Accepted Manuscript

Sous vide cook-chill mussel (*Mytilus galloprovincialis*): Evaluation of chemical, microbiological and sensory quality during chilled storage (3 Â°C)

Tiziana Bongiorno, Francesca Tulli, Giuseppe Comi, Alessandro Sensidoni, Debbie Andyanto, Lucilla Iacumin

PII: S0023-6438(17)30884-8

DOI: 10.1016/j.lwt.2017.12.005

Reference: YFSTL 6694

To appear in: *LWT - Food Science and Technology*

Received Date: 31 May 2017

Revised Date: 24 November 2017

Accepted Date: 3 December 2017

Please cite this article as: Bongiorno, T., Tulli, F., Comi, G., Sensidoni, A., Andyanto, D., Iacumin, L., *Sous vide* cook-chill mussel (*Mytilus galloprovincialis*): Evaluation of chemical, microbiological and sensory quality during chilled storage (3 Â°C), *LWT - Food Science and Technology* (2018), doi: 10.1016/j.lwt.2017.12.005.

1. Introduction

Due to socio-economic evolution and lifestyle, a growing number of single-person and small households (Byrne, 1998) have boosted the demand for convenience in meal preparation (Calderon, Iglesias, Laca, Herrero, & Diaz, 2010). Thermal processing is still one of the most common methods used to obtain safe convenience food with an extended shelf-life. Designing thermal processes for such products, typically in the range of 60-95°C for 10 to 30 min, is challenging since the heat treatments required for inactivating target microorganisms may cause undesirable quality changes in lipid and protein fractions. In seafood products, quality is severely reduced when the thermal process is designed for a shelf-life of more than 21 days under chilled conditions (Rosnes, Skåra, & Skipnes, 2011). New methods focusing on rapid or minimal heating able to maintain safety levels, are therefore fundamental for the future development of processed seafood. Recently, technological developments and new packaging materials have led to innovative food preservation strategies such as *sous vide* cook-chill (SVCC) processing.

SVCC processing consists of placing fresh, raw products in a vacuum-sealed bag or a semi-rigid tray, cooking it slowly under mild heating conditions i.e. pasteurization, immediate cooling and maintaining refrigeration at 3±1°C until serving. SVCC offers many advantages over traditional food processing such as the hermetic seal, which prevents moisture loss and contamination during and after treatments. In addition, the mild cooking temperature preserves the original flavor, texture and nutritional qualities. Moreover, vacuum packaging increases product shelf-life by inhibiting aerobic spoilage microrganisms, oxidative and other chemical spoilers (Ghazala, Ramaswamy, Smith, & Simpson, 1995) and is now widely used in food service establishments (catering, restaurant), fast food trades and supermarkets.

Despite the growing demand for and interest in safety of ready-to eat seafood, fast to prepare and easy to store seafood (Kennedy, Wall, Storrs, Devoluy, & Cruveiller, 2007), only a few studies are available regarding the application of *sous vide* cook-chilled processing to seafood (Diaz, Nieto, Banon, & Garrido, 2009; Espinosa, Diaz, Linares, Teruel & Garrido, 2015; Garcia-Linares, Gonzalez-Fandos, Garcia-Fernandez, & Garcia-Arias, 2004; Ghazala et al., 1995; Gonzalez-Fandos, Villarino-Rodrıguez, Garcıa-Linares, Garcıa-Arias, & Garcıa-Fernandez, 2005; Gonzalez-Fandos, Garcıa-Linares, Villarino-Rodrıguez, Garcıa-Arias, & Garcıa-Fernandez, 2004; Shakila, Jeyasekaran, Vijayakumar, & Sukumar, 2009). Moreover, such technology has not been applied to shellfish yet. While mussels have been subjected to chilled storage (Erkan, 2005; Gökoğlu, 2002), modified atmosphere packaging (Goulas, Nessi, Kontominas, & Savaidis, 2005a; Pastoriza, & Bernardez, 2011), freezing (Gökoğlu, Erkan, & Özden, 2000), cooking and vacuum cooling (Cavalheiro, Schmidt, Rodrigues, Siga, Leitempergher & Laurindo, 2013; De Lima, Siga, Leitempergher, Lerin, Soares, Tosati, Rodrigues & Monteiro, 2017), smoking (Turan, Sönmez, Çelik, & Kaya, 2008) and canning (Şengör, Gün, & Kalatofatoğlu, 2004) *sous vide* cook-chill processing has never been considered.

Bivalve mollusk, as filter feeders, can accumulate microorganisms, including pathogens, from seawater and the number and type of microorganisms present in the water depend on several seasonal, climatic and anthropogenic factors (Šolìć, Krstulović, Jozić, & Curać, 1999). Even if their harvesting and

commercialization is regulated by the EC directive 79/923 (Anonymous, 1979), which defines the classification of the rearing waters and by the EC directive 91/942 (Anonymous, 1991), which states the safety standards for live mollusk sale, the application of heat treatment according to the range of time/temperature proposals by EFSA (2015), ICMSF (1996) and European Commission (1997), may also ensure safety for bivalve mollusk, considering the risk of pathogenic microorganisms such as Hepatitis A virus (HAV), *Clostridium botulinum* and *Listeria monocytogenes.* Furthermore, heat treatments also seem effective in inactivating human norovirus (HuNov), considered to be the major common cause of acute epidemic nonbacterial human gastroenteritis (Bozkurt, D'Souza, & Davidson, 2014). So, the possibility of producing pasteurized mussels and new mussel-based ready-meals with extended shelf-life could become an important innovation and expansion opportunity for producers and give additional value to this shellfish product, while preserving fresh-like appearance, high nutritional value (Bongiorno et al., 2015), preventing product loss and reducing waste (Espinosa, Lopez, Diaz, Linares, & Garrido, 2016).

In this scenario, the aim of the present study was to evaluate the application of SVCC technology on *M. galloprovincialis* and to assess its stability over time in comparison to conventional home cooked mussels.

2. Materials and methods

2.1. Mussels and cooking treatments

Freshly harvested mussels (*M. galloprovincialis*, 20±1g and 6±1cm) were collected (80 kg) from a mussel farm site in the North Adriatic Sea (Gulf of Trieste, Italy) and subjected to depuration (CE IT 592 CDM) for 24 h (EU reg. 854/04). Then, animals manually selected to eliminate deformed or undersized individuals, were subjected to debyssing, brushing and transported (1 h under refrigerated conditions) to the laboratory of the University of Udine. Mussels were subjected to 4 different experimental treatments:

- *Sous Vide Cook Chill* (SVCC); mussels (15 specimens/pouch) were packaged in Oriented Polyamide/Polypropylene (OPA/PP) pouch (thickness 15/65 μm, Orved S.p.a., Musile di Piave-VE, Italy), and heat sealed under vacuum condition (VM 53,Orved S.p.a., Musile di Piave-VE, Italy). The pouches were subjected to heat treatment (85°C for 10 min in core), in a steam oven (HMG061X, Lainox Ali-s.p.a., TV, Italy) and immediately chilled at 3°C using a blast chiller (RCM051S, Lainox Ali-s.p.a., TV, Italy) (fig. 1A).
- *Brine and Sous Vide Cook Chill* (BSVCC) mussels (15 specimens/pouch) with the addition of brine (3% NaCl solution), in a ratio 1:2 to total mussel weight, were treated as SVCC (fig 1B).
- *Cooked and Chilled* (CC); mussels (15 specimens/pouch) were processed as SVCC and BSVCC without vacuum conditions (fig 1 D).
- *Conventional Cooking and Chill* (CMC), mussels were placed in a closed pan and cooked conventionally (90°C for 10 min in core), then placed in OPA/PP pouches (15 specimens/pouch) and immediately chilled as previously indicated (fig. 1 C).

The pasteurization value (P0) and Cook value (Cg) was determined in a previous test aimed at optimizing the thermal process and resulted in: Po = 1.08 min Cg = 3.46 min for SVCC; Po = 0.92 min Cg = 2.30 min for

BSVCC; Po = 0.32 min Cg = 1.65 min for CC. P0 and Cg values were not available for Conventional Cooking and Chilled treatment.

During all processes the internal temperature was continuously monitored by a data logger sensor (Tracksense Pro Val, Fasinternational s.r.l, Milano, Italy) inserted between the valves of one of the 15 mussels in the pouches. The management of probes and time-temperature data processing were performed with Valsuite basic Software (Ellab, Hilleroed, Denmark).

Raw mussels (RM) were characterized in terms of microbiological and chemical parameters before being processed.

2.2. Experimental design and sampling

Mussels subjected to the processing treatments were analyzed at 1, 7, 14, 21, 30 and 50 days of storage at 3±0.2°C. Two pouches of mussels for each sampling time were analyzed in duplicate for chemical and microbiological analysis, while 3 pouches of mussels per treatment were used for sensory analysis in each panel session to insure a suitable number of mussels for the panelist's evaluation. To determine Total Volatile Basic Nitrogen (TVB-N) content, for each sampling time, one pooled mussel sample (50 g) was frozen at -80°C till analyzed.

2.3. Chemical analysis

Moisture content was determined on RM and processed mussel meat according to method 934.01 (AOAC, 1997).

pH was measured in duplicate for each pouch in an aliquote (15 mL) of the homogenized mussel meat and shell liquor using a Basic 20 Crison pH-meter (Crison, Barcelona, Spain).

Total volatile basic nitrogen (TVB-N) content was determined according to Pearson (1976).

2.4. Microbiological analysis

The whole content, except the shells, of each pouch and for each treatment, was diluted 1:10 in a sterile stomacher bag with saline-peptone water (8 g/L NaCl, 1 g/L bacteriological peptone, Oxoid, Milano, Italy), and mixed for 1.5 min in the stomacher machine (PBI, Milano, Italy). The analyses were performed in duplicate agar plates on serial decimal dilutions of each mussel homogenate.

Total aerobic bacterial count (TBC), lactic acid bacteria (LAB), *Enterobacteriaceae* and anaerobic Sulphite-reducing clostridia were counted as described by Bongiorno et al. (2015). *Pseudomonas* were enumerated on PAB agar (Oxoid, Milan, Italy) and incubated at 30°C for 48 h.

2.5. Sensory analysis

The Sensory Analyses were performed, according to UNI-ISO standards (UNI-ISO 8589, 1990) by a trained seven-member panel that evaluated the quality of mussel meat at each sampling time. Before serving the mussels on a half shell in small aluminum trays to the panelists, mussels were steam-warmed (MP Julabo 19)

at 50 °C for 10 min. Each assessor rated the quality of five mussels per treatment (SVCC, BSVCC, CMC, CC) and per storage time, using characteristics to describe: color, odor, taste and texture of the mussel meat. Each characteristic was scored using a point scale (1-14) according to the scale range reported in Table 1. If the score was < 7, mussels were considered unacceptable. An overall sensory score was determined as the average value of the score of the attributes evaluated.

2.6. Statistical analysis

Data were subjected to two-way ANOVA to test the processing technology and the storage time on the quality of the product. If appropriate, means were compared by Tukey's multiple range test for $P<0.05$. SPSS-PC 17.0 statistical software (SPSS Inc. Chicago, IL, USA) was used for data analysis. Data are expressed as average value and standard deviations.

3. Results and discussion

3.1. Chemical analysis

3.1.1. Moisture

The moisture content of raw mussels was 78.9±2.2%. The cooking treatments (on day 1) resulted in a significant ($P<0.05$) reduction of moisture content in CMC mussels (73.5±1.0%), similar to values reported by Turan, Sönmez, Çelik, & Kaya (2007) on boiled mussels, while it increased significantly in BSVCC (81.4±1.3%) and was unaffected in CC (79.9±1.6%) and SVCC (79.7±1.1%) treatments (Fig. 2). As suggested by Voskresensky (1965) in his studies regarding the effect of salting in fish, this phenomena is attributable to the processes of osmosis. NaCl in solution dissociates into Cl^- and Na^+, which are involved in the solubilization of meat proteins by swelling. Swelling of the myofibrils reduces drip loss by increasing the spaces between myofibrils, which can retain more water (Aliño, Grau, Fernández-Sánchez, Arnold, & Barat, 2010). In fact, the higher NaCl content of mussel tissues, capturing water from the brine, resulted in an increase both in the moisture content and weight of mussels**,** whereas the reverse phenomena was observed when an hyperosmotic salt brine (26.4% vs 3.0%) caused the diffusion of water from the mussels into the surrounding brine (Turan et al., 2007).

Different from frozen stored mussels, where a loss of 5.21% at the end of 4 month storage period was observed (Gökoğlu et al., 2000), the moisture content remained unaltered in all processed mussels for the entire 50 chilled storage days of the present trial.

3.1.2. TVB-N

TVB-N content for raw and treated mussels are presented in Fig. 3. The TVB-N value of raw mussels registered in this study resulted in an average value of 15.3±0.2 mg N/100g, slightly higher than the figure (11.5-9.07 mg N/100g) reported by other authors (Erkan, 2005; Turan et al., 2007). The processing method significantly affected TVB-N content, which significantly increased after CMC (16.7±0.5 mg N/100g) and CC (15.3±0.3 mg N/100g) treatments, while it decreased ($P<0.001$) after SVCC and BSVCC treatments to

11.9±0.8 and 10.4±0.4 mg N/100g, respectively. During cold storage, TVB-N increased in CMC and CC from 14 to 21 storage days until the end of the storage period (50 days), reaching values of 31.9±0.3 and 44.4±0.3 mg N/100g, respectively. On the other hand, in both *sous vide* processed mussels, SVCC and BSVCC, TVB-N content remained constant during the whole storage period with final values of 13.9±0.8 mg N/100g (SVCC) and 12.6±0.4 mg N/100g (BSVCC), while in Gökoğlu et al., (2000), TVB-N concentration increased to 21.53 mg N/100g after 60 storage days, even in frozen mussels. Total volatile basic amine represents all nitrogen fractions that are formed in the tissues during the *post-mortem* processes due to bacterial degradation**,** which is the main cause of seafood spoilage (Ólafsdottir & Jónsdóttir 2010). For this reason TVB-N is one of the most widely used measurements of seafood freshness and quality. Regarding acceptable limits, different opinions have been reported in the literature. For example, according to Ludorff & Meyer (1973), TVB-N content of 25 mg N/100 g is expected for high quality product**s**, 30 mg N/100 g for good, 35 mg N/100 g for marketable ones, while TVB-N values > 35 N/100g are attributed to spoiled seafood. Goulas, & Kontominas (2005b) and Erkan (2005) suggest one acceptability limit for mussels of TVB-N content of 22-25 and 15 mg N/100g, respectively, although the European Union indicates a limit of 35 mg N/100 g (EEC, 1995).

In this study, TVB-N content of *sous vide* cook and chill mussels (with or without brine supplementation) remained constant and lower than the threshold limit suggested in the above mentioned studies for the entire storage period.

3.1.3. pH

The pH value registered in RM was 6.2±0.04, similar to values reported by other authors (Gökoğlu et al., 2000; Turan et al., 2008). pH was unaffected by the applied treatments (6.3±0.02); the mean values registered for pH in BSVCC, CC, CMC and SVCC are shown in Table 2. Processing treatment significantly affected pH value from day 7 onward ($P<0.001$) when the pH value increased to 7.05±0.16 and maintained similar values during the 50 chilled storage days. According to Goulas & Kontominas (2005b), such an increase in pH is probably due to the production of volatile basic components, such as ammonia, trimethylamine, etc., by fish-spoiling bacteria.

3.2. Changes in microbiological quality

The average total bacterial count in processed mussels and the relative changes during the storage period are presented in Table 3.

A TBC of 2.2±0.33 log CFU/g was detected in RM. The processing treatments did not significantly affect the TBC growth until 21 days of storage ($P>0.05$). Thereafter, CMC processed mussels always exhibited the highest value relative to SVCC and BSVCC up to 50 days ($P<0.05$), while in comparison with CC up to 30 days.

After processing, the mean value of TBC in CMC, SVCC and BSVCC was below the detection limit of the method and remained constant until day 7 of storage showing **in**significant differences ($P>0.05$). The *sous*

vide processed mussels (BSVCC and SVCC) remained almost stable until 30 days; statistical differences**,** observed in SVCC at day 14 and 21, can be considered as negligible from a microbiological point of view. Thereafter, TBC significantly increased in SVCC and BSVCC (1.9±0.20 and 3.1± 0.01 log CFU/g, respectively, $P<0.001$) at 50 storage days.

Regarding CMC, the initial low TBC counts remained constant until 14 storage days and then increased significantly reaching a value of 2.6±0.4 and 2.0±0.08 log CFU/g after 21 and 30 storage days, respectively with a further increase (4.2 log CFU/g, $P<0.001$) after 50 storage days.

In CC processed mussels, the initial value of TBC was 0.9± 0.06 log CFU /g and didn't show significant differences until 30 storage days ($P>0.05$), then increased to 4.5± 0.08 log CFU/g after 50 storage days.

Cooked foods are generally considered acceptable for human consumption, if TBC results in values below 5 log CFU/g (Huss, 1995); in this study SVCC and BSVCC processed mussels always maintained values below this limit, while in CMC and CC values close to the limit of acceptability were detected after 50 days (4.2 log and 4.5 log CFU/g, respectively). These results were consistent with Rybka, Kailasapathy, & Bergam (1999), who indicate a shelf-life of 54 days for cook-chill fish when stored at 0-3°C.

In addition**,** microbiological safety and sensory quality of rainbow trout fillets (*O. mykiss*) and salmon (*S. salar*) slice processed by sous vide method resulted in a shelf-life of 45 days under optimal storage conditions (+2°C) (Gonzalez-Fandos et al. 2004; 2005). Can (2011), in his study on carp fillets processed by sous vide, with or without sauce, demonstrated that the treated products could guarantee good microbiological quality for 56 storage days when preserved at 2°C.

Vacuum condition produces an ecosystem favorable to the growth of LAB, able to grow under microaerophilic/anaerobic conditions and associated with the spoilage of sous vide products, resulting in swelling and/or development of off-flavors and off-odors (Carlin, Guinebretiere, Choma, Schmott, & Nguyen, 1999). Guerzoni, Gianotti, & Lopez (1999) observed that LAB, undetectable immediately after the sous vide treatment in meat products, could sporadically be recovered after storage. In this study, LAB were found in RM (2.00±0.09 log CFU/g) but always remained below the detection limit in SVCC, BSVCC and CC control mussels, while LAB appeared in CMC (2.4±0.06 log CFU/g) after 21 storage days. The cooking process at 85°C for 10 min resulted in the reduction of this group of bacteria, similar to the results reported by Rosnes, Kleiberg, Bergstein, & Vidvei, (1999), where LAB in sous vide fish-based meals were not detected during a storage period of 42 days. Instead, a gradual increase in LAB counts was observed when the products were preserved at 10°C: after 3 storage days in trout fillets and after 14 in salmon slices (Gonzalez-Fandos et al., 2004; 2005) and carp fillets (Can, 2011), but these results can be due to the higher storage temperature (10°C) favorable to bacterial growth.

In RM, *Pseudomonas* were enumerated at a concentration of 5.32±0.21 log CFU/g. After treatments, the concentration of these bacteria was always below the detection limit of the method (10 log CFU/g) and was detected only in CMC (4.36±0.1 log CFU/g) after 30 storage days. According to Rhodehamel (1992), the normal spoilage microorganism, such as *Pseudomonas*, yeasts and molds are inhibited by vacuum packaging

in sous vide foods. As expected, the vacuum packaging and cooking prevented the growth of *Pseudomonas* in mussels, as well.

Enterobacteriaceae were counted in RM at levels of 2.00±0.23 log CFU/g; similar values were reported in fillet carp (Can, 2011), on raw trout (2.18±0.41 log CFU/g) and raw salmon (2.66±0.91 log CFU /g) (Gonzalez-Fandos et al.,2004; Gonzalez-Fandos et al., 2005). Enterobacteriaceae remained, for all treatments and control, below the detection limit of the method. Instead, Enterobacteriaceae were detected in the trout batches processed at 70°C after 45 days when storage temperature was 10°C (2.84±0.21 log CFU/g), highlighting the important role played by the storage temperature.

Anaerobic Sulphite-reducing clostridia were 2.00±0.17 log CFU/g and after treatment were always below the detection limit of the method (<1 CFU/g). Shakila et al. (2009) reported the presence of anaerobic sulphite-reducing clostridia in conventional packs of fish cakes and in conventional cook-chill fish cakes at the end of storage (3 MPN g-1), but not in sous vide cook chill fish cakes. Schmidt, Lechowich, & Folinazzo (1961) reported that the lowest temperature limit established for the growth and toxin production by strains of psychrotrophic *C. botulinum* is 3.3°C. However, recent studies have indicated that they may grow in vacuum packed meats at temperatures as low as 2°C (Moorhead & Bell, 2000). In addition, Gonzalez-Fandos et al. (2004 and 2005) showed that a temperature abuse of 10°C decreased the shelf-life of sous vide trout and salmon and allowed the growth of spore forming bacteria, thus implying a potential risk for the consumer's health. Due to the possible temperature abuses during distribution, retailing and consumption, additional hurdles should be included (Genigeorgis, 1993).

It must be highlighted that the microbiological shelf-life of *sous vide* mussels depends on different factors including the microbiological quality of raw material, efficacy of depuration, process parameters applied and maintenance of the cold chain (Schleining, 2007)

3.3. Changes in sensory quality

The score of sensory attributes i.e., color/appearance, odor intensity, meat turgidity, flavor, succulence and aftertaste of processed mussels during chilled storage are shown in Fig. 4.

On day 1, BSVCC, SVCC and CMC mussels obtained high scores in terms of overall acceptability; SVCC mussels obtained the highest score (10.54) mainly linked to odor intensity (Fig 4C), flavor (Fig 4E) and succulence (Fig 4F) reflecting its high sensory qualities. Since the first tasting, CC mussels were unacceptable for the majority of the attributes considered. According to panelists, the cooking process appeared visibly heterogeneous and the tissues of the mantle remained adherent to the mussels shell making the overall appearance unpleasant; despite similar heat treatment**s?** of pasteurization, probably in "*sous vide"*, the retention of intervalvar liquid ensured uniform heat transmission. On the contrary, when mussels were not subjected to vacuum two things occurred: heat expanded the packaged gases causing a reduction in thermal transmission and the opening of the valves that determined the release of intervalvar liquid. Overall this resulted in heterogeneous cooking, which negatively impacted the sensory analysis of the panelists (Skipnes, Oines, Rosnes, & Skara, 2002).

After 7 chilled storage days BSVCC, SVCC and CMC mussels obtained similar scores in all attributes considered; BSVCC and SVCC mussels decreased their scores, even if remaining acceptable for all attributes considered beginning at 14 storage days while CMC mussels resulted in being unacceptable. After 21 storage days, BSVCC mussels still obtained a score higher than the SVCC ones in terms of color/appearance, odor intensity, succulence, aftertaste and flavor while the latter were unacceptable. After 30 storage days, the overall score of BSVCC was unacceptable even if, in terms of odor intensity, meat turgidity and aftertaste, it still registered acceptable scores.

4. Conclusions

The application of mild heat treatments and chilled storage is desirable to maintain nutritional and sensorial properties of the mussels. *Sous vide* processing (SVCC and BSVCC) applied to fresh *M. galloprovincialis* mussels was able to preserve product quality and was beneficial in terms of extended shelf-life and increased product safety. At the end of the 50 storage days, the mesophiles reached a population > 5 log CFU/g, TVB-N < 35 mg/100g and the mussels obtained scores below 7. It was inferred that mussels cooked traditionally (90°C-10 min) had a shelf–life of about 14 days, while according to the conditions applied in the present experiment (85°C-10 min), mussels *sous vide* cooked and chilled exhibited a shelf–life of about 21 days with shelf-life extending to about 30 days when brine was added.

The absence of vacuum conditions resulted in the heterogeneous cooking of mussels while the inclusion of brine resulted in benefits in terms of moisture content, TVB-N and sensory attributes such as meat turgidity. Moreover, the reduction of water activity due to salt represented a further obstacle to bacterial proliferation and it could be a carrier of aromatic compounds. In addition, the introduction of brine prevents direct contact between the cutting margins of the shells and the packaging thus**,** preventing micro ruptures of the package and making the packaging operation easier.

Since the storage temperature plays a key role in ensuring the quality and safety of *sous vide* products, other tests are needed to evaluate the stability of these products during storage under refrigeration and thermal abuse conditions.

Acknowledgements

Research supported by Regione Friuli Venezia Giulia (L.R. 26/05). Authors gratefully acknowledge Dr. S. Micolini dr. A. Zentilin (Almar Soc. Coop. a.r.l., Marano Lagunare, Udine) for scientific collaboration and prof. E. Piasentier for having made available the sensory analysis laboratory.

References

Aliño, M., Grau, R., Fernández-Sánchez, A., Arnold, A., & Barat, J. M. (2010). Influence of brine concentration on swelling pressure of pork meat throughout salting. *Meat Science, 86(3)*, 600–606.

Anonymous (1979). Council Directive of 30 October 1979 on the quality required of shellfish water (79/923 EEC). *Official Journal of the European Communities*, no. L281 10.11.79, 47–52.

Anonymous (1991). Council Directive of 15 July 1991 laying down the health conditions for the production and the placing on the market of live bivalve molluscs (91/492/EEC). *Official Journal of the European Communities*, no. L268 24.9.91, 1–14.

Anonymous (2004). Council Directive of 29 April 2004 on the organisation of official checks on products of animal origin intended for human consumption (04/854/EC). *Official Journal of the European Communities*, no. L226/83 25.6.04, 1–45.

AOAC (1997). Association of Official Analytical Chemists International Publ. *Official methods of analysis* (16th ed.). USA, Arlington.

Bongiorno, T., Iacumin, L., Tubaro, F., Marcuzzo, E., Sensidoni, A., & Tulli, F. (2015). Seasonal changes in technological and nutritional quality of *Mytilus galloprovincialis* from suspended culture in the Gulf of Trieste (North Adriatic Sea). *Food Chemistry, 173*, 355-362.

Bozkurt, H., D'Souza, D.H., & Davidson, M. P. (2014). A comparison of the thermal inactivation kinetics of human norovirus surrogates and hepatitis A virus in buffered cell culture medium. *Food Microbiology, 42*, 212-217.

Byrne, D. (1998). In: Marketing, *The state we're*, February, (pp. 14–15).

Calderon, L. A., Iglesias, L., Laca, A., Herrero, M., & Diaz, M. (2010). The utility of Life Cycle Assessment in the ready meal food industry. *Resources, Conservation and Recycling, 54*, 1196–1207.

Can, Ö. P. (2011). Evaluation of the microbiological, chemical and sensory quality or carp processed by the *sous vide* method. *World Academy of Science, Engineering and Tecnology, 56*, 1225-1230.

Carlin, F., Guinebretiere, M.H., Choma, C., Schmott, P. & Nguyen, C. (1999). Spore forming bacteria in cooked chilled foods contained vegetables. *Proceedings of the Third European Symposium on Sous Vide*. Leuven, Belgium. Alma Sous Vide Competence Center, 55-67.

Cavalheiro, D., Schmidt, F.C., Rodrigues, L.G.G., Siga, C., Leitempergher, F. & Laurindo, J.B. (2013). Processing of *Perna perna* mussels using integrated process of cooking and vacuum cooling. *Journal of Food Process Engineering, 36*, 192–201.

De Lima, M., Siga, C., Leitempergher, F., Lerin,L.A., Soares, L.S., Tosati, J.V., Rodrigues, N.B. & Monteiro, A.R. (2017). Mussel (*Perna perna*) processing by an alternative method and packaging under modified atmosphere to improve physicochemical and microbiological characteristics. *Journal of Food Processing and Preservation, 41*, 1-9.

Diaz, P., Nieto, G., Banon, S., & Garrido, M. D. (2009). Determination of shelf-life of sous vide salmon (*Salmo salar*) based on sensory attributes. *Journal of Food Science, 74*, 371–376.

EEC (1995). Total volatile basic nitrogen (TVB-N) limit values for certain categories of fishery products and specifying the analysis methods to be used (Commission Decision 95/149/EEC of 8 March 1995). *Official Journal European Communication, 97*, 84–87.

EFSA (2015). EFSA BIOHAZ Panel. Scientific opinion on the evaluation of heat treatments, different from those currently established in the EU legislation, that could be applied to live bivalve mollusks from B and C production areas, that have not been submitted to purification or relaying, in order to eliminate pathogenic microorganisms. *EFSA Journal,* 13(12): 4332, 76 pp. doi: 10.2903/j.efsa.2015.4332.

Erkan, N. (2005). Changes in quality characteristics during cold storage shucked mussels and selected chemical decomposition indicators. *Journal Science Food Agricolture, 85*, 2625–2630.

Espinoza, M.C., Lopez, G., Diaz, P., Linares, M.B., & Garrido, M.D. (2016). Development of a convenience and safety chilled sous vide fish dish: Diversification of aquacultural products. *Food Science and Technology International, 22 (3),* 185-195.

Espinosa, M.C., Díaz, P., Linares, B., Teruel, M.R. & Garrido, M.D. (2015). Quality characteristics of *sous vide* ready to eat seabream processed by high pressure. *LWT - Food Science and Technology, 64,* 657-662.

European commission (1997). Harmonization of safety criteria for minimally processed foods. Martens, T. and Lucchetti, A. *European Commission report:* FAIR Concerted action CT96-1020, 36-37.

Garcia-Linares, M. C., Gonzalez-Fandos, E., Garcia-Fernandez, M. C., & Garcia-Arias, M. T. (2004). Microbiological and nutritional quality of sous vide or traditionally processed fish: Influence of fat content. *Journal of Food Quality*, *27*, 371–387.

Genigeorgis, C.A.(1993). Additional hurdles for "*sous vide"* products, In: *First European European Symposium on sous-vide proceedings,* 25-26 March, Leuven, Belgium.

Gonzalez-Fandos, E., Garcıa-Linares, M.C., Villarino-Rodrıguez, A., Garcıa-Arias, M.T., & Garcıa-Fernandez, M.C. (2004).Evaluation of the microbiological safety and sensory quality of rainbow trout (*Oncorhynchus mykiss*) processed by the *sous vide* method. *Food Microbiology, 21,* 193–201.

Gonzalez-Fandos, E., Villarino-Rodrıguez, A., Garcıa-Linares, M.C., Garcıa-Arias, M.T., & Garcıa-Fernandez, M.C. (2005). Microbiological safety and sensory characteristics of salmon slices processed by the sous vide method. *Food Control, 16,* 77–85.

Goulas, A.E., Nessi, C.E., Kontominas, M.G., & Savaidis, I.N. (2005a). Microbiological, biochemical and sensory assessment of mussels (*Mitylus galloprovincialis*) stored under modified atmosphere packaging. *Journal of Applied Microbiology, 98,* 752-760.

Goulas, A.E. & Kontominas, M.G. (2005b). Effect of salting and smokig-method on the keeping quality of chub mackerel (*Scomber japonicus*): Biochemical and sensory attributes. *Food Chemistry, 93,* 511-520.

Gökoğlu, N., Erkan, N., & Özden, O. (2000). The effect of frozen storage on the proximate composition and quality of mussels (*Mytilus galloprovincialis*). *Journal of aquatic food product technology, 9 (2),* 83-88.

Gökoğlu, N. (2002). A descriptive method for sensory evaluation of mussels. *Lebensmittel-Wissenschaft und -Technologie, 35,* 563–567.

Guerzoni, M.E., Gianotti, A., & Lopez, C.C. (1999). Effect of some process variables on safety and shelf-life of ‘‘sous vide’’ cooked foods. In: Third *European Symposium on Sous-vide Proceedings*, Leuven, Belgium 253–266.

Huss, H.H. (1995). Quality and quality changes in fresh fish. FAO *Fisheries Technical Paper*, Italy, No. 348, p. 195.

ICMSF (1978). Microorganisms in foods. 1. Their significance and methods of enumeration. *International Commission on Microbiological Specifications for Foods* (ICMSF), second ed. University of Toronto Press, Toronto.

ICMSF. (1996). Microrganism in foods, Microbiological Specifications of Food Pathogens. London: Blackie Academic and Professional.

Kennedy, J., Wall, P., Storrs, M., Devoluy, M.C., & Cruveiller, P. (2007). Food safety challenges. In Safety Handbook: Microbiological Challenges (pp. 8–19). France: Bio-Mérieux Education.

Ludorff, W. & Meyer, V. (1973). Fische und Fischerzeugnisse, Verlag Paul Parey, Hamburg-Berlin, Germany (Cited by Turan 2006).

Moorhead, S.M., & Bell, R.G. (2000). Botulinal toxin production in vacuum and carbon dioxide packaged meat during chilled storage at 2 and 4°C. *Journal Food Safety* 20, 101–110.

Ólafsdottir, G., & Jónsdóttir, R. (2010). Volatile aroma compounds in fish in *Handbook of Seafood and Seafood Products Analysis*. Edited by Nollet L.M.L. Toldrà F. pp 97-115.

Pastoriza, L., & Bernardez, M. (2011). Quality of live packaged mussels during storage as a function of size and oxygen concentration. *Food Control 22,* 257-265.

Pearson, D. (1976). The Chemical Analysis of Foods, 7th ed. Churchill Livingstone, London.

Rhodehamel, E.J. (1992). FDA’s concerns with sous-vide processing. *Food Technology, 46,* 73–76.

Rosnes, J.T., Kleiberg, H., Bergstein, H. & Vidvei, J. (1999). Microbiological safety of two sous vide fish based meals. In:*Third European Symposium on sous-vide proceedings,* Leuven, Belgium, 195–204.

Rosnes, J. T., Skåra, T. & Skipnes, D. (2011). Recent advances in minimal heat processing of fish: effect on microbiological activity and safety. *Food Bioprocess Technology, 4,* 833-848.

Rybka, S., Kailasapathy, K., & Bergam, J. (1999). Storage characteristics of selected cook-chill meals with an extended shelflife.In: *Third European Symposium on sous-vide proceedings*. Leuven, Belgium, 317–330.

Schmidt, C.F., Lechowich, R.V. & Folinazzo, J.F. (1961). Growth and toxin production by type E Clostridium botulinum below 40°F. *Journal of Food Science, 26,* 626–630

Şengör, G.F., Gün, H. & Kalatofatoğlu, H.(2004). The determination of microbial flora water activity and chemical analyses in smoked, canned mussel (*Mytilus galloprovincialis*). *Turkish Journal Veteterinary Animal Science, 28,* 793–797.

Schleining, G. (2007). Preventive Measures for Food Safety in *Food Safety A Practical and Case Study Approach.* Edited by Anna McElhatton and Richard J. Marshall, pp 50-67.

Shakila, R. J., Jeyasekaran, G., Vijayakumar, A., & Sukumar, D. (2009). Microbiological qualità of sous-vide cook chill fish cakes durino chilled storage (3°C). *International Journal of Food Science an Technology, 44*, 2120-2126.

Skipnes, D., Oines, S., Rosnes, J.T., & Skara, T. (2002). Heat transfer in vacuum packed mussels (*Mytilus edulis*) during thermal processing. *Journal of Aquatic Food Product Tecnology, 11*, 5-18.

Turan, H., Sönmez, G., Çelik, M., Yalçin, Y., & Kaya, Y. (2007). Effects of different salting process on the storage quality of Mediterranean mussel (*Mytilus galloprovincialis* L.1819). *Journal of Muscle Foods, 18*, 380-390.

Turan, H., Sönmez, G., Çelik, M. Y., & Kaya, Y. (2008). The effects of hot smoking on the chemical composition and shelf-life of Mediterranean mussels (*Mytilus galloprovincialis* L.1819) under chilled storage. *Journal of Food Processing and Preservation, 32,* 912–922.

UNI-ISO 8589 (1990). Sensory Analysis - General Guidance For The Design Of Test Rooms.

Voskresensky, N.A. (1965). Salting of herring. Fish as Food Borgstrom, G. Vol III. New York: Academic Press.

Table 1. Quality scale used in sensory analysis.

Characteristic	Score	
	1	14
Colour/appearence	Opaque/old	Very bright/fresh
Odour intensity	Rotten seaweed	Fresh
Meat turgidity	Flaccid	Very firm
Flavour	Insipid	Very tasty
Succulence	No juicy	Very juicy
Aftertaste	No persistent	Very persistent

Table 2. Mean values and standard deviation for pH of processed mussels during 50 days of storage at 3±1°C.

Treatment	**BSVCC**	**CC**	**CMC**	**SVCC**	Significance
Days					
1	6.27±0.035x	6.26±0.021x	6.24±0.014x	6.25±0.014x	NS
7	6.84±0.035y	6.71±0.014y	6.74±0.001y	6.79±0.000y	NS
14	6.90±0.141y	6.76±0.021yz	6.84±0.212yz	6.87±0.106y	NS
21	7.00±0.000ayz	6.84±0.014cz	6.98±0.141aw	6.91±0.000byz	***
30	6.93±0.042by	6.94±0.021bw	7.24±0.064aj	6.97±0.014byz	***
50	7.25±0.212az	6.98±0.212bcw	6.88±0.028cz	7.08±0.042bz	***
Significance	***	***	***	***	

a.b.c: different letters indicate statistical differences among treatments.
x. y. z. w. j: different letters indicate statistical differences among days of storage.
*** P<0.001.
Data are reported as average ± standard deviation (n=2 pouch for each sampling time analysed in duplicate). Legend: BSVCC. Brine and *Sous Vide* Cook-Chill mussels; SVCC. *Sous Vide* Cook-Chill mussels; CMC. Conventional Cooking and Chilling; CC. Cooked and Chilled mussels.

Table 3. Mean values (log CFU/g) and standard deviation for total bacterial counts of processed mussels during 50 days of storage at 3±1°C.

Treatment	**BSVCC**	**CC**	**CMC**	**SVCC**	Significance
Days					
1	$<10^{§}$ y	0.88±0.67y	$<10^{§}$ w	$<10^{§}$ z	NS
7	$<10^{§}$ y	1.30±0.75y	$<10^{§}$ w	$<10^{§}$ z	NS
14	0.70±0.01y	1.00±0.75y	$<10^{§}$ w	1.01±0.57y	NS
21	0.25±0.01by	0.88±0.50by	2.57±0.38ay	1.00±0.00by	***
30	$<10^{§}$ cy	1.00±0.00by	2.04±0.05ay	$<10^{§}$ cz	***
50	3.06±0.01bx	4.47±0.08ax	4.24±0.08az	1.90±0.20bx	**
Significance	***	***	***	***	

a.b.c: different letters indicate statistical differences among treatments.
x. y. z. w. j: letters indicate statistical differences among days of storage.
** P<0.05; *** P<0.001.
§ : data expressed as CFU/g
Data are reported as average of log tbc ± standard deviation (n=2 pouch for each sampling time analyzed in duplicate). Legend: BSVCC. Brine and *Sous Vide* Cook-Chill mussels; SVCC. *Sous Vide* Cook-Chill mussels; CMC. Conventional Cooking and Chilling; CC.

Figure 1.

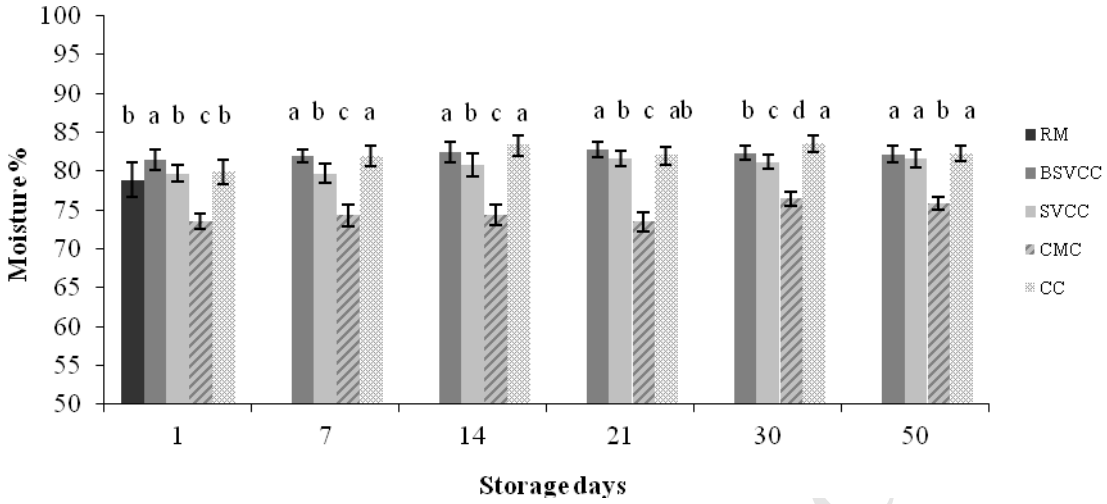

Figure 2.

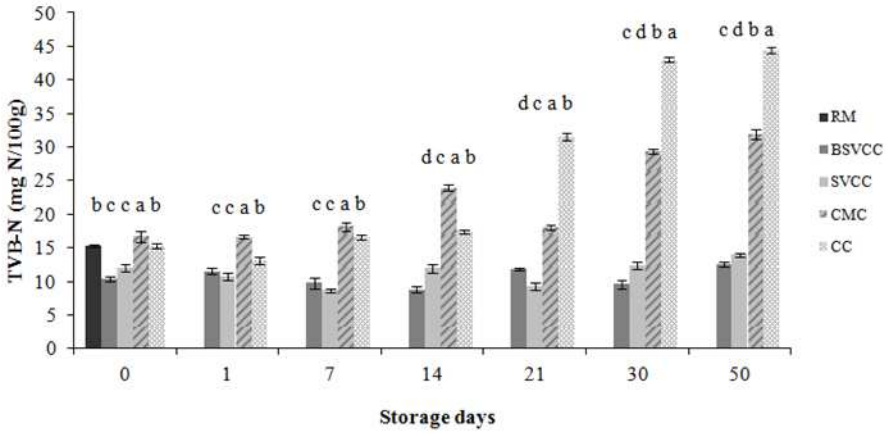

Figure 3.

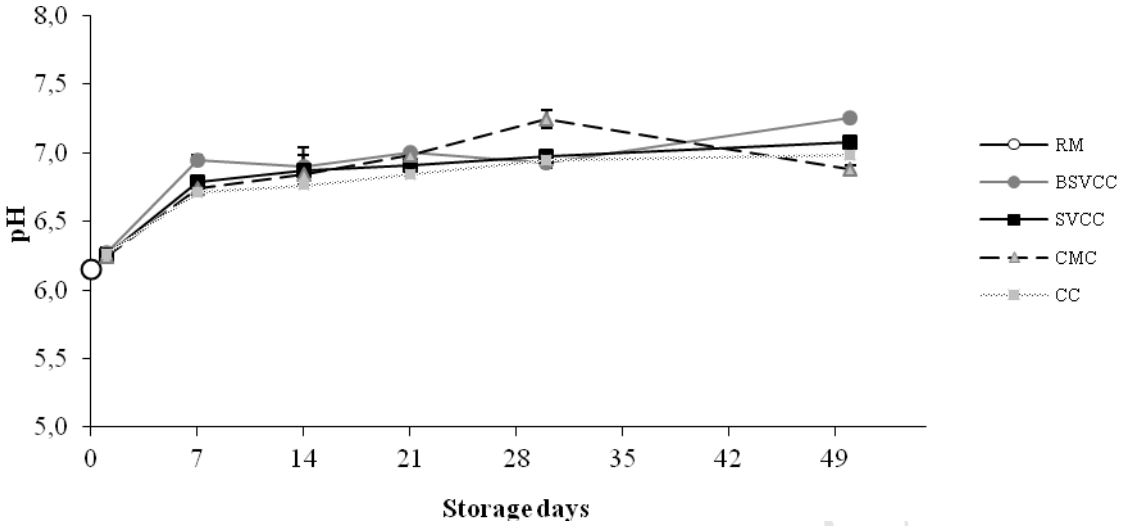

Figure 4. deleted in the revised MS

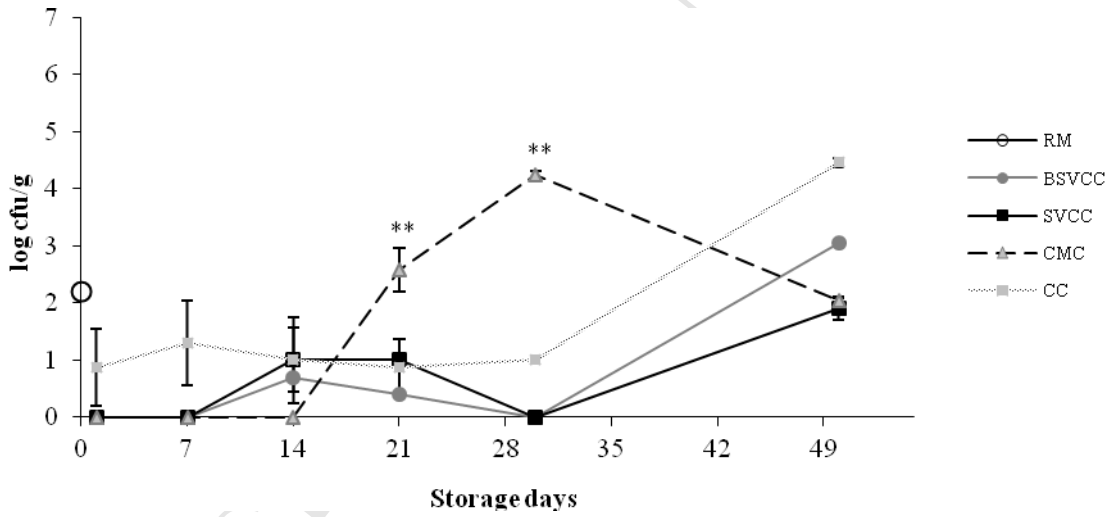

Figure 5. deleted in the revised MS

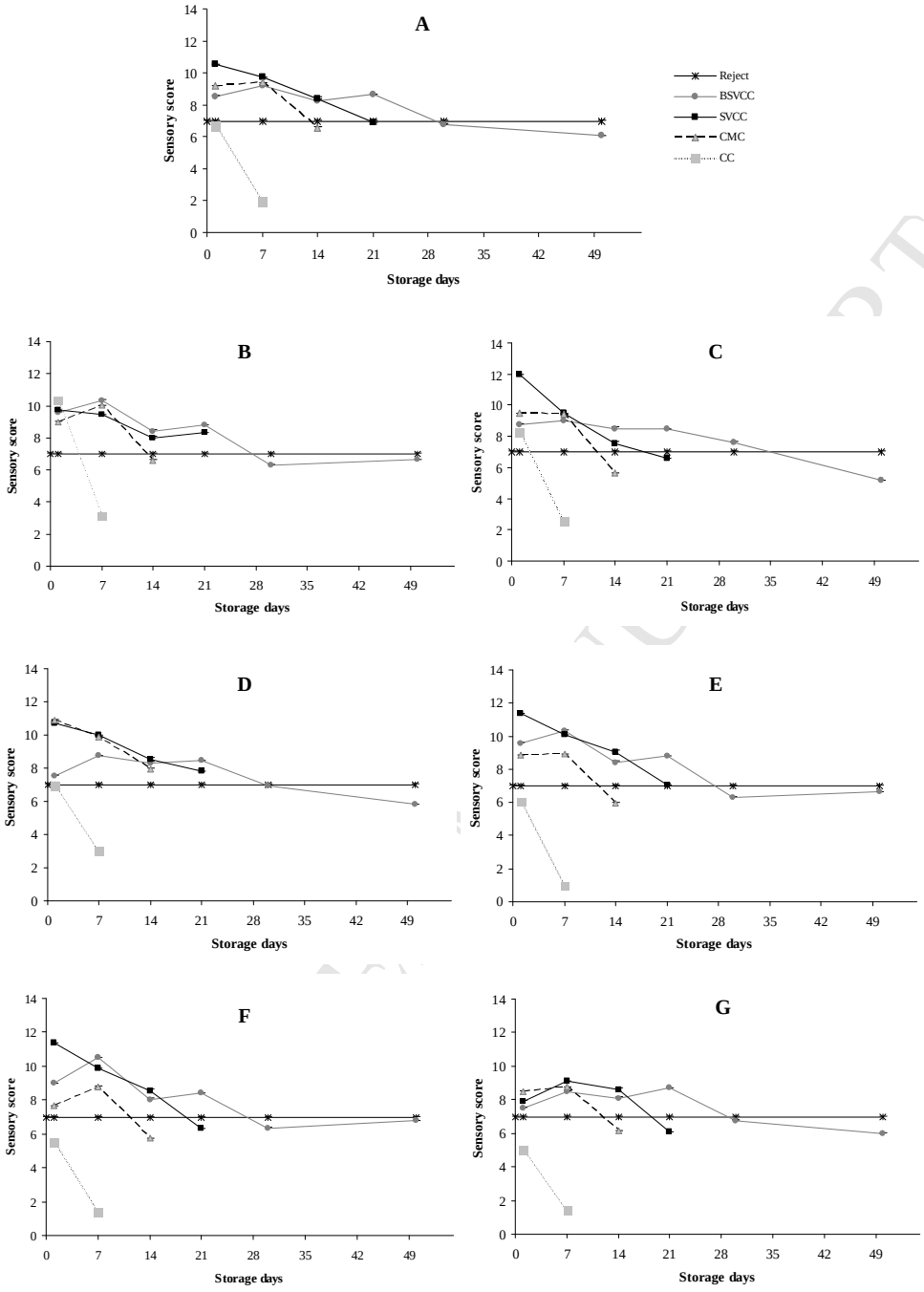
A
B
C
D
E
F
G
Sensory score
Storage days
Reject
BSVCC
SVCC
CMC
CC

Figure 4.

Figure 1. Mussels after treatment: SVCC, *Sous Vide* Cook and Chill (A); BSVCC, Brine and *Sous Vide* Cook & Chill (B); CMC, Conventional Cooking and Chilling (C); CC, Cooked and Chilled (D).

Figure 2. Changes in moisture content of mussels subjected to different cooking treatments and chilled, during storage at 3±1°C. Data are reported as average ± standard deviation (n=2 pouch for each sampling time analyzed in duplicate), different letters indicate significant differences among treatments for each sampling time, $P<0.05$. Legend: RM, Raw Mussels; BSVCC, Brine and *Sous Vide* Cook-Chill mussels; SVCC, *Sous Vide* Cook-Chill mussels; CMC, Conventional Cooking and Chilling; CC, Cooked and Chilled mussels.

Figure 3. Changes in total volatile basic nitrogen (TVB-N) content of mussels subjected to different cooking treatments and chilled, during storage at 3±1°C. Data are reported as average ± standard deviation (n=2 pouch for each sampling time analyzed in duplicate), different letters indicate significant differences among treatments for each sampling time, $P<0.05$. Legend: RM, Raw Mussels; BSVCC, Brine and *Sous Vide* Cook-Chill mussels; SVCC, *Sous Vide* Cook-Chill mussels; CMC, Conventional Cooking and Chilling; CC, Cooked and Chilled mussels.

Figure 4. Changes in the overall sensory scores (A), color/ appearance (B), odor intensity (C), meat turgidity (D), flavour (E), succulence (F) and aftertaste (G) scores of mussels subjected to different cooking treatments and chilled, during storage at 3±1°C. Data are reported as average ± standard deviation, different letters indicate significant differences among treatments for each sampling time, $P<0.05$. Legend: BSVCC, Brine and *Sous Vide* Cook-Chill mussels; SVCC, *Sous Vide* Cook-Chill mussels; CMC, Conventional Cooking and Chilling; CC, Cooked and Chilled mussels.

Highlights

- Use of *sous vide* processing (85°C, 10 min) with/ without salt brine on mussels
- Microbiological quality of processed mussels preserved beyond 15 days
- Mussels processed by *sous vide* extended shelf-life to 21 days
- Brine extended shelf-life to 30 days for *sous vide* processed mussels
- Sensory quality of processed mussels acceptable over 14 days