Physicochemical and sensory changes of vacuum-packed, salt-ripened anchovy fillets (Engraulis ringens) stored at 8 and 20°C

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Abstract: Anchovy (Engraulis ringens) fillets obtained from traditional salt-ripened for 8 weeks showed 26.9 mg/100g total volatile bases nitrogen (TVB-N), 2.9 mg/100g trimethylamine, 7.1, proteolytic activity index, 3.3% hydrolysis degree, 2.6 mg malonaldehyde/kg (TBA test) and 22.4 g KOH/100g total ester index. Water activity (Wa) values suggest osmotic equilibrium at 8th week (0.743±0.007).

Physicochemical changes when fillets were vacuum-packed and stored for 14 weeks at 8°C/20°C were 32.4 / 75.7 TVB-N; 3.3 / 4.2 mg trimethylamine /100g; 9.8 /13.2 proteolytic activity index; 4.2 / 7.4% hydrolysis degree; 17.9/6.5 mg malonaldehyde/kg; and 20.1 / 21.7 total ester index respectively. Sensory scores corresponded to attractive reddish color and soft pleasant aroma of the product until 18th week at both temperatures, at the end of the study unpleasant odors corresponding to non-typical ripened fish (particularly in samples stored at 20°C) affected adversely the acceptability of the product for human consumption.

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PUBLIC INTEREST STATEMENT
Peruvian anchovy (Engraulis ringens) represents the largest marine biomass in the world, it is widely used for oil and fishmeal manufacture. However, for direct human consumption, this high valuable protein resource is also intended for salting-ripened products which are exported under different presentations such as vacuum-packed salting-ripened fillets.

Manufacture of this product is artisanal. Salting-ripened takes 4 months; further, vacuum packaged refrigerated fillets arrive industrial markets after 2 months in average, sometimes with undesirable quality changes.

Considering the commercial interest in the product, the present paper describes the physicochemical and sensory changes affecting quality of vacuum refrigerated salting-ripened anchovy fillets.
1. Introduction

Salt-ripened anchovy is a traditional product in Mediterranean countries, it is made from European anchovy (*Engraulis encrasicolus*) subjected to a process of salting and ripening lasting for about 3–12 months. The product obtained is firm and has juicy texture, pink color and intense flavor (ham-like taste). Due to a great decrease of anchovy stocks in the Mediterranean Sea and fishery controls (FAO, 2016), other *Engraulidae* are currently used (*Engraulis anchoita* and Peruvian species *Engraulis ringens*) (Czerner, 2011).

*E. ringens* represents the largest fish biomass (6%) in the world intended for fishmeal and fish oil production (FAO, 2017). In Peru, salt-ripened anchovy production is not large—approximately 0.7% of fishery products—nevertheless, during the last 5 years, volumes exported show an increasing tendency: from 7 to 11 t/year reported by United Nations Comtrade Database during last years (https://comtrade.un.org/data/); some commercial presentations are anchovy salted in barrels, semi-preserved in glass or metal containers and salted-ripened fillets packed in vacuum bags (INDECOPI, 2013).

Salt-ripened fish packed in vacuum bags and stored in refrigeration is an interesting commercial option because of low manufacture and transportation costs (Chouliara, Savvaidis, Panagiotakis, & Kontominas, 2004). At present, anchovies are ripened for around 4 months to obtain the fillets.

Traditionally, salt-ripened Peruvian anchovy is processed beheaded in barrels for about 5–6 months followed by light scalding to remove manually bones and skin and obtain the salt-ripened fillets. Semi-preserved final product is obtained by filling the fillets into glass or metal containers covered with vegetal oil, no heat treatment is applied.

During the preparation of traditional salt-ripened anchovy, a set of complex biochemical and physical reactions occur. These changes contribute especially to the development of sensory characteristics in fish meat—mainly own proteases and lipases action (Fernández, & Vitancurt, 1999; Steffanson & Gudmundsdottir, 1995). Many studies show chemical results associated with the ripening time and sensory changes, it is the case of a linear increment of proteolysis index (PI) (Besteiro, Rodríguez, & Pascual, 2000; Czerner, 2011; Durand, 1982; Hernández-Herrera, Roig-Sagues, Lopez-Sabater, Rodriguez-Jerez, & Mora-Ventura, 1999, Pons-Sánchez-Cascado, 2005). Ripening time in fish has also been correlated with total content of total volatile bases nitrogen (TVB-N) (Czerner, 2011; Hernández-Herrero et al., 1999), other changes have been observed in lipolysis activity and oxidation associated to increases of ester index (Filsinger, Barassi, Lupin, & Trucco, 1982) and thiobarbituric acid (TBA) values (Czerner, Tomás, & Yeannes, 2010). Fernández and Vitancurt (1999) mention a remarked increase in ripening speed in salted anchovies containing all or part of their viscera explained by the presence of flora proteases and lipases.

Even though refrigeration does not stop enzymatic changes or bacterial growth during ripening process, it can retard them (Pons Sánchez-Cascado, 2005). Nevertheless, for travel periods of around 2 months, vacuum-packed anchovy fillets must be refrigerated considering that the product reaches the market after 4–5 months and sometimes consumers complain on sensory quality.

Information about physicochemical and sensory data in vacuum-packed salt-ripened Peruvian anchovy is scarce. The aim of the study is to present information on physicochemical and sensory changes observed in vacuum-packed fillets obtained from traditional salt-ripening in barrels for 2 months; evaluations were made on stored fillets at 8 ± 2 and 20 ± 1°C.
2. Materials and methods

2.1. Sample preparation
Two tons of Peruvian anchovy (E. ringens) were caught on the coast of Chimbote, Peru (9° 4' S, 78° 35' W), and kept in ice until arrival to the factory for immediate process. This raw material was immersed in saturated brine for 16–24 h at room temperature (20–22°C), then manually beheaded, partially gutted and placed in 250 kg plastic barrels. Ripening of the anchovies was made according to the traditional procedure applied to Engraulidae species. A bottom layer of salt was spread on the bottom of barrels, then, fillets were treated with granular salt, layered alternately (fish and salt layers); salt was spread at top and barrels were covered with a plastic disk and 120 kg concrete for pressure. The ratio of fish to salt was 4:1 (w/w). Salt ripening developed at room temperature (20 ± 4°C) for 2 months. When salt-ripening was completed, anchovies were immersed in saturated brine to eliminate grains of salt; backbone and skin were manually removed and dried by manual pressure using absorbent cloths. Three hundred and fifty grams of fillets were vacuum-packed in 20 × 30 cm nylon-polyethylene thickness 90 µm bags and stored at 8 ± 2°C, reference samples at room temperature (20 ± 1°C) were additionally prepared.

2.2. Physicochemical analysis
Proximate composition was determined according to analytical techniques described by FAO (1986) in raw material and salt-ripened fillets stored at 8 and 20 ± 1°C. Fat content was determined using a Soxtherm® equipment (C. Gerhardt, Germany) with hexane as solvent, ash content was obtained in a muffle furnace at 600°C and moisture by oven drying at 102 ± 1°C both until constant weight, and protein quantity was obtained by multiplying total nitrogen content by 6.25 (Kjeldahl methodology).

In salt-ripened fillets stored at both temperature conditions, salt content was verified by chloride content analysis according to Mohr methodology (referred by Nielsen, 2010) and water activity (Wa) was measured using a CX-22 Aqualab equipment (NMKL 168, 2001).

Ripening development was measured by quantitative analysis of nitrogen fractions: nonprotein nitrogen determined by Kjeldahl in deproteinized sample using 5% trichloroacetic acid (TCA) (Hashimoto, Watabe, Kono, & Shiro, 1979), total volatile bases nitrogen (TVB-N) (Official Journal of the European Communities, 2014–2017) and trimethylamine (TMA) by Conway’s micro-diffusion technique (Clancy, Beames, Higgs, & Donsanjh, 1995).

PI value obtained from nonprotein nitrogen/total nitrogen relation, and HD, represented by the ratio hydrolyzed peptide bonds/total peptide bonds, was used to estimate proteolysis activity. To determine HD, dinitrofluorobencene was used to quantify free amino groups. Hydrolyzed peptides in salt-ripened sample were obtained from a slurry (1 g/100 mL water), total hydrolysis was achieved in 0.5 g sample incubated at 102°C in 20 mL 7 N H₂SO₄ for 24 h. Quantification was performed spectrophotometrically at 410 nm (Perkin Elmer lambda 950), standard curve was prepared using glycine (Dumay, Barthomeuf, & Berge, 2004).

Malondialdehyde (MDA) as fat deterioration product was measured by TBA test using 5% TCA deproteinized extracts at 532 nm using tetraethoxypropane for the standard curve (Tironi, Tomás, & Añón, 2007). For the EI, a methodology AOAC No. 9.125 referred by Filsinger et al. (1982) was carried out, results were expressed in g of KOH/100 g of desalted dry matter.

2.3. Sensory evaluation
Sensory analysis was determined in vacuum-packed salt-ripened anchovy fillets according to the method proposed by Filsinger et al. (1982) and Vásquez (1997) modified to evaluate flesh color (internal side), consistency, odor and flavor attributes (Table 1). Scale for each attribute was set from 2 to 8 and the sum of scores was considered the final score. Five trained panelists evaluated samples at the Sensory Laboratory. A good product was considered into a range from 22 to 26 points.
Table 1. Sensory characteristics evaluated on salt-ripened fillets of *E. ringens*, packed in vacuum bags

| Score | 2 | 4 | 6 | 8-Over ripened | Deteriorated |
|-------|---|---|---|----------------|--------------|
| Muscle color (inner side of fillets) | Beige to the edges, slightly darker and reddish in the middle | Nonuniform distribution of brown and/or dark red in central part and light beige to edges | Uniform distribution of reddish on the center line, beige at the edges | Nonuniform distribution. Dark brown coloration | Dark brown, brownish-green color |
| Odor | Salted fish. Brine smell. Non-intense aroma | Light to cured meat. Soft aroma | Characteristic and pleasant of the ripened anchovies. Soft-pleasant aroma | Strong aroma to cured | Rancid, acid, ammoniac or sulfurous off-odors. Unpleasant aroma |
| Flesh consistency | Elastic. Slightly moist | Slightly elastic, slightly moist and firm | Nonelastic. Firm and resistant | Flimsy and easily melts | Soft, it melts |
| Flavor (disregarding salt) | Strong salted dry fish | Slightly characteristic of cured meat | Characteristically to cured-madures meat (ham-like) | Intense taste of cured fish | Rancid, stale, nasty |

*Adapted from Filsinger et al. (1982) and Vásquez (1997).*
2.3.1. Statistical analysis

Analysis of variance was applied on data to determine the temperature effects on physicochemical and sensory changes during storage time. Tukey test was used to establish differences ($p < 0.05$). Statgraphics Centurion XVII program was used.

3. Results and discussion

Proximate composition values of raw material were 70.6% moisture, 9.0% crude fat, 16.5% protein, and 2.3% ash. At the 8th week, salt-ripened fillet values showed a considerable decrease in moisture (52.05%) and fat (3.10%). This could be explained as a result of the osmotic dehydration caused by the high salt concentration and removal of the skin with subcutaneous fat besides the pressure exerted into the barrel. Observation was made on the fatty layer suspended on top of the saturated brine. On the other hand, the influence of this behavior was the significant increase in protein and ash values. No significant differences ($p > 0.05$) were observed when comparing proximate composition values in salt-ripened vacuum-packed fillets at $8 \pm 2°C$ and $20 \pm 1°C$ (Table 2) suggesting no additional effect of osmotic mechanisms and a steady salt content into the product.

Wa values obtained during ripening in barrels went down from the initial 0.995–0.826 at the first week, up to the 6th week, a continuous decrease was observed reaching 0.786. Smaller Wa values corresponded to the lapse between 8th and 11th week (around 0.74), this last decrease seems to indicate that equilibrium starts at this time. Regarding Wa less than 0.80 related to control on putrefactive bacteria growth (Ababouch & El Marrakchi, 2009), the values obtained in the study suggest that putrefactive microbial action would be stopped (Figure 1).

Table 3 shows a higher increase in TVB-N values of samples stored at $20 \pm 1°C$ compared with a no significant increase showed at $8 \pm 2°C$, this would explain the rate of protein degradation to nonprotein molecules by proteases activity; this trend has been reported during traditional salt-ripening process of Engraulis encrasicolus (Czerner, 2011; Hernández-Herrero et al., 1999). When storage temperature data are correlated with TVB-N contents, a direct relationship is established. Higher values were observed at $20 \pm 1°C$ demonstrating a steady production of nonprotein nitrogen (from an initial value of 26–75.7 mg TVB-N/100 g at week 22).

TMA fish freshness indicator is related to a group of volatile basic compounds accumulated formed by bacterial reduction of trimethylamine oxide (TMAO) (Huss, 1995). Initial value in salt-ripened samples to be vacuum-packed was 2.95 mg TMA/100 g. Storage temperature seems to influence directly the increase of this value up to a final 4.15 mg TMA/100 g at $20 \pm 1°C$ and 3.31 mg TMA/100 g at $8 \pm 2°C$; differences at refrigeration storage were less significant than at $20 \pm 1°C$ (Table 3).

Table 2. Proximate composition, NaCl content, Wa and pH values in vacuum-packed, salt-ripened anchovy (E. ringens) fillets(*)

|                      | Fillets anchovy before vacuum packaging—week 8 | Fillets anchovy after vacuum packaging (8 ± 2°C)—week 22 | Fillets anchovy after vacuum packaging (20 ± 1°C)—week 22 |
|----------------------|-----------------------------------------------|----------------------------------------------------------|----------------------------------------------------------|
| Moisture             | 52.05 ± 0.12                                  | 51.93 ± 0.43                                             | 52.00 ± 0.53                                             |
| Protein              | 25.48 ± 0.56                                  | 25.93 ± 0.99                                             | 26.49 ± 0.94                                             |
| Crude fat            | 3.10 ± 0.58                                   | 3.12 ± 0.5                                               | 3.02 ± 0.39                                              |
| Ash                  | 16.93 ± 0.45                                  | 17.57 ± 0.31                                             | 17.58 ± 0.31                                             |
| NaCl                 | 19.11 ± 1.32                                  | 19.39 ± 1.64                                             | 19.73 ± 1.07                                             |
| Aw                   | 0.743 ± 0.003                                 | 0.740 ± 0.007                                            | 0.746 ± 0.005                                            |
| pH                   | 5.76 ± 0.04                                   | 5.73 ± 0.07                                              | 5.70 ± 0.09                                              |

(*) Mean ± standard deviation.
The direct correlation between TMA with TVB-N in fresh fish has been associated to microbial spoilage by Beirão (1976); however, the reduction of TMAO to TMA could be linked to nonspecific endogenous enzymatic processes. Then again the low increase of TVB-N in vacuum-packed fillets could be related with the muscular enzymatic systems—more than with the bacterial activity—mainly cathepsins which play a non-negligible role during storage of meat at room temperature (Sen, 2005).

The gradual increase of PI and HD during storage points toward a muscular proteases activity acting significantly on the protein fraction (Table 4). During storage at 20 ± 1°C, higher and significant PI values were obtained compared to refrigerated samples condition. Similar

| Week | 8 ± 2°C | 20 ± 1°C | 8 ± 2°C | 20 ± 1°C |
|------|---------|----------|---------|----------|
| 8    | 26.91 ± 0.26<sup>a</sup> | 26.91 ± 0.26<sup>a</sup> | 2.95 ± 0.11<sup>a</sup> | 2.95 ± 0.11<sup>a</sup> |
| 9    | 26.25 ± 0.78<sup>a</sup> | 34.43 ± 0.21<sup>b</sup> | 2.53 ± 0.11<sup>a</sup> | 3.01 ± 0.22<sup>a</sup> |
| 10   | 28.54 ± 3.23<sup>ab</sup> | 34.60 ± 0.32<sup>b</sup> | 2.87 ± 0.01<sup>a</sup> | 3.03 ± 0.22<sup>b</sup> |
| 12   | 29.82 ± 2.35<sup>bc</sup> | 38.25 ± 0.67<sup>b</sup> | 3.39 ± 0.13<sup>b</sup> | 3.82 ± 0.01<sup>b</sup> |
| 14   | 32.49 ± 3.41<sup>ab</sup> | 42.82 ± 0.99<sup>c</sup> | 3.18 ± 0.02<sup>b</sup> | 3.35 ± 0.23<sup>bc</sup> |
| 16   | 32.88 ± 2.00<sup>abc</sup> | 43.63 ± 2.32<sup>c</sup> | 3.83 ± 0.19<sup>abc</sup> | 3.82 ± 0.23<sup>c</sup> |
| 18   | 31.62 ± 3.42<sup>abc</sup> | 53.00 ± 1.37<sup>c</sup> | 2.66 ± 0.22<sup>b</sup> | 3.82 ± 0.24<sup>b</sup> |
| 22   | 32.43 ± 0.80<sup>c</sup> | 75.74 ± 1.95<sup>c</sup> | 3.31 ± 0.01<sup>b</sup> | 4.15 ± 0.20<sup>c</sup> |

Different lower-case letters (a, b, c, d, e, f) in the same col indicate significant differences (p < 0.05). At 20 ± 1°C, assays show an increasing linear tendency.

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increases tendency in proteolysis activity due to temperature has been reported in traditional ripening of *E. anchoita*: PI values of 19 and 26 at 5 and 15°C, respectively, in Ababouch and El Marrakchi (2009) and Czerne (2011), as well as 14.5 in *E. ringens* at room temperature (Maza & Salas, 2004).

Results of HD increased accordingly to the storage time showing no significant difference at 8 ± 2°C while at 20 ± 1°C, the values showed statistical significance (\(p < 0.05\)). At the end of the study (22nd week), the values were 4.19% and 7.38%, respectively (Table 4). Hernández-Herrero et al. (1999) and Ababouch and El Marrakchi (2009) demonstrated that partial viscera absence in traditional ripened fish prolongs the ripening time.

Vacuum represents a barrier to fat oxidation in anchovy fillets; however, metabolic activities can remain active and consume residual oxygen generating oxidation processes (Parry, 1993). Performance of fat oxidation measured by TBA values in the study was very variable. At 20 ± 1°C, the initial value increased significantly until week 12, at week 14 the values got to 7.6 mg MDA/kg but kept almost constant until the 22nd week. In refrigerated samples, the final value represented almost seven times the initial value and showed significant differ-

### Table 4. PI and GH changes in vacuum-packed salt-ripened fillets of *E. ringens* stored at 20 ± 1°C and 8 ± 2°C

| Week | 8 ± 2°C | 20 ± 1°C | 8 ± 2°C | 20 ± 1°C |
|------|---------|---------|---------|---------|
| 8    | 7.10 ± 0.09 | 7.10 ± 0.09\(^a\) | 3.28 ± 0.20\(^a\) | 3.28 ± 0.20\(^a\) |
| 9    | 6.99 ± 1.87 | 8.47 ± 2.10\(^ab\) | 3.33 ± 0.04\(^a\) | 4.21 ± 0.06\(^a\) |
| 10   | 8.37 ± 1.79 | 9.12 ± 0.82\(^b\) | 3.96 ± 0.36\(^ab\) | 4.53 ± 0.21\(^b\) |
| 12   | 8.55 ± 0.57 | 11.28 ± 0.56\(^c\) | 4.09 ± 0.39\(^ab\) | 5.25 ± 0.05\(^c\) |
| 14   | 9.00 ± 0.34 | 11.44 ± 1.06\(^c\) | 4.31 ± 0.34\(^bc\) | 5.73 ± 0.09\(^c\) |
| 16   | 9.17 ± 0.48 | 11.97 ± 0.21\(^c\) | 4.38 ± 0.08\(^bc\) | 5.56 ± 0.07\(^c\) |
| 18   | 9.63 ± 1.03 | 13.70 ± 0.30\(^c\) | 4.19 ± 0.10\(^b\) | 6.16 ± 0.20\(^c\) |
| 22   | 9.77 ± 0.45 | 13.20 ± 0.03\(^c\) | 4.19 ± 0.12\(^b\) | 7.38 ± 0.10\(^c\) |

Different lower-case letters (a, b, c, d, e, f) in the same col indicate significant differences (\(p < 0.05\)). Rows without letters indicate similar results (\(p > 0.05\)).

At 20°C, assays show an increasing linear tendency.

### Table 5. TBA values and esterification index in vacuum-packed salt-ripened fillets of *E. ringens* stored at 8 ± 2 and 20 ± 1°C

| Week | 8 ± 2°C | 20 ± 1°C | 8 ± 2°C | 20 ± 1°C |
|------|---------|---------|---------|---------|
| 8    | 2.6 ± 0.1\(^a\) | 2.6 ± 0.1\(^a\) | 22.4 ± 0.2 | 22.4 ± 0.2 |
| 9    | 4.8 ± 0.4\(^ab\) | 6.2 ± 0.3\(^c\) | 21.4 ± 0.9 | 22.8 ± 0.4 |
| 10   | 3.4 ± 0.4\(^ab\) | 4.5 ± 0.1\(^b\) | 21.1 ± 1.0 | 22.7 ± 2.3 |
| 12   | 4.4 ± 0.1\(^c\) | 5.7 ± 0.5\(^c\) | 21.7 ± 0.3 | 22.0 ± 1.3 |
| 14   | 6.9 ± 0.1\(^c\) | 7.6 ± 0.2\(^d\) | 20.9 ± 1.0 | 22.2 ± 0.3 |
| 16   | 9.1 ± 0.1\(^b\) | 6.9 ± 0.4\(^c\) | 21.9 ± 0.8 | 22.2 ± 4.1 |
| 18   | 10.5 ± 0.4\(^b\) | 7.7 ± 0.1\(^d\) | 21.6 ± 0.4 | 22.3 ± 1.1 |
| 22   | 17.9 ± 0.1\(^b\) | 6.5 ± 0.1\(^d\) | 20.2 ± 0.5 | 21.7 ± 0.5 |

Different lower-case letters (a, b, c, d, e, f, g) in the same col indicate significant differences (\(p < 0.05\)). Values without letters indicate similar results (\(p > 0.05\)).
ences along the storage time. The pattern observed is not in agreement with TBA data obtained in studies of refrigerated vacuum-packed salt-ripened catfish (*Pseudoplatystoma* sp.) that demonstrated advantages of refrigeration to control oxidation in vacuum samples (Rodríguez, Barrero, & Kodaira, 2009). Discrepancies could be explained by the sensitivity of TBA as a method and its nonspecific reaction with some proteins conducting to values lower than expected (Pereira & Tenuta-Filho, 2005; Schaich, 2016) (Table 5).

At both temperatures, EI in samples oscillated between 20.2 and 22.8 g KOH/g and showed no significant differences during all the storage time (Table 5). Lower results were obtained in salt-ripened *E. anchoita* that increased from 4 to 9 g KOH/g when stored at room temperature (Filsinger et al, 1982); this difference could be attributed to the characteristics of each species.

Sensory evaluation for the initial eight weeks before vacuum reached score 15. A low increase up to 18.5 was observed on 22nd week in samples stored at 8 ± 2°C, suggesting that a longer salting-ripening time is needed to obtain better scores. At 20 ± 1°C, development of reddish color and nonelastic and firm consistency at week 18th was observed. Rancid and ammonia odors of the samples at 22nd week triggered scores over 28 indicating unpleasant characteristics (Figure 2). In this regard, results of no typical sensory characteristics due to slow ripening have been reported in *E. encrasischolus* processed without viscera confirming the importance of digestive enzymatic action in this type of product (Ababouch & El Marrakchi, 2009; Hernández-Herrero et al., 1999). Vacuum is an additional hurdle to salting-ripening and refrigeration of samples.

Figure 2. Evolution of sensory score of vacuum-packed salt-ripened fillets of *E. ringens*, stored at 8 ± 2 and 20 ± 1°C.
4. Conclusions

- Non-significative variations were observed in proximate composition, sodium chloride content and water activity value of salt-ripened anchovy fillets before and after vacuum. Moreover, refrigeration storage at temperatures 8 ± 2 and 20 ± 1°C did not affect results.

- Moisture (51.7 ± 1.0%), sodium chloride (17.1 ± 1.1%) and Wa (0.75 ± 0.02) average values in vacuum packed salt-ripened anchovy fillets are in accordance with commercial quality specifications (INDECOPI (Instituto Nacional de Defensa de la Competencia y de la Protección de la Propiedad Intelectual, Perú), 2013). In spite of that, the absence of viscera seems to influence no typical odor changes reminding the importance of traditional ripening into the barrel.

- Increase of TVB-N, TMA, PI and DH values in the products stored at 20 ± 1°C was notoriously higher compared to refrigerated conditions.

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