI) increased during the ripening process in both batches of *E. ringens* (Table 3), indicating ongoing proteolysis reactions. Although samples with lower fat content (batch 2) exhibited slightly higher PI values at the same time intervals compared to samples with higher fat content, these differences were not significant. PI values started around 7 at week 1 and gradually increased during ripening, reaching values of approximately 13–16 at week 24. These values were lower compared to other

Table 2. Proximate composition (%) and water activity (aw) of fresh-whole anchoveta *Engraulis ringens* and its skinless fillets during ripening in salting.

Week	Protein (%)		Moisture (%)		Ash (%)		Crude fat (%)		Water activity (aw)	
	Batch 1	Batch 2	Batch 1	Batch 2	Batch 1	Batch 2	Batch 1	Batch 2	Batch 1	Batch 2
0*	16.49	16.51	70.64	73.67	3.34	3.2	9.07	6.4	1.00 ± 0.00^{a}	0.99 ± 0.00^{a}
1	27.4 ± 0.3	25.3 ± 0.0	51.9 ± 0.4	53.0 ± 0.0	15.7 ± 0.4	18.3 ± 0.3	3.3 ± 0.2	1.5 ± 0.0	0.80 ± 0.01^{b}	0.76 ± 0.00^{b}
4	26.8 ± 0.5	26.1 ± 0.2	55.3 ± 1.2	52.0 ± 0.7	14.1 ± 1.1	17.2 ± 0.8	3.7 ± 0.5	1.8 ± 0.3	0.79 ± 0.02^{c}	0.76 ± 0.01^{c}
6	26.7 ± 0.5	27.9 ± 1.4	51.1 ± 0.2	50.9 ± 0.8	18.1 ± 0.3	17.6 ± 0.4	3.1 ± 0.1	2.2 ± 0.1	0.74 ± 0.01^{d}	0.76 ± 0.01^{d}
8		28.4 ± 0.9		52.7 ± 1.5		17.7 ± 0.4		2.4 ± 0.2	0.73 ± 0.00^{e}	0.76 ± 0.00^{d}
16	27.1 ± 0.7	24.2 ± 0.4	50.4 ± 0.3	53.0 ± 0.9	17.8 ± 0.1	17.5 ± 0.3	3.5 ± 0.2	2.5 ± 0.1	0.73 ± 0.00^{f}	0.77 ± 0.02^{d}
20	27.6 ± 1.9	24.4 ± 0.2	49.8 ± 0.8	55.9 ± 0.9	17.6 ± 0.4	16.2 ± 0.3	2.8 ± 0.2	2.2 ± 0.1	0.72 ± 0.01^{g}	0.78 ± 0.01^{d}
28	28.4 ± 0.2	24.6 ± 0.7	50.7 ± 0.8		17.2 ± 0.8		2.4 ± 0.3	2.2 ± 0.2	0.73 ± 0.01^{h}	0.78 ± 0.02^{d}

Values are mean ± SD. Different lower-case letters (a, b, c, d, e, f, g, h) in the same col indicate significant differences ($P < 0.05$).
0*: The proximate composition was measured in fresh, gutted, and headless fish (raw material). The water activity (AW) measurement was performed on fillets obtained from this raw material.

Table 3. Changes in the proteolysis index (PI), total volatile base nitrogen (mg TVB-N.100 g^{-1}), trimethylamine nitrogen (mg N-TMA.100 g^{-1}), and total ester index (g KOH.100 g^{-1}) in skinless fillets of anchoveta *Engraulis ringens* during ripening in salting.

Week	Proteolysis index (PI)		Total volatile base nitrogen mg TVB-N.100 g^{-1}		Trimethylamine nitrogen mg N-TMA.100 g^{-1}		Total ester index g KOH.100 g^{-1}	
	Batch 1	Batch 2	Batch 1	Batch 2	Batch 1	Batch 2	Batch 1	Batch 2
1	8.03 ± 0.45^{a}	7.09 ± 0.05^{a}	31.87 ± 3.70^{a}	22.83 ± 0.00^{a}	1.22 ± 0.16^{a}	1.16 ± 0.00^{a}	17.96 ± 1.50^{a}	26.97 ± 3.53^{a}
4	10.33 ± 1.18^{b}	9.09 ± 0.42^{b}	31.43 ± 1.41^{a}	25.21 ± 1.71^{a}	2.45 ± 0.84^{b}	1.70 ± 0.33^{ab}	20.31 ± 1.13^{b}	25.29 ± 0.86^{a}
6	9.73 ± 0.42^{bc}	11.86 ± 1.99^{bc}	37.02 ± 2.01^{b}	32.26 ± 2.66^{c}	2.82 ± 0.46^{b}	1.78 ± 0.15^{ab}	14.70 ± 0.35^{c}	24.90 ± 6.23^{ab}
9	11.59 ± 1.83^{bd}	10.49 ± 0.63^{bc}	36.26 ± 1.78^{b}	33.77 ± 0.53^{c}	4.18 ± 0.42^{d}	2.24 ± 0.32^{b}	17.55 ± 3.60^{abc}	19.06 ± 1.22^{bc}
16	10.33 ± 2.98^{abcd}	11.25 ± 1.76^{bc}	41.65 ± 7.27^{bc}	39.83 ± 2.54^{d}	3.40 ± 0.98^{bc}	1.84 ± 0.14^{ab}	22.13 ± 3.22^{abd}	19.11 ± 0.62^{c}
20	12.71 ± 2.29^{e}	16.59 ± 0.95^{e}	42.00 ± 7.94^{bc}	55.90 ± 2.49^{f}	3.66 ± 1.10^{cd}	2.25 ± 0.35^{b}	22.52 ± 2.84^{cd}	19.45 ± 2.09^{bc}
24	13.02 ± 2.87^{bcde}	15.69 ± 2.13$^{d, e}$	51.90 ± 4.18^{d}	48.85 ± 4.23^{e}	4.04 ± 1.26^{d}	1.89 ± 0.16^{ab}	19.46 ± 1.82^{ab}	23.15 ± 1.22^{b}
28	13.54 ± 2.17^{e}	14.65 ± 1.47^{d}	50.51 ± 6.91^{d}	42.54 ± 2.54^{de}	3.20 ± 0.65^{c}	1.86 ± 0.28^{ab}	20.03 ± 2.01^{abd}	17.70 ± 0.22^{c}

Values are mean ± SD. Different lower-case letters (a, b, c, d, e) in the same col indicate significant differences ($P < 0.05$).

ripened anchovies, such as those from *E. anchoita* and *E. encrasicolus*, which reached PI values around 25 and 20 at weeks 29 and 9, respectively (Hernández-Herrero et al., 1999a; Czerner, 2011).

Total volatile basic nitrogen (TVB-N) serves as an indicator of fish freshness, measuring the byproducts of protein breakdown, particularly ammonium and trimethylamine (TMA). However, when salting anchovies for ripening, the TVB-N value may not solely reflect freshness; rather, it can also signify endogenous enzymatic activity and the presence of halophilic microorganisms during extended salting (Hernández-Herrero et al., 1999b). In the ripening process of *E. ringens*, a progressive increase in TVB-N was observed, reaching approximately 40 mg TVB-N.100 g^{-1}, in mature samples (Table 3). Interestingly, no significant differences were noted between batches. These observations contrast with findings for *E. encrasicolus*, where a value of 35 mg TVB-N. 100 g^{-1} was

associated with maturity (Hernández-Herrero et al., 1999b).

Trimethylamine (TMA), a component of total volatile bases, exhibited varied and non-constant increases in *E. ringens*, with differences observed between batches. Levels ranged from 1.2 to 4.2 mg N-TMA. 100 g^{-1} in the first batch and from 1.1 to 2.3 mg N-TMA. 100 g^{-1} in the second batch (Table 3). In contrast, in *E. encrasicolus*, TMA demonstrated a consistent increase during the initial 8 weeks of ripening, starting at 1.2 and peaking at 6.3 mg N-TMA.100 g^{-1} (Ababouch and El Marrakchi, 2009). Subsequently, these values declined due to dissolution in brine.

The total ester index (TEI) serves as an indirect indicator of free fatty acid release through lipolysis, a key ripening process influencing the sensory attributes of the final product (Gandemer, 2009). During prolonged salting processes, endogenous or microbial lipases release free fatty acids, which react with alcohols to form esters—compounds that contribute to the distinctive aromas and flavours of ripened products. However, salt promotes lipid and protein oxidation, an inevitable process requiring careful control, as excess free fatty acids and oxygen can lead to rancid or unpleasant flavours, emphasising the need for temperature regulation during ripening (Andersen et al., 2007; Sampels, 2015). During brine ripening of *E. ringens*, TEI values ranged from 15 to 27 g KOH.100 g^{-1}, with no clear pattern related to ripening times or significant differences between batches throughout the study period (Table 3). In contrast, TEI values in *E. anchoita* steadily increased between weeks 15 and 40, ranging from approximately 4 to 8 g KOH.100 g^{-1} (Filsinger et al., 1982), likely due to the shorter ripening time and distinct characteristics of *E. anchoita*.

The high variability in the results of PI, TVB-N, TMA and TEI observed in the present work could be attributed to several factors: 1) The complexity of the sampling process in barrels that contained approximately 400 kg of anchovy and saturated brine. 2) The processing conditions of 4 tonnes of anchovies, matured in two different factories; each with its own environmental and working conditions. Considering that pressure, temperature and ventilation (oxygen) can influence ripening and its characteristics (Filsinger, 1987; Karaçam et al., 2002; Rojas-De-Los-Santos et al., 2018) and 3) The physico-chemical characteristics of the samples, including proximal composition, endogenous proteolytic activity and the concentration of proteases (mainly visceral ones) in the brine (Yatsunami and Takenaka, 2000; Siringan et al., 2006). All of these variables can influence proteolysis and lipolysis reactions.

The free amino acid content in anchovy fillets during ripening results from both osmotic processes due to immersion in brine and endogenous proteolytic activity. Histidine (His) emerges as the predominant amino acid in *E. ringens*; fresh fillets contain 3.9 g.100 g^{-1} of total His (dry weight), with 53 % of this existing as free His (2.1 g.100 g^{-1}). Free His constitutes 81 % of the total free amino acids (Tables 4a, 4b). During the initial 2 weeks in the barrel, a rapid decrease in free His occurs due to osmotic diffusion into the brine and the pressure exerted on the fillets. Subsequently, a slight decrease is observed, likely attributed to ongoing proteolytic processes Free histidine present in muscle and brine may be vulnerable to endogenous and microbial histidine decarboxylase activity, potentially resulting in histamine formation and associated toxicity risks. Therefore, monitoring this parameter is crucial to ensure product quality. European legislation recommends histamine levels below 200 ppm for salt-ripened anchovies. In our study, histamine levels were maintained below 40 ppm.

Except for His, the rise in other free amino acids during ripening underscores the impact of endogenous proteolytic activity, directly contributing to the product's flavour (Table 4a). Notably, leucine, lysine, and glutamic acid exhibited the greatest increases among the free amino acids, a trend consistent with findings in other pelagic fish species (Stefánsson and Guðmundsdóttir, 1995; Mendes et al., 1999).

In the case of *E. ringens*, the ratio of (∑ lys, arg) / (∑ asp, glu, pro, ser, thr) decreased from 1.5 to 0.6, whereas in ripening herring (*Clupea harengus*), a similar ratio decreased from 4.0 to 0.5 over a comparable period (Stefánsson & Guðmundsdóttir). The total amino acid content in fresh anchovy fillets was approximately 73 g.100 g^{-1} (Table 4b). However, during the initial weeks of ripening, this value decreased to around 60 g.100 g^{-1}. These results are consistent with those reported for anchovies by Kari et al. (2022). Although analyses were performed in duplicate only, the observed trend indicates a measurable reduction. Due to the limited number of replicates, no statistical analysis was conducted for the amino acid data. This decline is primarily attributed to the release of peptides and amino acids induced by osmotic effects and the solubilisation of certain proteins by the brine. Additionally, proteolytic processes occurring throughout ripening contribute to the liberation of peptides into the medium. Studies on *E. encrasicolus* ripened in brine have indicated endogenous proteolytic activity, leading to the hydrolysis of the myosin heavy chain (Hernández-Herrero et al., 2000), suggesting similar processes might occur in *E. ringens*. While proteolytic processes occur during ripening, they did not significantly impact the total sum of amino acids.

Conclusion

This study demonstrated that fat content influenced the rate of water activity decline but did not significantly affect the sensory attributes or key physico-chemical parameters during industrial-scale ripening of *E. ringens*. Proteolysis, nitrogenous compound formation, and amino acid diffusion followed expected trends, with histamine levels

Table 4a. Free amino acid content in anchoveta: raw fillets and fillets at weeks 3 and 28 of salt ripening. Results are expressed as mg amino acid.100 g^{-1} of dry weight.

Free amino acids	Batch1			Batch 2		
	Raw fillet	Week 3	Week 28	Raw fillet	Week 3	Week 28
Ile	10 ± 0.00	56.67 ± 0.02	203.3 ± 0.06	10.0 ± 0.00	45.0 ± 0.01	250.0 ± 0.05
Leu	16.67 ± 0.01	63.33 ± 0.02	393.33 ± 0.10	23.33 ± 0.01	100.0 ± 0.01	370.0 ± 0.06
Lys	106.7 ± 0.01	103.33 ± 0.02	370.0 ± 0.12	113.33 ± 0.01	85.0 ± 0.01	333.33 ± 0.03
Met	10.0 ± 0.00	16.67 ± 0.01	110.0 ± 0.05	13.33 ± 0.01	25.0 ± 0.01	103.33 ± 0.01
Phe	16.67 ± 0.01	40.0 ± 0.00	206.67 ± 0.01	20.0 ± 0.00	55.0 ± 0.01	203.33 ± 0.04
Thr	26.67 ± 0.01	36.67 ± 0.01	150.0 ± 0.01	30.0 ± 0.00	35.0 ± 0.01	153.33 ± 0.03
Trp	NR	NR	NR	NR	NR	NR
Val	23.33 ± 0.01	46.67 ± 0.01	206.67 ± 0.05	26.67 ± 0.01	45.0 ± 0.01	190.0 ± 0.02
His	2090.0 ± 0.16	636.67 ± 0.08	310.0 ± 0.02	2053.33 ± 0.12	355.0 ± 0.04	383.33 ± 0.03
Arg	10.0 ± 0.00	80.0 ± 0.01	216.67 ± 0.05	10.0 ± 0.00	80.0 ± 0.00	270.0 ± 0.03
Ala	116.67 ± 0.01	86.67 ± 0.02	276.67 ± 0.07	120.0 ± 0.00	75.0 ± 0.01	266.67 ± 0.03
Tyr	30.0 ± 0.00	30.0 ± 0.00	120.0 ± 0.02	30.0 ± 0.00	40.0 ± 0.00	136.67 ± 0.02
Asp	< 0.01	36.67 ± 0.01	240.0 ± 0.07	< 0.01	30.0 ± 0.00	243.33 ± 0.03
Cys-Cys	NR	NR	NR	NR	NR	NR
Glu	6.67 ± 0.01	60.0 ± 0.01	346.67 ± 0.08	10.0 ± 0.00	65.0 ± 0.01	306.67 ± 0.04
Gly	36.67 ± 0.01	26.67 ± 0.01	80.0 ± 0.03	36.67 ± 0.01	20.0 ± 0.00	76.67 ± 0.01
Pro	20.0 ± 0.00	26.67 ± 0.01	83.33 ± 0.01	20.0 ± 0.00	20.0 ± 0.00	73.33 ± 0.01
Ser	23.33 ± 0.01	36.67 ± 0.01	146.67 ± 0.05	16.7 ± 0.01	40.0 ± 0.00	180.0 ± 0.03
∑ Total (%)	2.55	1.38	3.46	2.53	1.12	3.54
∑ Total - His (%)	0.44	0.69	2.95	0.47	0.72	2.91
A: ∑ Lys, Arg (%)	0.12	0.18	0.59	0.12	0.17	0.60
B: ∑ Asp, Glu, Pro, Ser, Thr (%)	0.08	0.20	0.97	0.08	0.19	0.96
A/B ratio	1.52	0.93	0.61	1.61	0.87	0.63

Values are mean ± SD. NR = unreported data.

Table 4b. Total amino acid content in anchoveta from raw fillets and fillets at weeks 3 and 28 of salt ripening. Results are expressed as g amino acid.100 g^{-1} of dry weight.

Total amino acids	Batch1			Batch 2		
	Raw fillet	Week 3	Week 28	Raw fillet	Week 3	Week 28
Ile	3.48 ± 0.21	2.83 ± 0.07	3.01 ± 0.07	3.4 ± 0.10	3.12 ± 0.10	2.97 ± 0.02
Leu	5.93 ± 0.03	4.79 ± 0.06	4.99 ± 0.05	6.15 ± 0.05	5.34 ± 0.16	4.98 ± 0.17
Lys	6.76 ± 0.05	5.34 ± 0.11	5.21 ± 0.01	6.88 ± 0.02	6.74 ± 0.18	6.10 ± 0.17
Met	2.33 ± 0.14	1.31 ± 0.01	1.37 ± 0.02	2.51 ± 0.03	1.40 ± 0.06	1.36 ± 0.066
Phe	3.15 ± 0.01	2.51 ± 0.03	2.70 ± 0.04	3.37 ± 0.01	2.77 ± 0.08	2.65 ± 0.09
Thr	3.37 ± 0.04	2.69 ± 0.04	2.80 ± 0.07	3.50 ± 0.01	3.00 ± 0.08	2.82 ± 0.10
Trp	0.97 ± 0.01	0.81 ± 0.03	0.87 ± 0.03	0.99 ± 0.02	0.94 ± 0.05	0.84 ± 0.03
Val	3.90 ± 0.02	3.17 ± 0.06	3.31 ± 0.06	3.97 ± 0.02	3.43 ± 0.11	3.27 ± 0.13
His	3.89 ± 0.03	2.08 ± 0.11	1.77 ± 0.012	3.92 ± 0.03	1.94 ± 0.04	1.77 ± 0.04
Arg	4.38 ± 0.02	3.59 ± 0.03	3.54 ± 0.07	4.46 ± 0.05	3.90 ± 0.12	3.56 ± 0.10
Ala	4.37 ± 0.04	3.40 ± 0.03	3.68 ± 0.53	4.47 ± 0.05	3.85 ± 0.10	3.53 ± 0.13
Tyr	2.51 ± 0.05	2.04 ± 0.03	2.20 ± 0.05	2.57 ± 0.04	2.27 ± 0.08	2.17 ± 0.08
Asp	7.41 ± 0.04	5.92 ± 0.09	5.94 ± 0.06	7.58 ± 0.12	6.41 ± 0.16	5.93 ± 0.23
Cys-Cys	0.71 ± 0.01	0.56 ± 0.02	0.56 ± 0.02	0.78 ± 0.02	0.61 ± 0.02	0.53 ± 0.03
Glu	10.21 ± 0.16	8.24 ± 0.11	8.17 ± 0.08	10.37 ± 0.04	9.12 ± 0.23	8.17 ± 0.03
Gly	3.471 ± 0.01	2.73 ± 0.01	2.76 ± 0.02	3.50 ± 0.01	2.87 ± 0.08	2.68 ± 0.10
Pro	2.56 ± 0.01	2.07 ± 0.01	2.13 ± 0.03	2.58 ± 0.02	2.26 ± 0.13	2.10 ± 0.11
Ser	2.89 ± 0.02	2.35 ± 0.02	2.38 ± 0.02	2.99 ± 0.01	2.49 ± 0.06	2.32 ± 0.07
∑ Total	72.80	56.42	56.81	73.45	62.47	57.76
∑ Total - His	68.42	54.34	54.90	69.60	60.53	55.99

Values are mean ± SD.

remaining within safe limits. The high variability observed underscores the need for further controlled studies to assess the specific impact of fat content under standardised conditions.

Acknowledgements

We are sincerely grateful to Eng. Alberto Salas-Maldonado for his support in coordinating our work with various salted anchovy processing plants. This work was supported by CIENCIACTIVA—CONCYTEC-PERÚ: [Grant Number 226-2015-FONDECYT.

Conflict of interest: The authors declare that they have no conflict of interest.

Author contributions: Verónica Valverde-Vera: Conceptualised the study, conducted laboratory analyses, performed statistical analysis, compiled the data, drafted the manuscript. Estrellita Rojas De Los Santos: Methodology and conducted laboratory analyses. Jessica Vela: Assisted in methodology development and initial data visualisation. Aarón Mondragón-Martínez: Methodology and conducted analyses in the factory setting. Fabiola Olivares: Designed experiments, reviewed, and edited the manuscript. Miguel Albrecht-Ruiz: Conceived and conceptualised the study, wrote the original draft, acquired funding, supervised the project.

References

Ababouch, L., El Marrakchi, A. 2009. Élaboration des semi-conserves d'anchois: aspects économiques, techniques et hygiéniques. In *FAO*. Document technique sur les pêches et l'aquaculture 525. FAO, Rome. 90 pp. (in French).

Albrecht-Ruiz, M., Salas, M.A. 2015. Chemical composition of light and dark muscle of Peruvian anchovy (*Engraulis ringens*) and its seasonal variation. Journal of Aquatic Food Product Technology 24:191–196. https://doi.org/10.1080/10498850.2012.762705

Andersen, E., Andersen, M.L., Baron, C.P. 2007. Characterization of oxidative changes in salted herring (*Clupea harengus*) during ripening. Journal of Agricultural and Food Chemistry 55:9545-9553. https://doi.org/10.1021/jf071369b

AOAC. 2006. Official methods of analysis of AOAC international. Association of Official Analytical Chemists International. Washington, D.C. USA.

Astruc, T., Vénien, A., Clerjon, S., Favier, R., Loison, O., Mirade, P.S., Portanguen, S., Rouel, J., Lethiec, M., Germond, A. 2022. Effect of dry salt versus brine injection plus dry salt on the physicochemical characteristics of smoked salmon after filleting. Heliyon 8:e11245 https://doi.org/10.1016/j.heliyon.2022.e11245

Ayala-Galdós, M.E., Albrecht-Ruiz, M., Salas-Maldonado, A., Paredes-Minga, J. 2002. Fat content of Peruvian anchovy (*Engraulis ringens*), after "El Niño" phenomenon (1998-1999). Journal of Food Composition and Analysis 15:627–631. https://doi.org/10.1006/jfca.2002.1059

Bradley, R.L. 2010. Moisture and total solids analysis. In: Food analysis. Springer, Boston. https://doi.org/10.1007/978-1-4419-1478-1_6

Chang, S.K.C. 2010. Protein analysis. In: Food analysis. Springer, Boston. https://doi.org/10.1007/978-1-4419-1478-1_9

Clancy, G.S., Beames, R.M., Higgs, D.A., Dosanjh, B.S. 1995. Effect of methodology on the determination of total volatile nitrogen and trimethylamine levels in previously frozen Pacific Herring (*Clupea harengus pallasii*) stored at 2-5°C for up to 15 days. Canadian Technical Report of Fisheries and Aquatic Sciences 2047:1–15.

Czerner, M. 2011. Aspectos tecnológicos de la maduración de la anchoíta (*Engraulis anchoita*) salada. Efecto de la composición química y otras variables tecnológicas [Doctor en Ingeniería]. Universidad Nacional de La Plata. 225 pp. (in Spanish).

Czerner, M., Agustinelli, S.P., Guccione, S., Yeannes, M.I. 2015. Effect of different preservation processes on chemical composition and fatty acid profile of anchovy (*Engraulis anchoita*). International Journal of Food Sciences and Nutrition 66:887–894. https://doi.org/10.3109/09637486.2015.1110687

Czerner, M., Tomás, M.C., Yeannes, M.I. 2011. Ripening of salted anchovy (*Engraulis anchoita*): Development of lipid oxidation, colour and other sensorial characteristics. Journal of the Science of Food and Agriculture 91:609–615. https://doi.org/10.1002/jsfa.4221

Czerner, M., Yeannes, M.I. 2013. Modelling the effect of temperature and lipid content on anchovy (*Engraulis anchoita*) salting kinetics. Journal of Food Engineering 115:164–172. https://doi.org/10.1016/j.jfoodeng.2012.10.004

Czerner, M., Yeannes, M.I. 2014. Bacterial contribution to salted anchovy (*Engraulis anchoita* Hubbs & Marinni, 1935) ripening process. Journal of Aquatic Food Product Technology 23:102–114. https://doi.org/10.1080/10498850.2012.697537

De la Torre Boronat, M., Soler Segón, C. 1984. Anchoas en salazón: Parámetros químicos de calidad. In ARXIUS de l'Esc. Sup. d'Agricultura pp. 59–79.

Filsinger, B., Barassi, C., Lupin, H., Trucco, R. 1982. An objective index ripening for evaluation of the ripening of salted anchovy. Journal of Food Technology 17:193–200.

Filsinger, B.E. 1987. Effect of pressure on the salting and ripening process of anchovies *(Engraulis anchoita*). Journal of Food Science 52:919–921. https://doi.org/10.1111/j.1365-2621.1987.tb14242.x

Gandemer, G. 2009. Dry cured ham quality as related to lipid quality of raw material and lipid changes during processing: A review. Grasas y Aceites 60:297–307. https://doi.org/10.3989/gya.130908

Hall, G.M. 2011. Preservation by curing (drying, salting and smoking). In: Fish processing – Sustainability and new opportunities, First edition, Hall, G.M. (Ed.), Wiley-Blackwell, pp. 51–75.

Hernández-Herrero, M.M., Roig-Sagués, A.X., López-Sabater, E.I., Rodríguez-Jerez, J.J., Mora-Ventura, M.T. 1999a. Protein hydrolysis and proteinase activity during the ripening of salted anchovy (*Engraulis encrasicholus L.*). A microassay method for determining the protein hydrolysis. Journal of Agricultural and Food Chemistry 47:3319–3324. https://doi.org/10.1021/jf980809j

Hernández-Herrero, M.M., Roig-Sagués, A.X., López-Sabater, E.I., Rodríguez-Jerez, J.J., Mora-Ventura, M.T. 1999b. Total volatile basic nitrogen and other physico-chemical and microbiological characteristics as related to ripening of salted anchovies. Journal of Food Science 64:344-347. https://doi.org/10.1111/j.1365-2621.1999.tb15897.x

Hernández-Herrero M.M., Roig-Sagués A.X., López-Sabater E.I., Rodríguez-Jerez J.J., Mora-Ventura M.T. 2000. SDS-PAGE of salted anchovies (*Engraulis encrasicholus* L) during the ripening process. European Food Research and Technology 212:26–30. https://doi.org/10.1007/s002170000197

Instituto Nacional de Defensa de la Competencia y de la Protección de la Propiedad Intelectual, Perú (INDECOPI). 2013. Norma Técnica Peruana NTP 204.056-2013. Lima, Perú: Anchovies salted and packed. Requirements. 21 pp. (in Spanish).

Karaçam, H., Kutlu, S., Köse, S. 2002. Effect of salt concentrations and temperature on the quality and shelf-life of brined anchovies.

International Journal of Food Science and Technology 37:19–28. https://doi.org/10.1046/j.1365-2621.2002.00526.x

Kari, N.M., Ahmad, F., Ayub, M.N.A. 2022. Proximate composition, amino acid composition and food product application of anchovy: a review. Food Research 6:16-29. https://doi.org/10.26656/fr.2017.6(4).419

Lam, R. 1968. Estudio sobre la variación del contenido de grasa en la anchoveta peruana (*E. ringens* J.). Informe N° 24, Instituto del Mar del Perú (IMARPE). 31 pp. https://repositorio.imarpe.gob.pe/bitstream/20.500.12958/252/1/INF%2024.pdf (in Spanish).

Marshall, M.R. 2010. Ash analysis. In: Food analysis. Springer, Boston. https://doi.org/10.1007/978-1-4419-1478-1_7

Mendes, R., Goncalves, A., Nunes, M.L. 1999. Changes in free amino acids and biogenic amines during ripening of fresh and frozen sardine. Journal of Food Biochemistry 23:295-306. https://doi.org/10.1111/j.1745-4514.1999.tb00021.x

Min, D.B., Ellefson, W.C. 2010. Fat analysis. In: Food analysis. Springer, Boston. https://doi.org/10.1007/978-1-4419-1478-1_8

Ministerio de Agricultura y Pesca, Alimentación y Medio Ambiente - MAGRAMA. 2017. El mercado de la anchoa en España, Madrid, España. 49 pp. https://www.mapa.gob.es/es/pesca/temas/mercados-economia-pesquera/informeanchoaene2016-5agosto_tcm30-291087.pdf (in Spanish)

Ministerio de la Producción – PRODUCE. 2022. Anuario estadístico pesquero y acuícola 2022. Ministerio de la Producción, Lima, Perú. https://ogeiee.produce.gob.pe/index.php/en/shortcode/oee-documentos-publicaciones/publicaciones-anuales/item/1116-anuario-estadistico-pesquero-y-acuicola-2022 (in Spanish).

Mood, A., Brooke, P. 2024. Estimating global numbers of fishes caught from the wild annually from 2000 to 2019. Animal Welfare 33:e6. https://doi.org/10.1017/awf.2024.7

Neves-Martins, M.J., Ribeiro-Sanches, M., Carregari-Polachini, T., Basilio de Oliveira, E., Dos Reis Coimbra, J., Telis-Romero, J. 2023. Solubility of different salts used in the control of the water activity of foods. Ciencia e Agrotecnologia 47:e018722. https://doi.org/10.1590/1413-7054202347018722

Nordic Committee on Food Analysis - NMKL. 2001. Water activity: Instrumental determination by Novasina electronic hygrometer and Aqua Lab dew point instrument. NMKL No. 168. Oslo, Norway.

Norma Chilena Oficial NCh 2637. 2001. Fish and marine products – histamine and others biogen amines. HPLC with UV detector method. Instituto Nacional de Normalización INN, Chile. 14 pp. https://es.scribd.com/document/479129054/NCh-2637-0f2001-pdf (in Spanish).

Official Journal of the European Communities. 1995. Determination of the concentration of volatile nitrogenous bases (TVB-N) in fish and fish products: a reference procedure. In: Official Journal of the European Communities, 95/149/EC (annex II), 95/149/EC. pp. 85–87. https://eur-lex.europa.eu/legal-content/en/ALL/?uri=CELEX%3A31995D0149

Rojas-De-Los-Santos, E., Valverde-Vera, V., Del-Aguila-Moyano, S., Vela-Rosas, J., Molleda-Ordoñez, A., Ayala-Galdos, M., Albrecht-Ruiz, M. 2018. Physicochemical and sensory changes of vacuum-packed, salt-ripened anchovy fillets (*Engraulis ringens*) stored at 8 and 20°C. Cogent Food and Agriculture 4:1549194. https://doi.org/10.1080/23311932.2018.1549194

Salas-Maldonado, A., Ayala-Galdós, M., Albrecht-Ruiz, M. 2002. Contenido de EPA y DHA en aceite crudo de pescado producido en el Perú durante el periodo 1996-2000. Ciencia y Tecnología Alimentaria 3:283–287. https://doi.org/10.1080/11358120209487740 (in Spanish).

Sampels, S. 2015. The effects of processing technologies and preparation on the final quality of fish products, Trends in Food Science & Technology 44:131-146. https://doi.org/10.1016/j.tifs.2015.04.003

Siringan, P., Raksakulthai, N., Yongsawatdigul, J. 2006. Autolytic activity and biochemical characteristics of endogenous proteinases in Indian anchovy (*Stolephorus indicus*). Food Chemistry 98:678-684. https://doi.org/10.1016/j.foodchem.2005.06.032

Stefánsson, G., Guðmundsdóttir, G. 1995. Free amino acids and their relationship to taste in (salt) ripened pelagic fish species. Report 91 of Icelandic Fisheries Laboratories. http://www.matis.is/media/utgafa/Skyrsla91.pdf

The Observatory of Economic Complexity (OEC). 2022. Salted/brined anchovies, not dried/smoked. https://oec.world/en/profile/hs/saltedbrined-anchovies-not-driedsmoked

Yatsunami, K., Takenaka, T. 2000. Characterization of brine proteases as agents of hydrolysis during the ripening of fermented sardine with rice-bran. Fisheries Science 66:569-573. https://doi.org/10.1046/j.1444-2906.2000.00088.x