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The Microbiology of Brown Crab (*Cancer pagurus*)

Key external stakeholders:
Seafood Processors & Bord Iascaigh Mhara

Practical implications for stakeholders:
The outcome/technology or information/recommendation are;

- Irish brown crab processors should continue to use high barrier films to process at 90°C for 10 minutes.
- Adding binders like sodium caseinate or potato starch increases yield and acetic acid extends microbial shelf-life.
- This new knowledge may be used to increase profits and open new, more distant, markets.

Main results:

- The shelf-life of crab meat may be extended by up to 3 days using lactic acid and more than doubled using acetic acid.
- Binders such as sodium caseinate or potato starch increase yield during processing.
- Milder processing conditions (70°C for 2 minutes) would assure the destruction of *Listeria monocytogenes* but the shelf-life would be adversely affected.
- Using a film with lower barrier properties would also reduce shelf-life.

Opportunity / Benefit:

This research, the first of its kind in the Food Safety Department at Teagasc, explored the use of different ingredients, processing conditions and packaging films to provide science based solutions to reduce losses during processing and maximize shelf-life. The knowledge generated and expertise acquired should be used by the crab processors to maximize export opportunities and profit.

Collaborating Institutions:

University College Dublin

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1. Project background:

Brown crab (*Cancer pagurus*) is a predominant species of crustacean in Irish waters and a valuable commodity to the Irish economy, with an annual net worth of over €43 Million (BIM, 2017). Further expansion into more distant geographical markets will require a better understanding of the bacterial populations, especially spoilage bacteria on crab and how novel ingredients and/or interventions may be applied to minimize losses during processing and maximise shelf-life.

2. Questions addressed by the project:

1. Can natural ingredients be used to enhance shelf-life?
2. Would clean label ingredients (sodium caseinate and potato starch) reduce processing losses, without adversely affecting shelf-life or promoting the growth of spoilage bacteria.
3. What bacteria are present in Irish brown crab?
4. Do milder processing conditions kill *Listeria monocytogenes*?
5. Would the application of milder processing conditions (70°C for 2 minutes instead of 90°C for 10 minutes) adversely affect shelf-life?
6. Would the use of a medium barrier film instead of the current high barrier film adversely affect shelf-life?

3. The experimental studies:

1. The effect of 5% organic acids (lactic acid acetic acid and citric acid) and 5% sodium chloride (NaCl) on the bacteria found in crab during storage (2°C for 12 days).
2. The effect of 5% (w/v) sodium caseinate and (5%, w/v) potato starch, with and without (0.5%, w/v) ascorbic acid on losses during processing and shelf-life (28 days storage at 4°C) were investigated.
3. Culture based methods and high-throughput 16S rRNA sequence analysis were used to examine the bacteria present in Irish brown crab.
4. Thermal inactivation studies were undertaken to provide much needed thermal inactivation data for *L. monocytogenes* in crab meat and to establish if milder processing conditions (70°C for 2 minutes) would assure the destruction of any *L. monocytogenes* present.
5. The effect of milder processing conditions (70°C for 2 minutes instead of 90°C for 10 minutes) on the shelf-life of brown crab.
6. The effect of the barrier properties of the packaging film on the shelf-life of brown crab.

4. Main results:

Enhance shelf-life using chemical treatments: Crab (*Cancer pagurus*) meat (white and brown) has a short shelf-life. Chemical treatments may inhibit microbial spoilage and extend shelf-life. Acetic acid (5%) was the most effective treatment for white meat, reducing the initial bacterial counts by up to 100 bacteria per gram and extended the shelf life to 8-11.5 days, compared to 5 days for untreated control samples. Lactic acid treatment also reduced the initial bacterial counts but the shelf life was only increased by 3 days. Citric acid and salt (sodium chloride) treatments had no effect. A similar pattern was observed for brown meat samples, although the shelf life was increased by a maximum of 1-3 days. The growth of spoilage bacteria was also reduced on acetic acid treated samples. It was concluded that the shelf-life of crab meat may be extended by up to 3 days using lactic acid and more than doubled using acetic acid.

Reduce processing losses using binders: On average 11.1% of the crabs were lost during processing. This was reduced to 8.0% when treated ascorbic acid and to 3.5%, 4.7%, 5.8% and 2.3% with sodium caseinate, potato starch, sodium caseinate plus ascorbic acid and potato starch plus ascorbic acid, respectively. None of these treatments negatively impacted on shelf-life and

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similar growth curves were observed for different bacteria, regardless of treatment. It was therefore concluded that, subject to sensory evaluation and validation under commercial conditions, these natural ingredients could be used to substantially increase the yield and hence commercial value of crab meat, without adversely affecting shelf-life.

Bacteria present: Culture based methods: Thirty brown crabs (*Cancer pagurus*) (10 crabs on 3 separate occasions) were collected from 3 different processing plants, and the external surfaces (carapace) were swabbed. *Post mortem* the hemolymph, gut and muscle (claw meat) were removed aseptically, and tested for a range of different bacteria. The bacterial count in the gut, haemolymph, muscle and carapace samples ranged from approximately 100 to 100,000 cells per cm^2, with the latter having the highest counts. Overall the carapace yielded the highest bacteria counts.
Molecular methods: The data obtained suggested that the carapace, gut and haemolymph support the survival and growth of bacteria belonging to a group (phyla) called the Proteobacteria. Other bacteria were also detected belonging to other groups such called the Firmicutes, Actinobacteria and Bacteriodetes.

Thermal inactivation of L. monocytogenes: It was concluded that current pasteurisation conditions (eg. 70°C for 2 min) would achieve the complete destruction of any *L. monocytogenes* present in crab meat and TSB could be used as a model matrix for assessing the thermal inactivation of *L. monocytogenes* in crab meat.

Thermal inactivation of *L. monocytogenes*: Thermal inactivation studies were undertaken to provide much needed thermal inactivation data for *L. monocytogenes* in crab meat and to investigate if tryptone soya broth (TSB) is representative of crab meat in thermal inactivation studies involving *L. monocytogenes*. *Listeria monocytogenes* from brown crab and smoked salmo were used in these experiments. It was concluded that mild pasteurisation conditions (eg. 70°C for 2 min) would achieve the complete destruction of any *L. monocytogenes* present in crab meat and TSB could be used as a model matrix for assessing the thermal inactivation of *L. monocytogenes* in crab meat.

Milder processing conditions: Studies were undertaken to assess the effect of milder pasteurisation conditions (70°C for 2 minutes) instead of the conventional 90°C for 10 minutes on the shelf-life of processed brown crabs (*Cancer pagurus*). The microbial shelf-life was monitored during chilled (4 °C) storage for 32 days as were spoilage bacteria. The bacterial counts were the same on samples cooked at 70°C for 2 minutes and 90°C for 10 minutes up to 12 days but thereafter lower counts were obtained in the crab processed using the higher temperature (Table 1).

Barrier properties of the packaging film and shelf-life: Studies were undertaken to investigate the effect of the barrier properties of the packaging film, specifically medium barrier (O_2 permeability rate of <60 cm^3/m^3) versus high barrier O_2 permeability rate of <20 cm^3/m^3) on bacterial growth and the shelf-life of pasteurised crabs. The microbial shelf-life was monitored during chilled (4 °C) storage for 32 days. General bacteria and spoilage bacteria were tested. Lower levels of bacteria were obtained in the high barrier packs, suggesting this film should continue to be used by the crab processing industry.

5. Opportunity/Benefit:

Teagasc is interested in collaborating with seafood processing companies in the use of different ingredients, processing conditions and packaging films to increase yield, enhance the sensory properties and maximize shelf-life.

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6. Dissemination:

1. McDermott, A., Whyte, P., Brunton, N., Lyng, J., Fagan, J. and Bolton, D.J. (2018) The effect of organic acids and sodium chloride on the shelf-life of Irish brown crab (*Cancer pagurus*) meat. *Food Science and Technology* 98: 141-147.

2. McDermott, A., Whyte, P., Brunton, N., Lyng, J., Fagan, J. and Bolton, D.J. (2018). Thermal inactivation of *Listeria monocytogenes* in Irish brown crab (*Cancer pagurus*) meat. Journal of Food Protection, 81, 12, 2003-2006.

3. McDermott, A., Whyte, P., Brunton, N., Lyng, J., Fagan, J. and Bolton, D.J. (2018) Increasing the yield of Irish Brown Crab (*Cancer pagurus*) during processing without adversely affecting shelf-life. FOODs 2018, 7, 99; doi:10.3390/foods7070099, available at file:///C:/Users/Declan.Bolton/Downloads/foods-07-00099.pdf

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