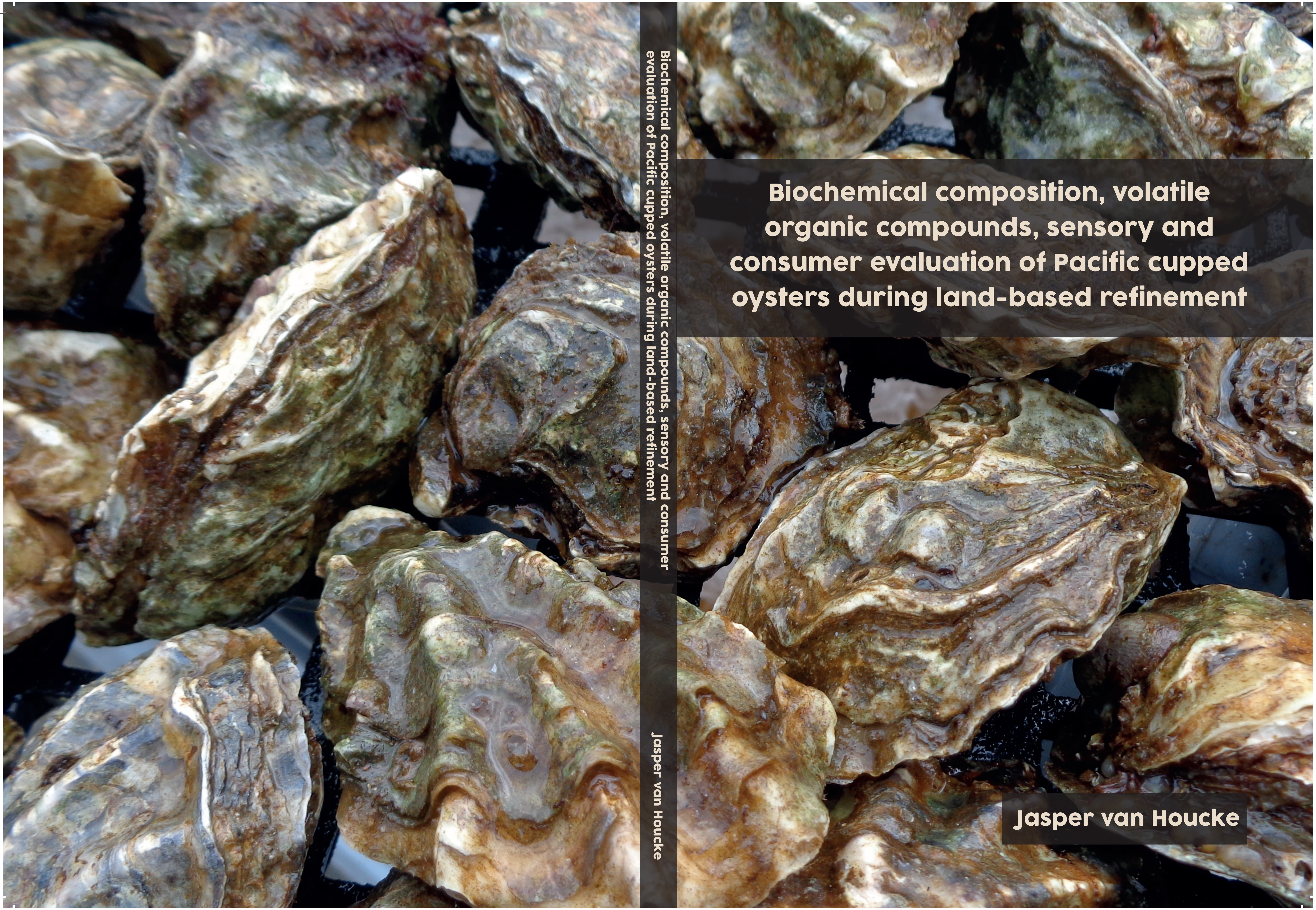

Biochemical composition, volatile organic compounds, sensory and consumer evaluation of Pacific cupped oysters during land-based refinement

Jasper van Houcke

Biochemical composition, volatile organic compounds, sensory and consumer evaluation of Pacific cupped oysters during land-based refinement
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Propositions

1. Native European flat and Pacific cupped oysters significantly differ in their polyunsaturated fatty acid composition and profile of volatile aldehydes, ketones and alcohols.
   (this thesis)

2. Refinement of Pacific cupped oysters with algae results in a decrease in dominant volatile alcohols and in odor, however, less when using algae rich in polyunsaturated fatty acids.
   (this thesis)

3. Profitable aquaculture in the Netherlands is only possible when focusing on quality aspects.

4. Sustainable aquaculture should focus on increasing the utilization of nutrients.

5. While scientific research is aimed and expected to be new and cutting edge work, the method of dissemination is the opposite.

6. New digital communication tools do not always contribute to a better understanding of the message.

Propositions belonging to the thesis, entitled

Biochemical composition, volatile organic compounds, sensory and consumer evaluation of Pacific cupped oysters during land-based refinement

Jasper van Houcke
Wageningen, 8 October 2018

Biochemical composition, volatile organic compounds, sensory and consumer evaluation of Pacific cupped oysters during land-based refinement

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This research was conducted under the auspices of the Graduate School VLAG (Advanced studies in Food Technology, Agrobiotechnology, Nutrition and Health Sciences)

Biochemical composition, volatile organic compounds, sensory and consumer evaluation of Pacific cupped oysters during land-based refinement

Jasper van Houcke

Thesis

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Chapter 1: Introduction

"Oysters are the most tender and delicate of all seafoods. The stay in bed all day and night. They never work or take exercise, are stupendous drinkers, and wait for their meals to come to them."

Hector Bolitho 'The Glorious Oyster' (1960)

Oysters are considered a delicacy throughout Western Europe. Fresh oysters are highly valued and are often marketed as being an exclusive product. In Western Europe the traditional season starts in autumn and ends in late spring. The peak in sales and consumption is during Christmas and New Year celebrations (Piveteau, Gandemer, Baud and Demaimay, 1999; Soletchnik et al., 2001). Oysters are mostly eaten as a fresh raw product. Live oysters are opened by breaking the hinge of the shell, the adductor muscle is cut on both sides, the internal liquid is drained, the oyster tissue is flipped and placed back and presented in the cupped shaped shell. The oysters are then eaten raw with lemon juice and ground pepper.

1.1 Oyster production

The worldwide production of oysters has increased in the last decade (figure 1.1) mostly due to the increase in production in Asia and the increasing popularity of oysters in Asian countries (FAO, 2016).

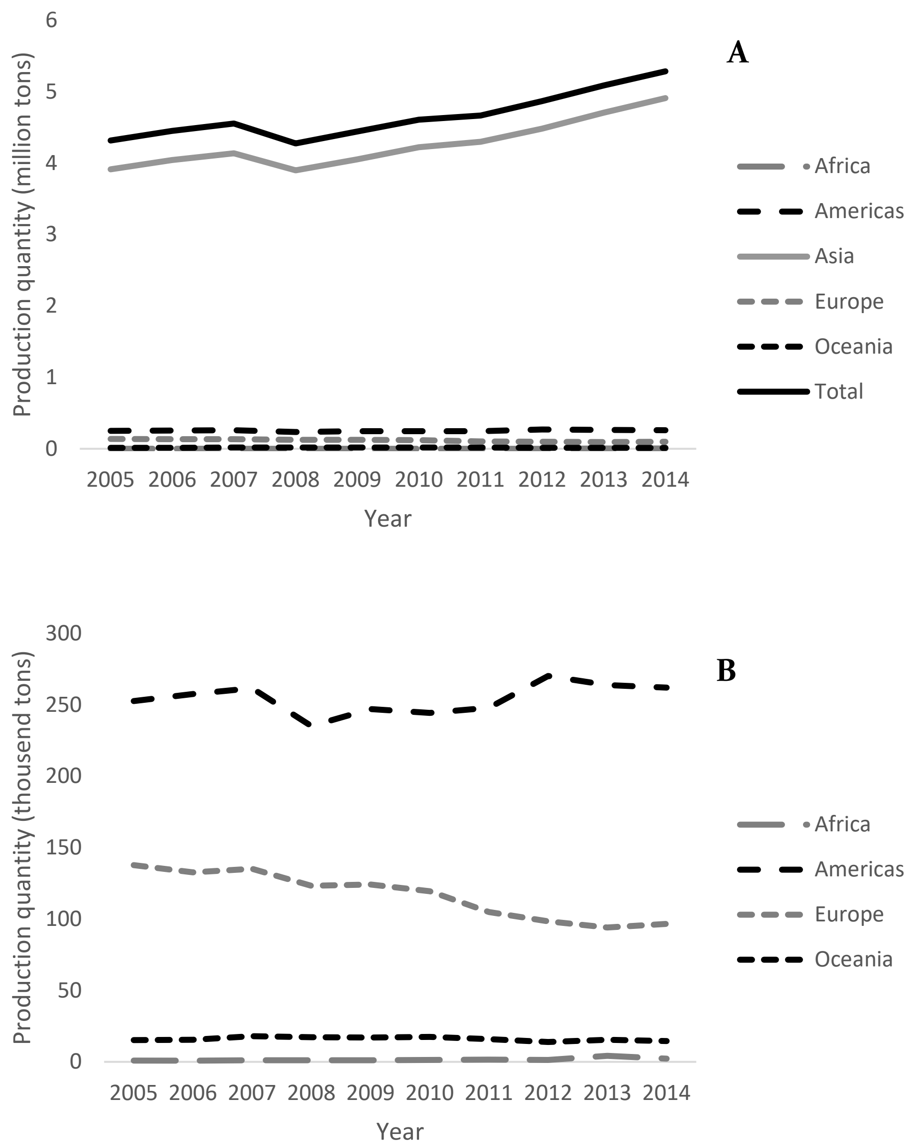

Figure 1.1 Oyster production during the last decade. A = including Asian production. B = excluding Asian production (FAO, 2016).

The total production of oysters in Europe however decreased during the last decade. In 2014 the total production of oysters in Europe was estimated at 96 603 tons of which the vast majority was produced in France (76 705 tons) and Ireland (9 777 tons). The estimated production in the Netherlands was 2 500 tons which makes the Netherlands Europe's third largest producer of oysters (FAO, 2016).

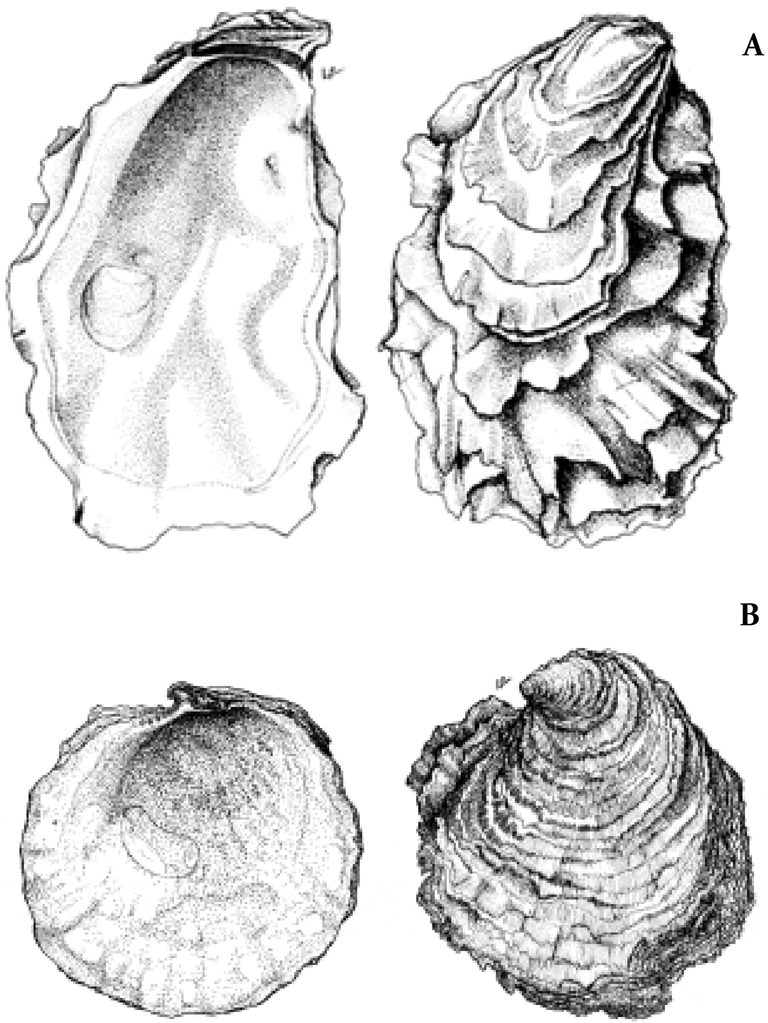

Figure 1.2 Oyster species cultivated in the Netherlands. A=Pacific cupped oysters (*Crassostrea gigas*). B=European flat oyster (*Ostrea edulis*). (FAO, 2016).

In the Netherlands two different oyster species (figure 1.2) are cultivated: The Pacific cupped oyster (*Crassostrea gigas*) and the native European flat oysters (*Ostrea edulis*). Nowadays there are two main areas in the Netherlands where oyster cultivation takes place: The Eastern Scheldt and Lake Grevelingen (figure 1.3). The European flat oyster is mainly cultivated in Lake Grevelingen while the Pacific cupped oyster is cultivated in both areas. The market volume of European flat oysters is rather low compared to the quantity of Pacific cupped oysters (respectively 200 and 2 300 tons in 2014 in the Netherlands) (FAO, 2016). Due to the scarcity of the European flat oyster and its highly-appreciated flavor the value of these oysters is more than four times the value of Pacific cupped oysters (€ 5.67 kg^{-1} and € 1.22 kg^{-1} in 2014 in the Netherlands, respectively) (FAO, 2016).

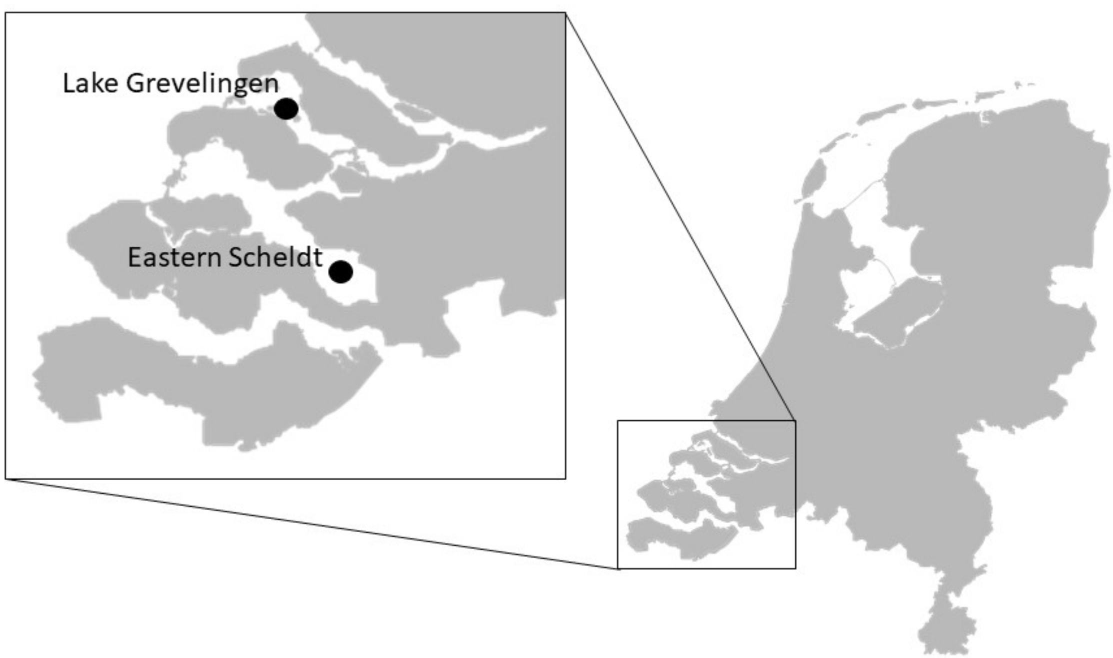

Figure 1.3 Main oyster cultivation areas in the Netherlands.

Production of the European flat oyster declined between 1940 and 1950 due to overfishing. Thereafter severe winters, mixing of broodstock and the introduction of

Bonamiasis due to the parasite *Bonamia ostreae* further decreased the natural populations and the production of the European flat oyster (Troost, 2010).

Production of Pacific cupped oyster is nowadays hampered by mortality due to the oyster herpes virus (Ostreid herpesvirus; OsHV-1 μvar) and the Japanese oyster drill (*Ocinebrellus inornatus*). The OsHV-1 μvar virus was present in the Dutch cultivation areas from 2010 onwards (Engelsma, Roozenburg, Voorbergen-Laarman and Haenen, 2010). Mortality of infected oysters occurs when water temperatures rise above 16°C (Dégremont, 2013). The first presence of the Japanese oyster drill in Dutch cultivation area of the Eastern Scheldt was recorded in 2007 (Goud, Titselaar and Mulder, 2008) and their numbers have increased ever since. The Japanese oyster drill preys on oysters and due to the, up until now, constricted dispersion in the Eastern Scheldt mainly effects Pacific cupped oyster production.

In addition to production related problems it is striking to see that the value of Pacific cupped oysters in the Netherlands is relatively low compared with the value of Pacific cupped oysters produced in other main producing (France and Ireland) or consuming countries (Belgium) (see figure 1.4). The price of oysters in France is € 9,10 per kg while oysters in the Netherlands have a price of € 4.97 per kg (FAO, 2016).

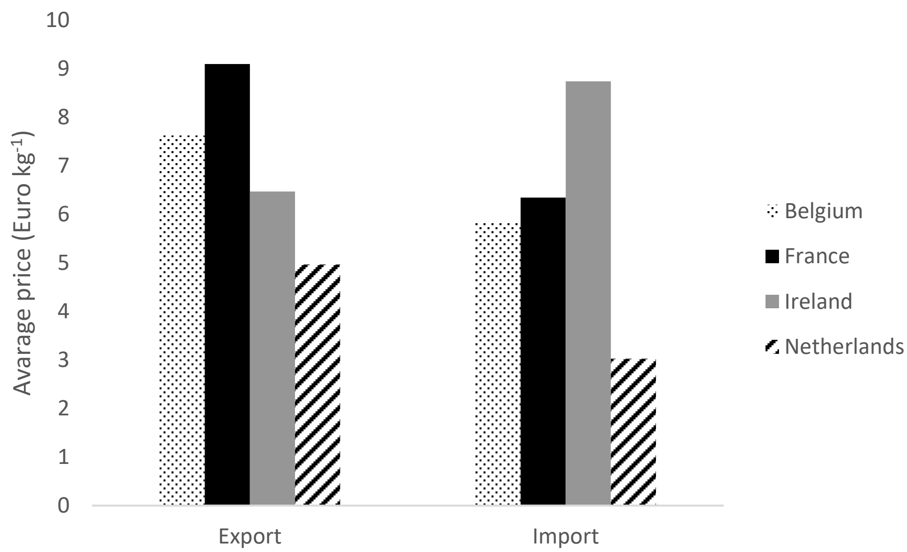

Figure 1.4 Average export- and import prices of live Pacific cupped oysters in Belgium, France, Ireland and the Netherlands in 2013 (FAO, 2016).

There are several differences between the French and Dutch oyster markets which could explain the differences in the market value of the Pacific cupped oyster. France is a net importer of oysters, only a small volume of the production is exported (Girard and Mariojouls, 2003). The Netherlands on the contrary mainly export their oysters to Belgium (approx. 50% of the total production). In the Netherlands Pacific cupped oysters are only sorted by weight and the different weight categories are marketed as different products. The weight categories range from 0 (total weight above 151 g per oyster) up to 5 (total weight between 30 and 45 g per oyster). The French have similar weight categories, but also have important additional quality approaches, product differentiations and certification schemes. For instance, in France oysters are branded according to production area (i.e. Huîtres Île de Ré, Fines de Normandie, Fines de Bretagne – Cancale).

Several quality labels such as the ‘Label Rouge’ for oysters have been developed (Girard and Mariojouls, 2003). Furthermore, several French regions are well-known for their high quality oysters and their so-called refinement process. During refining market sized oysters are kept for a certain period of time in land-based ponds and are fed with naturally occurring microalgae. The goals of the refinement process are to increase the oyster tissue weight and to alter the flavor of the oyster (Piveteau et al., 1999). Soletchnik et al. (2001) stated that the refinement process of oysters in land-based pond systems allows oysters to acquire a more complex taste, outer appearance and the right color thus yielding a higher quality product.

The revenues in the Dutch oyster sector are low as compared to other countries. The national consumption is low while export prices for Dutch oysters are also low compared to oysters exported from other countries. In order to increase revenues both domestic consumption and the value of the Dutch oyster should be increased. Therefore, there is a strong need in the Dutch oyster sector for more product innovation.

1.2 Consumers and oysters

In order to increase domestic consumption and the value of Pacific cupped oyster in the Netherlands a better understanding of Dutch consumers is required. Liu, Kow, Grewal and FitzGerald (2006) and Kow, Yu, FitzGerald and Grewal (2008) mentioned that consumer preferences, consumption patterns and specific marketing are critical in the development of a strong oyster sector and good economic returns.

Gempesaw, Bacon, Wessels and Manalo (1995) showed that consumers with a relatively higher educational background were more likely to buy oysters when compared to consumers with a lower educational background. Also, consumption patterns were significantly affected by the place of residence, age and seafood consumption frequency of the consumers. For instance, consumers were more likely to buy oysters when they lived relatively close to the seashore and were living in bigger cities. Furthermore, likelihood of oyster purchase increased with the age of the consumers. Purchase decision drivers such as health and taste play an important role in the buying behavior of oyster consumers. Consumers considering health as an important aspect in their purchase decision were six times more likely to buy oysters. Consumers considering taste as an important aspect in their purchase decision were 56 times more likely to buy oysters.

Flavor, variety of diet, availability of fresh oysters, health, nutrition and tradition are all mentioned by consumers as reason for oyster consumption (Hanson, House, Sureshwaran, Posadas and Liu, 2002; House, Hanson and Sureshwaran, 2003). Alternatively, important reasons for not consuming oysters are high prices, food safety concerns, lack of availability of fresh oysters, allergy, lack of preparation knowledge, taste, smell and texture of the oysters (Hanson et al., 2002; House et al., 2003).

Kow et al. (2008) and Mueller Loose, Peschel and Grebitus (2012) stressed the factor of convenience as a driver for purchase decisions. Australian consumers preferred, in their studies, pre-opened half-shell oysters over unopened oysters. Other important choice drivers in the study of Mueller Loose et al. (2012) were price, geographical origin of the oyster and oyster species. Kow et al. (2008) showed that Australian consumers had a preference for specific purchase places. Restaurants and specialist fish shops were the most popular purchase places while the supermarket was considered the least popular. Liu et al. (2006) suggested several marketing techniques based on their results on oyster consumption frequency and the place of oyster consumption by Australian consumers. The place of oyster consumption (dine in or out) is dependent on age, gender, residence and labelling while the frequency of consumption is dependent on age, packaging and price. In order to target the younger generations Liu et al. (2006) suggested educating young potential consumers by means of TV advertisements, promotions by role models and new recipes. Marketing strategies should focus on introducing information on different growing regions, growing methods, handling procedures, storage knowledge, nutritional benefits, quick and easy recipes and flavor profiles.

There is a lack of information on Dutch oyster consumers. Consumer profiles are unknown as are the preferences of these consumers. In order to assess which factors determine consumer appreciation of oysters and to identify opportunities for product innovations more insight into Dutch consumer preferences, behavior and attitude is required.

1.3 Sensorial aspects of oysters

Sensorial aspects of oysters (and other food products) are essential in consumer acceptance of new products or product innovations. A good understanding of the sensorial aspects of oysters and how these are influenced is therefore required.

Most of the sensory research regarding oysters is focused on the shelf life and storage conditions of these oysters. Aaraas et al. (2004) used a team of chefs and experienced oyster tasters in the development of attributes for sensory profiling of the European flat oyster in relation to freshness. For odor the attributes sea, seaweed, fishy, shellfish, crab, mud and mire were considered important. The appearance of the oysters was described using the attributes hydration of the gills, hydration of the mantle, color of plum and form of plum. The attributes describing texture were chewiness (1st impression), hardness (after some mastication) and juiciness. For the description of taste the attributes sea-like, salty, pungent, sweet, rich, creamy, seaweed, bitter/bitterness and metallic aftertaste were used.

In the study of Buzin, Baudon, Cardinal, Barillé and Haure (2011) odor attributes used in the freshness assessment of Pacific cupped oysters included global intensity, seaweed and mud. The attributes for the appearance of the oysters were the water and flesh quantity in the shell and green color of gills. Texture attributes in this study included crunch, fleshy and humidity while taste attributes included overall intensity, seaweed, iodized, salty, sweetened, hazelnut, astringency and bitter.

Josephson, Lindsay and Stuiber (1985) used 20 panelists for comparing the sensory properties of both the Pacific cupped oyster (*Crassostrea gigas)* and the American cupped oyster (*Crassostrea virginica)*. The intensity of the attributes fresh melon-like flavor and overall preference were scored on a semi-structured 7 point scale. The Pacific cupped oyster scored significantly higher ($p < 0.05$) for the fresh melon-like flavor than its counterpart the American cupped oyster. Even though it was said by the panelists that differentiation between true melon-like flavors and heavy plant-like and green-seaweed flavors was difficult.

Pennarun, Prost and Demaimay (2002b) established a list of seven descriptors for the odor of fresh raw Pacific oysters. These descriptors were oyster, seaside, seaweed, grass, cucumber, floral and mud. The descriptor cardboard was added to evaluate the odor of vacuum steam distillation oyster extract. Intensity was assessed on a 10 cm scale. The raw oyster and oyster vacuum steam distillation extract showed the same sensory characteristics. Cardboard odor in the oyster extract was attributed to the odor of the blotter strips or a taint caused by oxidation of oyster. Cucumber odor was more profound in the oyster extract as well and was likely to be produced in the extraction process by oxidation of fatty acids. These results were in agreement with earlier work of Pennarun, prost and Demaimay (2002a) where the same descriptors were used in a study comparing two extraction methods (vacuum hydro distillation and dynamic headspace). Pennarun et al. (2002a) found that the intensity of cardboard odor was more intense for vacuum hydro distillation. The authors also found a significant difference ($p < 0.05$) between fresh oyster (reference) and the vacuum hydro distillation extract, whereas the intensity of the cucumber odor was higher in both extracts compared to the oyster reference.

Sensory analysis of triploid and diploid oysters was performed by Allen and Downing (1991). In their consumer test 32 untrained panelists assessed attributes such as flavor and texture on a 5 point scale and overall preference on a 9 point scale. The oysters were steamed for 4 minutes. In addition, a test with an 'expert' panel (19 panelists) being members of oyster farmers association was conducted. In this case the oysters were eaten raw and a reference (high quality oyster) was added to the test. Assessment was done using the same method as in the consumer test. The overall preference was highest for the triploid oysters (7.4 – 7.5) followed by the reference (6.7) and the diploid (5.8 – 6.4). The same pattern of results was found in the texture of the oysters. A score of 4.0 – 4.1, 3.5 and 3.2 – 3.3 for the triploid, reference and diploid oyster respectively

Nell (2002) assumed that the firmer texture and therefore the higher overall preference observed by Allen and Downing (1991) in triploid oysters could be attributed to higher glycogen levels. Regarding flavor, the triploid oyster and the reference (in the 'expert' test) from the study of Allen and Downing (1991) scored best, 3.9 – 4.2, compared with the diploid oyster 3.4. In the consumer test there was a significant difference between the diploid and triploid oyster regarding flavor. The authors speculate that this difference could be due to the glycogen content of the oysters, being higher in the triploid oyster, even though there was no real indication for that. In contradiction a study by Nell, O'Riordan and Ogburn (2006) did not find any significant differences on aspects like odor, flavor, saltiness, texture, aftertaste and overall liking between diploid and triploid oysters. In this study 61 consumers responded in individual sensory booths on a hedonic unstructured 10 cm scale. The only significant difference found in this study was in respect to the appearance of the oysters. The diploid oysters were favored. The authors attribute this difference to the high differences in condition index which is a measure for fullness of the shell. The diploid oysters showed a higher condition index.

Assessing the sensorial aspects of oysters is a complicated matter as different attributes have been used in the literature. Comparisons have been made between Pacific cupped oysters and American cupped oysters and between diploid and triploid oysters. However, no direct comparison of the sensorial aspects of both Dutch oyster species has been investigated and reported.

1.4 Oyster refinement

The French oyster sector applies the process of refinement in order to alter the flavor of oysters and therefore add value to the oysters (Piveteau et al., 1999;. Soletchnik et al., 2001).

Pennarun, Prost, Haure and Demaimay (2003) investigated the sensory properties of Pacific cupped oysters fed with different microalgae diets cultivated under controlled environmental conditions in land-based systems. The authors noted that the three groups of oysters (oyster control group, oysters fed with *Skeletonema costatum* and oysters fed with *Isochrysis galbana*) were differently assessed regarding odor, taste and texture. Both oysters fed with microalgae were characterized by a strong grass odor. A fresh fish odor was noted in the oysters fed with *Isochrysis galbana*, whereas the oysters fed with *Skeletonema costatum* had a seaside odor. Mud odor was weak in all oyster groups, however oysters fed with *Isochrysis galbana* were closest to it on the basis of the factorial correspondence analysis. Furthermore, the oyster control group (fed with naturally occurring phytoplankton in seawater for six weeks) was characterized by a salty and bitter taste. Oysters fed with *Skeletonema costatum* and oysters fed with *Isochrysis galbana* shifted to a sweeter taste. Oysters fed with *Skeletonema costatum* were positioned nearer towards having an astringent taste. And lastly the texture of oysters fed with *Isochrysis* *galbana* were assessed as being firmer, the opposite of the oysters fed with *Skeletonema costatum* which showed a more melting texture. In order to assess whether or not oyster refinement as a product innovation is able to increase value and national consumption levels more insight into and control over the process of refinement is required. The effect of feed on the biochemical composition and its relation to the flavor profile and sensory evaluation of oysters is not yet fully understood. Furthermore, Dutch consumers' attitude towards refined oysters is unknown.

1.5 Thesis aim and outline

During the cultivation period, oysters are exposed to different external factors. Some of these external factors, for instance cultivation location, season and the feed of the oysters are known to have an impact on the composition of the oysters. Naturally the composition of different oyster species also differs. The composition of the oysters and more particular odor- and taste active compounds such as volatile organic compounds (VOCs) and free amino acids (FAAs), have an effect on the flavor profile or product quality. Upon harvesting the product is processed (cleaned, sorted and packed) and afterwards distributed. Through different distributions channels the oysters find their way to consumers. Consumers evaluate the oyster quality in a subjective way. In this thesis we mainly focus on the role of feed during refinement on the biochemical composition of the oysters and the quality assessment thereof by trained experts and by consumers. In order to evaluate the product quality various methods were used in this research (see figure 1.5).

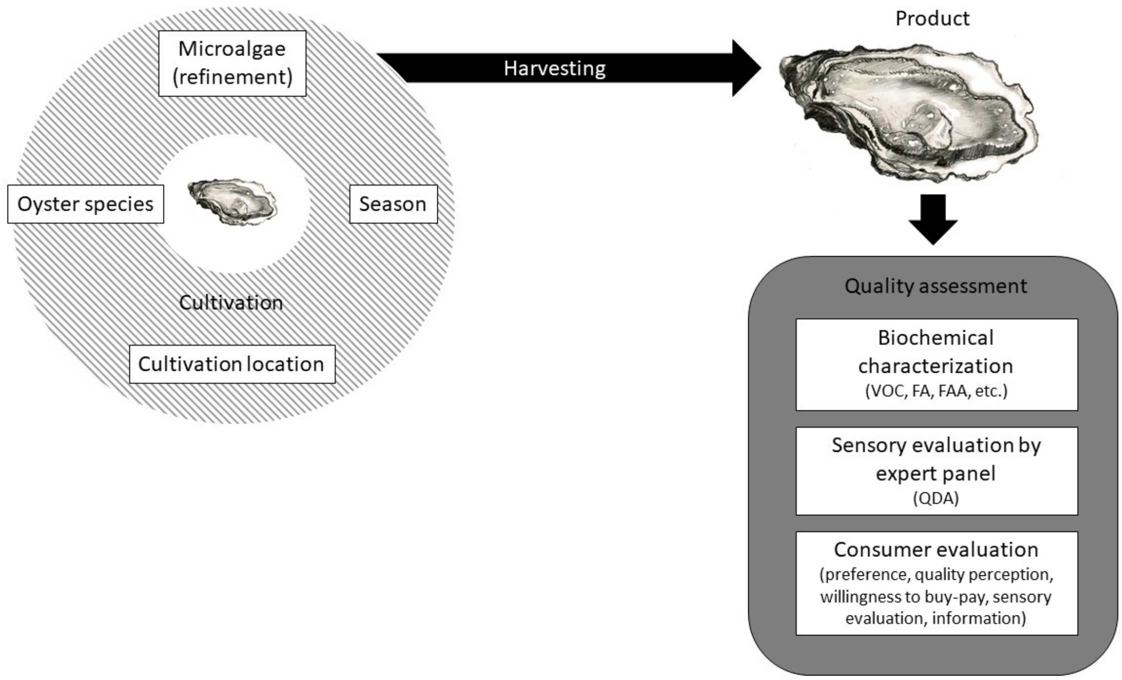

Figure 1.5 Schematic overview of factors determining oyster quality and the different quality assessments used in this thesis.

The overall aims of this thesis are:

1. To study the impact of refinement (feed) on the biochemical composition, flavor profile and sensory characteristics during the cultivation of Pacific cupped oysters.
2. To gain insight into Dutch consumers' preferences, quality perception and factors influencing their evaluation of Pacific cupped oysters.

In order to reach these aims a number of studies were carried out. In **chapter 2** a review on the important odor- and taste active compounds in fresh oysters is presented. The contribution of VOCs, FAAs, '5 nucleotides and bromophenols to the flavor profile of oysters and the origin of these compounds are discussed. A good understanding of the formation and presence of odor- and taste active compounds is necessary in order to identify possible correlations between the results of analytical measurements of these compounds and the results of sensory analysis.

Chapter 3 presents the consumer preference for either the Pacific cupped oyster or the European flat oyster and evaluates the perceived differences in the sensory characteristics for both oyster species. Consumer preference was assessed using two-alternative forced choice tests while the sensory properties were evaluated using a nine-attribute consumer evaluation. The research in this chapter was focused on assessing sensory differences between the Pacific cupped oyster and the European flat oyster and Dutch consumer preference.

In **chapter 4** a comparison of dynamic sensory perception of the Pacific cupped oyster and European flat oyster is presented. Consumers used the Temporal Dominance of Sensations (TDS) method in order to assess the temporal differences in the sensorial properties of both oyster species. As temporal effects are taken into account the outcome could give additional information to the sensory profile test results of chapter 3. In particular with respect to sensorial difference during consumption of oysters and the duration of taste perception.

Chapter 5 presents the biochemical composition and VOC profiles of Pacific cupped oysters and European flat oysters from two important cultivation sites in the Netherlands (Lake Grevelingen and the Eastern Scheldt). VOCs were studied using headspace solid phase micro extraction coupled to GC-MS analysis. Most of the VOCs found in fresh oysters are related to oxidation of the fatty acids of the microalgae eaten by the oysters. Potentially this may lead to differences in VOC profiles of oysters from different cultivation areas as the microalgae composition between areas differs. Furthermore, the VOC profiles of the two different oyster species were also studied to explain species differences.

The effects of refinement with the microalgae *Rhodomonas baltica* and *Skeletonema costatum* on the biochemical composition, VOCs, FAAs, sensory and consumer evaluation of Pacific cupped oysters is presented in **chapter 6**. The microalgae used in this study were selected on the differences in their fatty acid profile. Fatty acids, and in particular PUFAs, are known to be precursors of odor active compounds such as VOCs. Both microalgae were fed to the oysters for up to a period of seven weeks. Comparisons in biochemical composition, VOCs, FAAs of both refined oysters and reference oysters were made in order to assess potential differences in odor- and taste active compounds due to the diet of the oysters. Furthermore, sensory and consumer evaluations of refined and reference oysters were performed in order to assess the effect of the odor- and taste active compounds on the sensorial aspects of the oysters.

In **chapter 7** the insight into the quality perception, purchase intention and the impact of information on the evaluation of refined Pacific cupped oysters (*Crassostrea gigas*) by Dutch consumers is discussed. Using questionnaires the importance of oyster quality parameters to Dutch consumers and the willingness to buy-pay in relation to purchase intention drivers, such as refinement, was evaluated. Furthermore, the effect of product information on consumer evaluation of refined and non-refined oysters was studied. This chapter provides insight into the Dutch oyster consumers and their attitude towards refined oysters.

Chapter 8 discusses the main findings and interpretations of the research presented in this thesis. In addition, a commercial and a theoretical scenario for the cost price of refined oysters are presented. The theoretical scenario is based upon results from this thesis research combined with predicted decreases in the costs of microalgae cultivation. Lastly some practical considerations of this research and future prospects and suggestions are also presented in this chapter.

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Chapter 2: Odor and taste active compounds in oysters

"Small and rich, looking like little ears enfolded in shells, and melting between the palate and the tongue like salted sweets."

Guy de Maupassant (1850-1893) 'Bel Ami'

Jasper van Houcke, Jozef Linssen & Joop Luten

Odor- and taste active compounds play an important role in the overall flavor of food items such as oysters. Odor active compounds are usually small and volatile in nature (Shahadi, 1998). These so called volatile organic compounds (VOCs) are characterized by relative low boiling temperatures and are therefore often in the gas phase when oysters are consumed. Taste active compounds are generally non-volatile. Important taste-compounds in oysters include free amino acids (FAAs), inorganic salts, 5' nucleotides and bromophenols (Nguyen and Wang, 2012). The contribution of these compounds to the overall flavor profile of oysters is not always clear. The overall flavor profile of oysters is dependent on the odor- or taste threshold of the compounds and the concentration within the oyster tissue and the complexity of the matrix of the oyster tissue itself. Matrix compounds could have an enhancing, inhibiting or masking effect on the contribution of the individual odor- and taste active compounds.

In this chapter, an overview of odor- (VOCs) and taste active (FAAs, inorganic salts, 5' nucleotides and bromophenols) compounds present in fresh oysters is given. Further the contribution of these compounds to the overall oyster flavor is discussed.

2.1 Odor active compounds

Ronald and Thomson (1964) were the first to investigate VOCs of oysters. Their study revealed the presence of dimethyl sulfide in fresh Pacific cupped oysters. Furthermore, they showed that upon storage of the oysters thiols and other sulfur compounds, besides dimethyl sulfide, were detected. The dimethyl sulfide content increased during storage of the oysters.

Josephson, Lindsay and Stuiber (1985) studied the VOC profiles of two oyster species namely the Pacific cupped oyster (*Crassostrea gigas*) and the Atlantic oyster (*Crassostrea virginica*). They found 17 VOCs in the headspace of extracts from the Atlantic oyster and 29 VOCs in the headspace of Pacific cupped oyster extract. The following VOCs were only identified in Pacific cupped oysters: 1-penten-3-one, 2,3-pentanedione, 1,3,5-octatriene, 3-hexen-1-ol, (E,Z)-2,4-heptadienal, (E,E)-2,4-heptadienal, (E)-2-nonenal, dimethyl sulfoxide, (E,E)-3,5-octadien-2-one, (E,Z)-nonadiendal, 2-octen-1-ol, 3,6-nonadien-1-ol. About 31.9% and 31.4% of the total lipids in the Pacific cupped oyster and Atlantic oyster were n-3 polyunsaturated fatty acids (PUFAs). The total lipid concentration in the Pacific cupped oyster (2.8% of WW) was about 3-fold higher than in the Atlantic oyster (0.8% of WW). The authors attributed the differences in VOCs to the availability of appropriate enzyme systems in the different oyster species. This hypothesis was strengthened by the fact that the formation of certain VOCs could be suppressed by including selective enzyme inhibitors in the oyster extracts.

Nowadays it is known that most of the VOCs measured in oysters (or oyster products) are related to enzymatic lipid oxidation and the majority of these more specifically to oxidation of n-3 PUFAs (Josephson et al., 1985; Piveteau et al., 2000). Other VOCs measured in oysters arise from amino acid degradation, carotenoid degradation, polysaccharide degradation, Maillard reaction, Strecker degradation or unknown origin. Table 2.1 gives an overview of VOCs measured in fresh oysters.

Table 2.1 Volatile organic compounds (VOCs) measured in fresh oysters and their origin.

Compound	Reference	Origin
Aldehydes		
(E)-2-Butenal	[c]	unknown origin
(*E*)-2-Hexenal	[d,e,f,h,i]	*n*-3 PUFA oxidation
(*E*)-2-Nonenal	[b,d]	*n*-6 PUFA oxidation
(*E*)-2-Octenal	[d,f,h,i]	*n*-6 PUFA oxidation
(*E*)-2-Pentenal	[d,e,f,h,i]	*n*-3 PUFA oxidation
(*E,E*)-2,4-Heptadienal	[b,d,e,f,h,i]	*n*-3 PUFA oxidation
(E,E)-2,4-Octadienal	[d]	*n*-3 PUFA oxidation
(E,Z)-2,4-Heptadienal	[b]	unknown origin
(E,Z)-2,6-Nonadienal	[b,d,e,f,g,h,i]	*n*-3 PUFA oxidation
(*Z*)-2-Octenal	[d,e,f]	*n*-6 PUFA oxidation
(Z)-4-Heptenal	[e,f,h,i]	*n*-3 PUFA oxidation
2,4-Heptadienal	[d]	*n*-3 PUFA oxidation
2-Ethylpropenal	[d]	*n*-9 MUFA oxidation
2-Methyl-(E)-2-butenal	[c]	unknown origin
2-Methyl-2-pentenal	[b]	unknown origin
2-Methylbutanal	[c]	unknown origin
2-Methylpropanal	[c]	unknown origin
2-Pentenal	[b]	unknown origin
4-Ethylbenzaldehyde	[f]	carotenoid degradation
Benzaldehyde	[b,d,h,i]	amino acid degradation
Butanal	[c]	unknown origin
Decanal	[d,e,f]	*n*-9 MUFA oxidation
Heptanal	[h,i]	unknown origin
Hexanal	[c,d,h,i]	*n*-6 PUFA oxidation
Lilac aldehyde	[h,i]	unknown origin
Methional	[e,f]	Strecker degradation
Nonanal	[b,e,f]	*n*-9 MUFA oxidation
Octanal	[c,d,e,f]	*n*-9 MUFA oxidation
Pentanal	[c,h,i]	*n*-6 PUFA oxidation
Alcohols		
(*E*)-2-Octen-1-ol	[d,e,f]	*n*-6 PUFA oxidation
(*E*)-2-Penten-1-ol	[c,d,e,f,i]	*n*-3 PUFA oxidation
(E)-3-Hexen-1-ol	[e,f]	unknown origin
(E)-6-Nonen-1-ol	[f]	unknown origin
(E, Z)-3,6-Nonadien-1-ol	[e,f]	unknown origin
(Z)-2-Octen-1-ol	[i]	*n*-6 PUFA oxidation
(Z)-2-Penten-1-ol	[c,d,h]	*n*-3 PUFA oxidation
(Z)-3-Hexen-1-ol	[d]	*n*-3 PUFA oxidation

Compound	Reference	Origin
(Z,Z)-1,5-Octadien-3-ol	d,h	*n*-3 PUFA oxidation
1,5-Octadien-3-ol	b,i	*n*-3 PUFA oxidation
1-Hepten-3-ol	d,e,f	*n*-9 MUFA oxidation
1-Hexen-3-ol	c	unknown origin
1-Octanol	e,f	*n*-9 MUFA oxidation
1-Octen-3-ol	b,c,d,e,f	*n*-6 PUFA oxidation
1-Pentanol	b,e,f,i	*n*-6 PUFA oxidation
1-Penten-3-ol	b,c,d,e,f,g,h,i	*n*-3 PUFA oxidation
2-(2-Butoxyethoxy)ethanol	f	unknown origin
2,5-Octadien-1-ol	b	unknown origin
2-Butoxyethanol	e,f	unknown origin
2-Ethyl-hexanol	c,e,f	unknown origin
2-Hexanol	c	unknown origin
2-Nonanol	e,f	unknown origin
2-Octen-1-ol	b,h	unknown origin
3,6-Octadien-1-ol	b	unknown origin
3-Cyclohexene-1-ethanol	h,i	unknown origin
3-Hexanol	e,f	*n*-9 MUFA oxidation
3-Hexen-1-ol	b	unknown origin
3-Methyl-1-butanol	f	unknown origin
3-Methylthio-1-propanol	e,f	reduction product of Strecker degradation
3-Octanol	e,f	unknown origin
3-Octenol	g	unknown origin
3-Penten-2-ol	e,f	unknown origin
Cyclopentanol	b,d	*n*-3 PUFA oxidation
Dodecanol	f	unknown origin
Ethanol	b	unknown origin
Heptanol	d,e,f	*n*-9 MUFA oxidation
Hexanol	d	other fatty acid oxidation
Phenol	e,f	polysaccharide degradation
Ketones		
(E,E)-3,5-Octadien-2-one	b,e,f,i	*n*-3 PUFA oxidation
1,5-Octadien-3-one	b	unknown origin
1-Octen-3-one	b,d,h,i	*n*-6 PUFA oxidation
1-Penten-3-one	b,d,h,i	*n*-3 PUFA oxidation
2,3-Octanedione	i	unknown origin
2,3-Pentanedione	b,d,h,i	unknown origin
2-Nonanone	c,d,i	other fatty acid oxidation
2-Pentanone	c	unknown origin
2-Undecanone	c,e,f	carotenoid degradation
3-Hydroxy-2-butanone	c	unknown origin

Compound	Reference	Origin
3-Nonanone	e,f	unknown origin
3-Octanone	d,e,f,g,h	other fatty acid oxidation
3-Pentanone	d	unknown origin
4-Methyl-2-pentanone	c	unknown origin
6-Methyl-5-hepten-2-one	d,e,f	carotenoid degradation
Acetophenone	e,f	unknown origin
Alkenes		
(E,E,E)-1,3,6-Octatriene	d	other fatty acid oxidation
(*E,E,Z*)-1,3,5-Octatriene	d,e,f,g	*n*-3 PUFA oxidation
(Z,Z)-3,5-Octadiene	g	unknown origin
(Z,Z,Z)-1,3,5-Octatriene	d	other fatty acid oxidation
1,3,5-Octatriene	b	unknown origin
1,3,6-Octatriene	d	other fatty acid oxidation
1,3-Octadiene	d	other fatty acid oxidation
2,4-Octadiene	d	other fatty acid oxidation
2,5-Octadiene	g	unknown origin
4-Methyl-1,4-heptadiene	g	unknown origin
Pentanediene	d	unknown origin
1-Dodecene	e,f	unknown origin
1-Hexadecene	e,f	unknown origin
1-Octadecene	e,f	unknown origin
(E)-2-Octene	g	unknown origin
(Z)-7-Tetradecene	e,f	unknown origin
Cyclene		
1,3-Cyclooctadiene	g	unknown origin
Cedrene	g	unknown origin
Limonene	c,e,f	carotenoid degradation
α-Pinene	c,g	unknown origin
β-Pinene	c	unknown origin
Alkanes		
2,6,10,14-Tetramethylpentadecane	e,f	unknown origin
2-Thiopropane	i	unknown origin
Dodecane	e,f,g	unknown origin
Eicosane	e,f	unknown origin
Heneicosane	f	unknown origin
Heptadecane	e,f	unknown origin
Hexacosane	g	unknown origin
Hexadecane	e,f	unknown origin
Hexane	h,i	unknown origin

Compound	Reference	Origin
Nonadecane	e,f	unknown origin
Nonane	d	unknown origin
Octadecane	e,f	unknown origin
Pentacosane	g	unknown origin
Pentadecane	c	unknown origin
Tetradecane	e,f,g	carotenoid degradation
Tricosane	g	unknown origin
Tridecane	e,f	carotenoid degradation
Cyclane		
2-Propenyle-cyclopentane	g	unknown origin
7-Oxabicyclo[2.2.1]heptane	g	unknown origin
Furans		
2-Ethylfuran	h,i	*n*-3 PUFA oxidation
2-Methylfuran	i	unknown origin
2-Pentylfuran	c	unknown origin
Acids		
(Z)-11-Hexadecenoic acid	g	unknown origin
2-Methylpentanoic acid	c	unknown origin
3-Methylbutanoic acid	f	unknown origin
Acetic acid	b,c	unknown origin
Butonoic acid	g	unknown origin
Butyric acid	c	unknown origin
Decanoic acid	f	unknown origin
Isobutyric acid	c	unknown origin
n-Hexadecanoic acid	g	unknown origin
Nonanoic acid	f	*n*-6 PUFA oxidation
Octadecanoic acid	g	unknown origin
Octanoic acid	e,f	*n*-6 PUFA oxidation
Pentadecanoic acid	g	unknown origin
Pentanoic acid	e,f	*n*-6 PUFA oxidation
Propanoic acid	b,f,g	unknown origin
Propionic acid	c	unknown origin
Tetradecanoic acid	g	unknown origin
Valeric acid	c	unknown origin
Aromatic		
1,2,4-Trimethylbenzene	e,f	polysaccharide degradation
1,3-Diethenyl benzene	g	unknown origin
1,4-Diethenyl benzene	g	unknown origin

Compound	Reference	Origin
1-Ethenyl-3-ethyl-benzene	[g]	unknown origin
1-Ethenyl-4-ethyl-benzene	[g]	unknown origin
5-Ethyl-m-xylene	[g]	unknown origin
Butylated hydroxytoluene	[e,f]	unknown origin
Ethylbenzene	[d,e,f,g]	polysaccharide degradation
Isocyanato-benzene	[g]	unknown origin
m-Xylene	[f]	polysaccharide degradation
o-Xylene	[f]	polysaccharide degradation
p-Xylene	[d,e,f]	polysaccharide degradation
Styrene	[d]	unknown origin
Toluene	[d]	carotenoid degradation
Others		
2,4,6-Trimethylpyridine	[e,g]	unknown origin
3-Nonyne	[g]	unknown origin
Acetylpyrazine	[e,f]	Maillard reaction
Benzothiazole	[g]	unknown origin
Dimethyl disulfide	[b,d]	amino acid degradation
Dimethyl sulfoxide	[b,d]	unknown origin
Dimethyl sulfide	[a,e,f,g]	unknown origin
Dimethyl trisulfide	[b]	unknown origin
Ethylacetate	[c]	unknown origin
Ethylpyrazine	[e,f]	Maillard reaction
Methoxy phenyl oxime	[g]	unknown origin

[a] Ronald and Thomson, 1964. [b] Josephson et al., 1985. [c] Cha, 1995. [d] Piveteau et al., 2000. [e] Pennarun et al., 2002. [f] Pennarun et al., 2003. [g] Zhang et al., 2009. [h] Fratini et al., 2012. [i] Fratini et al., 2013.

A study by Cha (1995) identified 32 VOCs in raw Atlantic oyster. Eight aldehydes, five ketones, seven alcohols, six acids, three terpenes and three miscellaneous compounds were detected. Cha (1995) compared the VOCs of fresh oysters with oyster hydrolysate. VOCs such as 2-hexanol, 1-hexen-3-ol, (Z)-2-penten-1-ol, (E)-2-penten-3-ol, 2-ethyl-1-hexanol, acetic acid, propionic acid, isobutyric acid, butyric acid and 2-methylpentanoic acid were only detected in the fresh oysters. It was suggested that the terpenes found could originate from essential lipids of algae passed through the food chain.

Piveteau et al. (2000) identified 52 VOCs in Pacific cupped oysters. The VOCs found were alcohols (11), hydrocarbons (19), aldehydes (14), ketones (7) and one sulfur-containing compound. Two-thirds, predominately alcohols and carbonyl compounds, originated from n-3 PUFA oxidation. The compounds 1-penten-3-one, 1-penten-3-ol and (Z,Z)-1,5-octadien-3-ol were identified as the main volatiles originating from n-3 PUFA oxidation. The main oxidation products of n-6 PUFA oxidation were identified as 1-octen-3-ol and 1 –octen-3-one. In contradiction with n-3 PUFA, which are found in high concentrations (41.5% of the total lipids) in oysters, n-6 PUFA are usually present in low concentrations (4.4% of the total lipids) in oysters. The oxidation of n-9 mono unsaturated fatty acids (MUFA) led to aldehydes of which 2-ethylpropenal was reported as the most abundant. Compounds such as; 3-octanone, two isomers of 2,4-octadiene, two isomers of 1,3- and (Z,Z,Z)-1,3,5-octatriene originated from fatty acid oxidation as well, even though their exact origin remained unknown. The VOCs toluene and 6-methyl-5-hepten-3-one originated from carotenoid oxidation, while dimethyl disulfide originated from amino acid degradation. Origins of benzaldehyde, nonane, pentanediene, 3-pentanone, aromatic hydrocarbons and cyclic alkenes were reported unknown in this study.

Pennarun, Prost and Demaimay (2002) identified 59 VOCs in Pacific cupped oyster extract. The oyster extract contained 18 alcohols, 10 aldehydes, 6 ketones and 8 alkaloids. Four of the identified VOCs (Pennarun et al. 2002) were also detected in the study of Josephson et al. (1985); (E,E)-2,4-heptadienal, (E,Z)-2,6-nonadienal, 1-octen-3-ol and nonanal. However, comparison with the results of Piveteau et al. (2000) gives 14 identical VOCs in both studies; (E, E, Z)-1,3-5-ocatriene, (E)-2-pentenal, 1-penten-3-ol, (E)-2-hexenal, (E)-2-penten-1-ol, (E,E)-2,4-heptadienal, (E,Z)-2,6-nonadienal, (Z)-2-octenal, 1-octen-3-ol, (E)-2-octen-1-ol, octanal, decanal, 3-octanone and 6-methyl-5-hepten-2-one.

In a study regarding the influence of diet on the odorant composition and the sensory characteristics of raw Pacific cupped oysters, Pennarun,Prost, Haure and Demaimay (2003) found 59 VOCs before and 44 or 56 VOCs after six weeks of feeding oyster with either *Skeletonema costatum* or *Isochrysis galbana* for six weeks. The fatty acid profiles of oysters fattened with the microalgae were positively correlated with the fatty acid profiles of the microalgae which were fed to the oysters. As a consequence, a microalgae diet caused changes in the oyster's aroma composition. Oyster fed *Skeletonema costatum* showed less VOCs originating from n-6 PUFA, due to a lower level of n-6 PUFAs in the oysters. VOCs such as, (Z)-2-octenal, (E)-2-octen-1-ol and pentanoic acid were not found in oyster fed with *Skeletonema costatum*. Differences for VOCs originating from n-3 and n-9 PUFA were less clear between oysters fed with either *Skeletonema costatum* or *Isochrysis galbana*.

Zhang, Li, Wang, Zhang and Chen (2009) studied the VOCs of Pacific cupped oysters during storage of the oysters. In total 20 VOCs were found in fresh oysters. 1(R)-α-Pinene, 4-methyl-1,4-heptadiene, 2-propenyl-cyclopentane, 7-oxabicyclo[2.2.1]heptane, ethylbenzene, 5-ethyl-*m*-xylene and benzothiazole were only measured in the fresh Pacific cupped oysters. Comparing the results of Zhang et al. (2009) with the studies of Piveteau et al. (2000), Pennarun et al. (2002) and Pennarun et al. (2003), it is striking that only a few VOCs found by Zhang et al. (2009) in the fresh oysters were also detected by the other authors. (E,E,Z)-1,3,5-Octatriene, 1-penten-3-ol, (E,Z)-2,6-nonadienal, 3-octanone, dimethyl sulfide, ethylbenzene, dodecane and tetradecane were the only VOCs also reported in fresh Pacific cupped oysters in previous studies. Zhang et al. (2009) identified dimethyl disulfide, propanoic acid, 3-methylbutanoic acid and (E,Z)-3,6-nonadien-1-ol only in the deteriorated oysters, whereas Piveteau et al. (2000), Pennarun et al. (2002) and Pennarun et al. (2003) found these VOCs in fresh oysters.

Fratini, Lois, Pazos, Parisi and Medina (2012) measured 22 VOCs in fresh European flat oysters (*Ostrea edulis*). In total 11 aldehydes, five alcohols, four ketones, one hydrocarbon and one furan were identified. In comparison with other shellfish species like the common cockle (*Cerastoderma edule*), the pod razor shell (*Ensis ensis*), pullet carpet shell (*Venerupis pullastra*), mussels (*Mytilus galloprovincialis*) and goose barnacle (*Pollicipes cornucopia*), the European flat oysters showed the highest VOC content. The main VOCs found in the European flat oyster were the alcohols: 3-cyclohexene-1-ethanol, (Z)-1,5-octadien-3-ol and 2-octen-1-ol. Other VOCs also present in relative high concentrations were benzaldehyde and lilac aldehyde.

The VOCs of lagoon and sea-refined Pacific cupped oysters during storage were analyzed by Fratini et al. (2013). In total 28 VOCs were found including 12 aldehydes, six alcohols, six ketones, two alkanes and two furans. The VOC profile remained the same during storage however the content of the individual VOCs increased during a storage period of ten days. Furthermore Fratini et al. (2013) showed that fresh lagoon refined oysters have a higher overall VOC content in comparison with fresh sea refined oysters. Alcohols were most abundant due to high concentrations of 1,5-octadien-3-ol and 3-cyclohexene-1-ethanol. (E,Z)-2,6-Nonadienal, (E,E)-2,4-heptadienal, (E)-2-pentenal and hexanal were the main aldehydes in both lagoon- and sea refined oysters.

Only a very few studies regarding the odor of fresh oysters (Piveteau et al., 2000; Pennarun et al., 2002; Pennarun et al., 2003) actually identified the odor active VOCs (see table 2.2). Gas chromatography – olfactometry is often used in order to evaluate the odor descriptor and the potency of the VOCs as an odor active compound. Therefore, VOCs are extracted from the oyster tissue (Piveteau et al., 2000; Pennarun et al., 2002; Pennarun et al., 2003). Next the VOCs are separated by their polarity on a GC-column. Detection is performed by flame ionization and by sniffing by trained panelists at a separate detection port.

Panelists detected 42 odors in the extract of Pacific cupped oysters by olfactometry in a study described by Piveteau et al. (2000). Odors described as green/garlic/sulfur were followed by odors such as citrus and mushroom-like and odors described as green/cucumber and marine, respectively. Only 12 odors were definitely attributed to the identified VOC out of 42 detected odors. 1-Penten-3-one and hexanal were described by a green odor, (E,E)-2,4-heptadienal was described by a green, mushroom-like odor and (E,Z)-2,6-nonadienal by a cucumber odor. The VOCs 1-octen-3-ol and 1-octen-3-one were both described by a mushroom odor, (E)-2-octenal by an almond odor, octanal and 6-methyl-5-hepten-3-one by a citrus-like odor, decanal by a marine odor and dimethyl sulfide by a sulfur, crustacean and green odor. The odor description of green and cucumber was tentatively attributed to the volatile (E,E)-2,4-octadienal.

Table 2.2 Odor active compounds found in fresh Pacific cupped oysters and their odor description and detection threshold in water. Data from Piveteau et al., 2000; Pennarun et al., 2002; Pennarun et al., 2003.

Compound	Odor description	Detection threshold (ug kg^{-1})
n-3 PUFA oxidation		
(E)-2-Penten-1-ol	mushroom	
(E)-2-Pentenal	grass	1500
(E,E)-2,4-Heptadienal	mushroom, moss, green	
(E,E)-3,5-Octadien-2-one	fatty fruity	
(E,Z)-2,6-Nonadienal	fatty odor, green, cucumber, melon	0,01-1,0
(Z)-4-Heptanal	white boiled fish, cooked white fish	0,8-1,0
1-Penten-3-one	green	1,3
(*E,E,Z*)-1,3,5-Octatriene	plastic, green	
(E,E)-2,4-Octadienal	green, cucumber	
n-6 PUFA oxidation		
(E)-2-Octenal	citrus, cucumber, almond	
1-Octen-3-one	mushroom	0,09
Hexanal	oxidized fatty, green, grass, powerful, penetrating	
(*Z*)-2-Octenal	mushroom, marine	3
1-Octen-3-ol	boiled potato, mushroom	1-10
Octanoic acid	fresh, moss	3 000
n-9 MUFA oxidation		
Octanal	citrus	0,7
Decanal	marine, cucumber	10 000
1-Octanol	cucumber	110 - 130
Carotenoid degradation		
6-Methyl-5-hepten-2-one	ether, alcohol	50
2-Undecanone	cucumber, fresh	
4-Ethylbenzaldehyde	minty, aniseed	
Limonene	moss, green	10
Maillard reaction		
Ethylpyrazine	grilled	6 000 - 22 000
Acetylpyrazine	grilled	62

Compound	Odor description	Detection threshold ($\mu g\ kg^{-1}$)
Reduction product of Strecker degradation		
3-Methylthio-1-propanol	animal	
Unknown origin		
2,3-Pentanedione	buttery, milky	
(E)-3-Hexen-1-ol	moss, green, fresh	
Dimethyl sulfide	crustacean, green, sulfur	0,3 - 1
3-Octanol	moss, sulfury	
(Z)-7-Tetradecene	nc	
3-Nonyne	cucumber, marine	
2-Nonanol	cucumber	
(E)-6-Nonen-1-ol	green, fresh	
(E, Z)-3,6-Nonadien-1-ol	marine, cucumber	10

In a study by Pennarun et al. (2002) 25 odor-active VOCs were detected by at least 3 out of 10 panelists in a vacuum steam distilled Pacific cupped oyster extract. The compounds (E)-2-penten-1-ol (mushroom), (E)-3-hexen-1-ol (moss/fresh), decanal (marine) and 2-undecanone (cucumber/fresh) were detected by all panelists. Nine panelists detected (E)-2-pentenal (grass) and (E,E)-2,4-heptadienal (mushroom/moss). An unknown VOC (sulfurous/garlic) was detected by eight panelists. Limonene (moss/green) and an unknown compound (cucumber/green) were detected by seven panelists. The remaining 16 VOCs; (E,E,Z)-1,3-5-octatriene (plastic/green), (Z)-4-heptenal (boiled white fish), ethylpyrzine (grilled), 3-octanol (moss/sulfurous), unknown (mushroom), 1-octen-3-ol (boiled potato), methional (boiled potato), 2-nonanol (cucumber), 1-octanol (cucumber), (E,Z)-2,6-nonadienal (cucumber), acetophenone (animal), 3-methlthio-1-propanol (animal), acethylpyrazine (grilled), (E,Z)-3,6-nonadien-1-ol (marine), unknown (green) and octanoic acid (non-common descriptor) were detected by less than seven out of the ten panelists and considered less potent. Many of the odor-active VOCs found by Pennarun et al. (2002) had a fresh, marine and green odor. Some of the grilled odors (ethylpyrazine and acetylpyrazine) were attributed to the extraction and concentration process.

Pennarun et al. (2003) showed a marked difference in VOCs in Pacific cupped oyster fed microalgae diets. In oysters fed *Skeletonema costatum* 23 odor-active VOCs were detected, whereas 25 odor-active VOCs were found in oysters fed *Isochrysis galbana*. Most of the compounds were common to all the groups. However 6-methyl-5-hepten-2-one (ether /alcohol) and an unknown VOC (ether) are classified characteristic for the *Skeletonema costatum* diet. VOCs characteristic for the *Isochrysis galbana* diet are 3-nonyne (cucumber/marine), 6-(E)-nonen-1-ol (green /fresh), 4-ethylbenzaldehyde (minty/aniseed) and an unknown VOC (fresh). In the extract of oysters fed with *Skeletonema costatum* (E,E,Z)-1,3,5-ocatriene (plastic, green) was detected by six panelists, whereas in the extract of oysters fed with *Isochrysis galbana* only three panelist detected the VOC. In oysters fed with *Skeletonema costatum* (E)-2-pentenal (grass odor) was found in higher concentrations. The VOC (Z)-2-ocental was not detected in oysters fed with *Skeletonema costatum*. Octanoic acid (fresh, moss) was detected by eight panelists in oysters fed with *Isochrysis galbana* and by five panelists in oysters fed with *Skeletonema costatum*. In oysters fed with *Skeletonema costatum* the compound 1-octanol was not detected by the panelists. All of the above mentioned detected odor-active VOCs were in agreement with the amount of precursors found in the oyster tissue. In contradiction to these results (E)-2-ocental was detected in oysters fed with *Skeletonema costatum*, even though these oysters contained low n-6 PUFA concentrations.

Co-elution was considered a problem for quantification of odor-active compounds in most of the studies mentioned above. In Pacific cupped oysters fed with the microalgae diets (Pennarun et al., 2003) a boiled potato odor was perceived as more intense in opposition to the natural phytoplankton fed oysters. Methional is known to be responsible for a boiled potato odor and not only co-eluted with 1-octen-3-ol (mushroom), but it was also suggested that co-elution with another unknown (non-odorant or odorless) compound occurred, causing blur in the results obtained. Furthermore, because VOCs are detected individually using gas chromatography – olfactometry, as separated using their polarity, enhancing, inhibition or masking effects were not taken into consideration in these studies.

2.2 Taste active compounds

Numerous studies have shown the importance of taste active compounds in seafood, such as, free amino acids, 5'-nucleotides and bromophenols (e.g. Chen and Zhang, 2007; Kani, Yoshikawa, Okada and Abe, 2008; Whitfield, Helodoniotis and Smith, 2002).

In order to evaluate the contribution of individual taste-active compounds to the overall taste of the seafood product omission tests are usually used. Based on the composition of an extract from the seafood product synthetic equivalents are made. In case the synthetic equivalents resemble the natural extracts then the role of each compound can be determined by omitting the compound in question from the synthetic extract. Usually 3-

Alternative Forced Choice tests (3-AFC) are performed in order to evaluate if panelists taste differences. The results of these tests for both scallop and Manilla clam are shown in table 2.3.

Table 2.3 Decrease in flavor characteristics of scallop and Manilla clam according to omitted compounds in synthetic extracts (adapted from Fuke and Ueda, 1996).

Compound omitted	**Scallop[a]**	**Manilla clam[b]**
Glutamate	umami	umami
	sweetness	sweetness
	palatability	palatability
Glycine	sweetness	sweetness
	palatability	
Alanine	sweetness	
Arginine	character	character
AMP	umami	umami
	sweetness	sweetness
	palatability	palatability
Na^+	umami	sweetness
	character	umami
	palatability	sourness
Cl^-	umami	sweetness
	character	umami
	palatability	palatability

[a] data from Wantanabe et al., 1990. [b] data from Fuke and Konosu, 1991.

Taste active compounds involved in the taste formation of scallops (adductor muscle only) include: glutamate, glycine, alanine, arginine, adenosine-5'-monophosphate (AMP), sodium and chloride ions. The same taste active compounds, with the exception of alanine, are involved in the taste formation of Manilla clams. The results of these studies showed that glutamate plays a role in the formation of umami taste and sweetness in shellfish. Glycine seems to influence the perceived sweetness of shellfish while arginine contributes to the characteristic taste of both scallop and Manilla clam. Fuke and Konosu

(1991) suggest that the bitter taste of arginine is masked with other taste active compounds when present in low concentrations.

Specific omission studies on taste active compounds and their role in the taste formation of oysters are unfortunately missing. A more theoretical approach of calculating the, so called, Taste Activity Value (TAV) (Kato, Rhue and Nishimura, 1989) of the compounds present in oysters might give a better overview. The TAV is a ratio of the content of particular taste compounds and their taste threshold in water. A TAV value above one is considered to actively contribute to the overall taste of the oyster. The limitation in this method is that possible matrix effects such as masking or enhancing are not taken into account.

Chen et al. (2012) found that from the FAAs only glutamate, glycine and alanine showed a TAV above one for fresh Jinjiang oyster (*Crassostrea rivularis*). The authors concluded that glutamate contributes to the umami taste of oysters and glycine and alanine contribute to the sweet taste of oysters (table 2.4).

In the study by Liu, Zhang and Chen (2013) TAVs were determined in both visceral mass and adductor muscles of Jinjiang oyster (*Crassostrea rivularis*). The TAVs of glutamate, alanine, arginine, valine and lysine were all above one in adductor muscle tissue while in the visceral mass none of the FAAs showed a TAV above one (table 2.4). The differences in TAVs are explained by the total FAA content in the tissues. The total FAA content in the adductor muscle was about five times higher than in the visceral mass.

Table 2.4 Taste active compounds, their taste thresholds and Taste Activity Value (TAV) in visceral mass and adductor muscle of Jinjiang oyster (*Ostrea rivularis*) (Adapted from Chen et al., 2012; Liu et al., 2013).

Compound	Taste threshold	TAV Whole oyster	TAV Visceral mass	TAV Adductor muscle
Glutamate	0,30[a]	3,10	0,73	4,01
Glycine	1,30[a]	1,10	0,12	0,51
Alanine	0,60[a]	1,90	0,25	1,07
Arginine	0,50[a]	0,50	0,03	1,18
Valine	0,40[a]	0,30	0,15	1,19
Lysine	0,50[a]	0,30	0,14	1,14
IMP	25,00[b]	-	0,50	2,26
AMP	50,00[b]	-	1,65	13,49
Succinic acid	37,00[c]	-	3,40	1,50
Na+	180,00[c]	-	0,74	1,23
PO4-3	130,00[c]	-	2,72	0,95
Cl-	130,00[c]	-	2,92	2,99

[a] taste threshold in water expressed as mg ml^{-1} (Kubota et al., 2002; Hayashi et al., 1981). [b] taste threshold in water expressed as mg 100 ml^{-1} (Yamaguchi et al., 1971; Kato et al., 1989). [c] taste threshold in water expressed as mg 100 ml^{-1} (Rotzoll et al., 2006).

Sakaguchi and Murata (1989) also measured higher concentrations of FAA in the adductor muscle of Pacific cupped oyster (*Crassostrea gigas*) as compared to the whole tissue of the oysters. Sakaguchi and Murata (1989) suggest that the overall contribution of the adductor muscle to the taste of whole oysters is most likely limited as the adductor muscle weight comprises 5-8% of the total tissue weight of an oyster. Furthermore, these authors showed that the FAA content followed a seasonal pattern with the highest FAA content in spring. Taurine and alanine were exceptions in this case as the highest concentration of taurine was measured in oysters sampled in summer while the highest concentration of alanine was measured in winter time.

Liu et al. (2002) compared the main taste components between fresh triploid and diploid Pacific cupped oyster (*Crassostrea gigas*). Total FAA content was about three times higher in diploid oysters as compared with the triploid oysters. The reported taste active FAAs (glutamate, glycine, arginine, alanine and taurine) showed not only differences between diploid and triploid oysters, but also seasonal influences. While glutamate content in diploid oysters was higher in spring the opposite was true for triploid oysters with higher concentrations in summer. Glycine, taurine and alanine content were highest in both diploid oysters as compared to triploid oysters and in summer as compared to spring. Arginine was only found in diploid oysters in relative low concentrations. No apparent seasonal influence was noticed for the concentration of arginine as these were comparable in spring and summer.

Cochet, Brown, Kube, Elliott and Delahunty (2013) compared the sensorial characteristics and the FAA contents of Pacific cupped oysters cultivated in three different geographical locations in Australia. Even though the concentrations of taste-active compounds such as glutamate, glycine and alanine proved to be different between the oyster groups within this study, no direct sensory effect could be attributed to these compounds due to different taste attributes (no attributes regarding sweetness and umami) used in the study.

Adenosine-5'-MonoPhosphate (AMP) was reported to have a synergistic effect in the umami taste of scallops (Wantanabe, Lan, Yamaguchi and Konosu, 1990) and Manilla clam (Fuke and Konosu, 1991). Omission of AMP from both scallop and Manilla clam extract leads to lower perception of sweetness, saltiness and overall preference of the extracts in question (Fuke and Konosu, 1991).

The contribution of inosine monophosphate (IMP) in the overall taste of shellfish remains somewhat unclear. Both Wantanabe et al. (1990) and Fuke and Konosu (1991) showed that IMP did not influence the sensorial evaluation of scallop and Manilla clam. Liu et al. (2013) measured a TAV above one for IMP in the adductor muscle of Jinjiang oysters. However, the TAV was below one in the whole oyster. IMP interacts with FAA such as alanine, serine and glycine. IMP enhances the umami taste in seafood (Hong et al., 2002). The content on IMP and AMP were 4.5 and 8 times higher in the adductor muscle when compared with the visceral mass of oysters (Liu et al., 2013). Both AMP and IMP are degradation products of ATP. Yokoyama, Sakaguchi, Kawai and Kanamori (1992) studied the changes in ATP-related compounds in the adductor muscle and other parts of the Pacific oyster (*Crassostrea gigas*) during iced storage. The level of ATP related compounds in the adductor muscle was about twice that which was found in the other tissues tested. Both AMP and IMP content increased in post-mortem stored oysters. In fresh oysters IMP levels measured were relatively low which could explain the limited contribution of this compound on the taste profile of fresh oysters.

Succinic acid has been reported as being an important compound in the typical umami and sour taste formation in Manilla clam (*Venerupis philippinarum*) (Yuexin, 2001). Liu et al. (2013) also found TAV above one for succinic acid in both the visceral mass and adductor muscle of Jinjiang oysters (*Crassostrea rivularis*).

Inorganic ions were found in varied concentrations in the oyster tissues. In the adductor muscle of Pacific cupped oysters (*Crassostrea gigas*) PO_4^{-3} was found in higher concentrations while Na^+ is found in lower concentrations as compared to the visceral mass. In both cases only the high concentrations lead to a TAV above one. Cl^- was found in comparable concentrations and similar TAVs (approx. three) in both tissues (Liu et al., 2013). In an omission test by Fuke and Konosu (1991) differences were noted in scallop and Manilla clam extract without either Na^+, K^+ and Cl^-. Na^+ omission decreased the sweetness, saltiness, umami and characteristic flavor of both shellfish while the perceived bitterness increased. Omissions of both K^+ and Cl^- reduced the potency of the extract and lead to almost tasteless extracts.

Bromophenols are known to produce marine- or ocean-like flavors in seafood (Boyle, Lindsay and Stuiber, 1992). Table 2.5 shows the bromophenols found in fresh oysters, their taste threshold and taste description.

Table 2.5 Bromophenols found in fresh Jinjiang oysters (*Ostrea rivularis*), their taste description and taste threshold.

Compound	**Taste description**	**Taste threshold [a] (ng g^{-1})**
2-Bromophenol	sea-like	10
2,4-Dibromophenol	iodine-like	50
2,6-Dibromophenol	fish-, crab-, shrimp-like	0,1
2,4,6-Tribromophenol	salt, fish-like	10

[a] Taste threshold as defined in whitefish (Boyle et al., 1992).

Chung, Ma and Kim (2003) measured the seasonal variation in bromophenol content of Jinjiang oysters (*Ostrea rivularis*) in Hong Kong. Total bromophenol content in oyster tissue varied from 13.6 to 75.8 ng g DW^{-1} in summer and spring, respectively. This seasonal fluctuation coincided with the natural bromophenol concentration of algae in the study area. 2,4-Dibromophenol was the most abundant bromophenol compound found in oysters accounting for approximately 75% of all bromophenols measured. However only the most potent bromophenol: 2,6-dibromophenol was found in concentrations surpassing the taste threshold (in whitefish, Boyle et al., 1992) all year round. 4-Bromophenol was not detected in the oysters in this study. The total bromophenol content in oysters was relatively low as compared to the content found in other seafood products such as Manilla clams (*Venerupis phillipinarum*) or crab (*Charybdis feriatus*). The differences found were attributed to the living habitats and more particularly to the food sources of the seafood products in question. Other studies (Whitfield et al., 2000; Whitfield, Helidoniotis, Shaw and Svoronos, 1997; Whitfield, Helidoniotis, Shaw and Svoronos, 1998) also suggested that the origin of bromophenols in fish and crustacean is linked to their natural diets.

To summarize this chapter shows that factors such as oyster species, cultivation location, season and the feed of the oysters are known to have an impact on the concentrations of odor- and taste active compounds. The main odor- and taste active compounds found in oysters are VOCs, FAAs, '5 nucleotides and bromophenols. The VOCs found in oysters (aldehydes, alcohols, ketones, alkenes, cyclenes, alkanes, cyclanes, furans, acids and aromatic compounds) originate from n-3 PUFA, n-6 PUFA, n-9 MUFA oxidation or carotenoid, amino acid or polysaccharide degradation. The VOCs are known to be odor active compounds and are linked to odor descriptors in oysters such as mushroom, grass, green, cucumber, marine, citrus and fresh. FAA, 5' nucleotides and bromophenols on the other hand are known to be taste active compounds. The main FAA known to contribute to the overall taste of fresh oysters are glutamate, glycine and alanine. These FAA mainly contribute to the sweetness and umami taste in shellfish. The contribution of the 5' nucleotides to the overall flavor profile of oysters remains unclear as no data is available. The contribution of the bromophenols to the overall flavor profile of oysters seems to be limited as only the most potent bromophenol, 2,6-dibromophenol, was found in concentrations above the taste threshold of this compound.

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Chapter 3: Consumer preference and sensory properties of the Pacific cupped oyster (*Crassostrea gigas*) and the European flat oyster (*Ostrea edulis*)

"As I ate the oysters with their strong taste of the sea and their faint metallic taste that the cold white wine washed away, leaving only the sea taste and the succulent texture, and as I drank their cold liquid from each shell and washed it down with the crisp taste of the wine, I lost the empty feeling and began to be happy and to make plans."

Ernest Hemingway, 'A Moveable Feast'

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Abstract

Experts in the oyster supply chain (farmers, retailers and gourmet chefs) in the Netherlands have suggested that the European flat oyster (*Ostrea edulis*) has superior sensory qualities compared to the Pacific cupped oyster (*Crassostrea gigas*). However, scientific evidence is lacking. The aim of this study was to evaluate consumer preferences for these two oyster species and to determine the sensory properties of both species by consumers. Two-alternative forced choice tests were performed with the oysters involving a panel of 74 naïve consumers. No significant differences in preferences between the oyster species were observed by the consumers. Nine sensory attributes of both oyster species were evaluated. Consumers did not observe significant differences between species in the sensory attributes: overall odor intensity, sea odor, mud odor, sweetness, pungency and firmness. The Pacific cupped oyster was perceived as significantly saltier and more intense in greenness than the European flat oyster. We concluded that only a few sensory properties of the European flat oyster (*Ostrea edulis*) and the Pacific cupped oyster (*Crassostrea gigas*) are perceived by untrained consumers as being different.

3.1 Introduction

The Pacific cupped oyster (*Crassostrea gigas*) and the native European flat oyster (*Ostrea edulis*) are the main species of oysters cultured in the Netherlands. In the opinion of Dutch experts in the oyster supply chain (farmers, retailers and gourmet chefs) there is a distinct difference in flavor (taste and smell) between these species. These experts express their preference for the European flat oyster as being of superior quality. Jacobsen (2007) supported this view in his connoisseur's guide to oyster eating. In his opinion no oyster comes close to the taste of the European flat oyster. He described the taste as overwhelming, metallic zinc and like anchovy dipped in zinc. A stronger flavor than any other oyster species.

The price of the European flat oyster on the Dutch market was more than four times the price of the Pacific cupped oyster, 4.26 € per kg and 1.00 € per kg respectively in 2010 (FAO, 2013a). This price difference might be due to the suggested superior quality of the European flat oyster. It may also be related to the relative scarcity of the European flat oyster in the Dutch market compared to the more readily available Pacific cupped oyster (98 tons and 3 860 tons respectively in 2010) (FAO, 2013a). Dutch consumers consider oysters as a delicacy, but data are lacking regarding consumers preference between the two species. Distinct perceivable flavor difference has been suggested by experienced experts. It has not been determined whether the flavor differences between the species reported by experts are perceived by naïve consumers of oysters. Therefore, a consumer preference study combined with an evaluation of a limited number of sensory characteristics may provide insight into the quality perception of these products by consumers.

Few consumer preference studies with oysters have been conducted in which the consumers also assessed sensory characteristics. Allen and Downing (1991) used a consumer and oyster producer's panel to study overall preferences, flavor and texture in diploid and triploid Pacific cupped oysters. Both of the untrained panels rated triploid oysters significantly higher than diploid oysters for overall preference. The consumer panel gave also a significantly higher score for flavor and texture of the triploid oysters, while the oyster producer's panel did not. The effect of high pressure (HP) treatment on diploid and triploid Pacific cupped oysters on consumer appreciation (appearance, odor, texture and flavor) was studied by Nell, O'Riordan and Ogburn (2006). The appearance of the HP treated and diploid oysters was rated significantly higher than untreated triploid oysters.

A few sensory studies of Pacific cupped oysters by trained panels have been reported in literature. In the study of Buzin, Baudon, Cardinal, Barillé and Haure (2011), a trained sensory panel gave scores for the intensity of odor, appearance, texture and flavor of Pacific cupped oysters during cold storage. Cochet, Kube, Brown, Elliott and Delahunty (2011) and Cochet, Brown, Kube, Elliott and Delahunty (2013) developed a comprehensive descriptive vocabulary to objectively evaluate the sensory properties of raw Pacific cupped oysters. Twelve sensory attributes in four main categories (odor, flavor, texture and aftertaste) were used by a trained panel to determine relationships between sensory differences and the composition of the oysters.

The most common approach is to use consumers for hedonic testing and trained panels for descriptive sensory profiling. The aim of our study was to evaluate consumer preferences for the two oyster species (Pacific cupped oyster and European flat oyster) and to determine the sensory properties of both species as evaluated by consumers. Hedonic testing of foods should be performed with naïve, untrained users of the products. The analytical sensory evaluation was executed with the same naïve, untrained consumers since the objective was to determine whether the sensory differences mentioned by the experts are relevant and large enough to lead to significant perceptual changes in untrained assessors, i.e. regular users of the products. Therefore, the list of sensory attributes was limited and the consumer panel rather large in our study. It has been recently demonstrated that for particular products, including complex products such as perfumes, the use of untrained consumer panels appears to be a good alternative to the classical sensory profile provided by a trained panel (Worch, Lê and Punter, 2013). Also Ares, Bruzzone and Giménez (2011) showed that consumers and trained experts had very similar discriminative capability and reproducibility for five texture attributes of milk products.

3.2 Materials and methods

3.2.1 Oysters

Live Pacific cupped and European flat oysters were obtained from a Dutch shellfish company (Koninklijke Prins en Dingemanse, Yerseke, The Netherlands) in November 2012. Both oyster species were cultivated on oyster plot 81 (N 51°45'57.8", E 003°58'45.6") in Lake Grevelingen (The Netherlands) for approximately 2 years. The weight of the oysters varied from 80 to 120 g. The gross composition, as labeled on the packaging, was the same for both species: 6 g protein, 4 g carbohydrates and 1.9 g fat per 100 g of oysters . To ensure optimal quality of the oysters, the oysters were obtained daily from the wet storage area of the shellfish company. The oyster samples were stored refrigerated for a maximum of four hours at 4-6° C up until preparation for the consumer test. Oysters were opened by hand-shucking and the adductor muscles were cut with a knife on both sides. Oysters were either placed in a non-flipped way in non-transparent plastic cups or returned to a half-shell. The prepared samples were kept refrigerated (4-6 °C) for a maximum of two hours before the actual tests. Prior to serving the prepared samples the remaining internal liquid was drained from the oysters. All samples were coded with a randomized three-digit code.

3.2.2 Consumers

Consumers were recruited in the Province of Zeeland, The Netherlands. Recruitment was done at a local seafood fair in Vlissingen, by means of the website and intranet of the HZ University of Applied Sciences and through press releases in local newspapers. Consumer selection was based on the criteria that they were consuming oysters and had no allergies to shellfish or oysters. In total, n=78 consumers participated in this study. Consumers did not receive a financial reimbursement for participating in the study. No information on the actual aim or the experimental design was disclosed to the consumers.

3.2.3 Experimental design

The study included a socio-demographic questionnaire, two 2-alternative forced choice tests (2-AFC) and a sensory profile test.

The tests were carried out in four different sessions of 1.5 h during the period 20-24 November 2012. Tests were carried out at room temperature in different classrooms at the HZ University of Applied Sciences in Vlissingen, The Netherlands. Consumers were seated approximately 1 m apart in the classrooms.

Consumers were seated at random, received a participant number for anonymity and were instructed not to speak to each other during and between the tests.

Socio-demographic questionnaire

Consumers were asked to complete a short socio-demographic questionnaire about their age, gender, education level, income, seafood and oyster consumption frequency. The time to fill in this questionnaire was approximately 20 min.

Alternative force choice tests

Two 2-AFC tests were performed with n=78 consumers. Consumers were asked to indicate which sample of the pair of oysters offered they preferred most. In the first 2-AFC test, the oysters were presented in non-transparent plastic cups with a lid. The assessor could not see the oysters before tasting them (blind condition). In the second 2-AFC test, the oysters were presented as half-shell products. In that way the assessors saw the oysters in the shell before tasting them (non-blind condition). The aim was to evaluate whether viewing the oysters had an effect on the consumer preference for one of the oyster species. Each pair of samples in both 2-AFC tests consisted of one Pacific cupped oyster and one European flat oyster. The presentation order of the samples was completely randomized.

Before receiving the samples for the blind 2-AFC test, the assessors were instructed to remove the lid of the non-transparent plastic cups and to eat the oyster after sliding the contents of the cup into their mouth without looking at the oyster. The panel leaders controlled whether the consumers followed these instructions. The results from four consumers who looked into the cup before tasting the oysters were excluded from the study. The tasting sessions were of approximately 25 minutes duration. Each 2-AFC test took 10 minutes with a break of 5 minutes in between in which the consumers could take a sip of water.

Sensory Profile test

The sensory profile test was performed with n=78 consumers. The selected sensory attributes were derived from studies regarding the sensory profile of oysters (Cochet et al., 2011; Buzin et al., 2011; Aaraas et al., 2004; Pennarun, Prost and Demaimay, 2002; Pennarun, Prost, Haure and Demaimay, 2003). The comprehensibility of the selected attributes was discussed in a preliminary session with six consumers. The agreed attributes for the sensory profile test were: greenness, overall odor intensity, sea odor, mud odor, salt, sweet, pungent, creamy and firmness. The six consumers from the preliminary session did not participate in the actual tests.

In the actual test consumers were asked to score the perceived intensity of the nine different attributes on a nine-point scale, anchored with 'not at all' on the left-hand side and 'very much' on the right-hand side.

Oysters were presented in non-transparent plastic cups without a lid. The samples assessed consisted of one Pacific cupped oyster and one European flat oyster. The presentation order of the samples was randomized.

The consumers were asked to look first into the cup and to evaluate the appearance of the oyster on the attribute greenness. Next the consumers were asked to smell the sample and to evaluate the overall intensity, sea odor and mud odor. After eating the sample the consumers were asked to evaluate the attributes salty, sweet, pungent, creamy and firmness. Consumers were instructed to drink water and eat a cracker before evaluating the second sample.

The duration of a session was 35 minutes. Approximately 15 minutes for the evaluation of each sample and 5 minutes for a break in between in which the consumers were instructed to drink water and eat a cracker to clean the palate.

3.2.4 Salt content analysis

Sodium content was determined by flame photometer (Jenway Flame Photometer, Pfp7) as described by Castanheira et al. (2009). Samples were analyzed in triplicate. Results are expressed as % of NaCl (g/g of wet weight).

3.2.5 Statistical data analysis

Chi-square testing (SPSS version 20: IBM) was used to assess the consumer preference in the 2-AFC tests. One-way ANOVA was used to compare scores on the sensory attributes between oysters species. Data are expressed as mean ± standard deviation. Where $p < 0.05$ differences were deemed statistically significant.

3.3 Results and Discussion

3.3.1 Consumer panel characterization

The socio-demographic characteristics of the consumer panel are presented in Table 3.1. In this study 72% of the consumers (n=78) were male, 25% of the consumers were younger than 46 years, 28% between 46 and 55 years of age and 47% above 55 years of age. Approximately 64% of the consumers had a middle and about 30% a high educational level and their corresponding gross yearly household incomes (between €33 000 and €49 999 and above €50 000, respectively) are considered to be above the average income in the Netherlands (CBS, 2013). About 9% of the consumers consumed oysters once a year, while 29% of the consumers ate oysters two to three times a year. Up to 32% of the consumers ate oysters four to ten times a year and 29% of the consumers more than ten times a year. The consumers ate their oysters out of home (32% of all consumers) or both out of home and at home (64% of all consumers). Only 4% of the consumers in this study ate oysters only at home. Overall seafood consumption among the consumers in this study was relatively high (FAO, 2013b) as approximately 54% of the consumers consumed seafood one to two times per week and 18% of the consumers more than two times per week. The panel used in our study was considered as a group of regular oyster consumers which fits very well within the aim of this study.

Table 3.1 Sociodemographic characterization of the consumer panel (n=78).

Gender (%)		Oyster eater (%)	
Female	28	Yes	100
Male	72	No	0
Age (years) (%)		Yearly frequency oyster consumption (%)	
< 25	10	Once a year	9
26 - 35	8	2 - 3	29
36 - 45	6	4 - 10	32
46 - 55	28	> 10	29
> 55	47		
Highest educational level (%)*		Gross yearly income (%)	
n	*77*	*n*	*77*
Low	6	≤ €32 999	23
Middle	64	€33 000 - €49 999	43
High	30	≥ €50 000	34
Location of oyster consumption (%)		Weekly seafood consumption (%)	
At home	4	Less than once	28
Out of home	32	Once to twice	54
Both	64	More than twice	18

[a] Low educational level: primary school and secondary school; middle educational level: intermediate vocational education and bachelor's degree or equivalents and high educational level: master's degree and doctor of philosophy degree or equivalents.

3.3.2 Consumer preference

When presented blind, k=39 consumers preferred the Pacific cupped oyster (χ^2=0.054, n=74, p=0.642) over the European flat oyster. When the oysters were presented as a half-shelf product (non-blind condition), k=36 consumers preferred the Pacific cupped oyster (χ^2=0.216, n=74, p=0.816) over the European flat oyster. Therefore, no significant difference in Dutch consumer preference between the Pacific cupped and European flat oyster was found in blind and non-blind tasting. Both oyster species appeared to create similar eating quality experiences for the Dutch consumers not leading to a preference. This finding contrasts strongly with the suggestion that the European flat oyster is of superior quality expressed by experts in the oyster supply chain.

Comparing the results from our preference study with other oyster preference studies is difficult because different species (Josephson, Lindsay and Stuiber, 1985), di- or triploid (Allen and Downing, 1991) were compared with Pacific cupped oyster, other methodologies were applied, or the effect of a processing technology on consumer preference (Nell et al., 2006) was measured. However, all studies demonstrated the strength of consumer preference studies in obtaining a better insight into consumer's attitude to oyster.

3.3.3 Sensory evaluation

Figure 3.1 shows the results of the sensory evaluation of nine attributes by the untrained, naïve consumers (n=78). No significant differences were observed between the Pacific cupped oyster and the European flat oysters for the attributes overall odor intensity (3.8 ± 1.9 resp. 4.0 ± 1.9), sea odor (4.0 ± 2.2 resp. 4.2 ± 2.2), mud odor (2.8 ± 2.0 resp. 2.5 ± 1.7), sweet (3.9 ± 2.0 resp. 4.2 ± 1.9), pungent (4.6 ± 2.2 resp. 4.7 ± 2.0) and firmness (5.7 ± 1.5 resp. 5.5 ± 1.5). Greenness was found to be significantly higher (p=0.000) for the Pacific cupped oyster (3.0 ± 1.8) compared to the European flat oyster (1.7 ± 0.9) and saltiness was significantly higher (p=0.036) for the Pacific cupped oyster (6.2 ± 1.9) compared to the European flat oyster (5.5 ± 2.2). Creaminess of the Pacific cupped oyster (5.2 ± 1.8) tended to be higher (p=0.053, not significant) compared to the creaminess of the European flat oyster (4.7 ± 1.9). The significant difference found in the attribute greenness between the two species suggests that consumers can visually distinguish oyster species by the color of the oyster flesh.

The NaCl content of the Pacific cupped oyster was 1.07% ± 0.09% (g g^{-1} wet weight) while the European flat oyster had a NaCl content of 0.82% ±< 0.01% (g g^{-1} wet weight). This small, but significant difference (p=0.010) of 0.25% in total NaCl content between the oysters is large enough to lead to a significant difference in saltiness perception by naïve consumers. Pacific cupped oysters with higher salt content are also perceived by the consumer as being more salty than the European flat oyster.

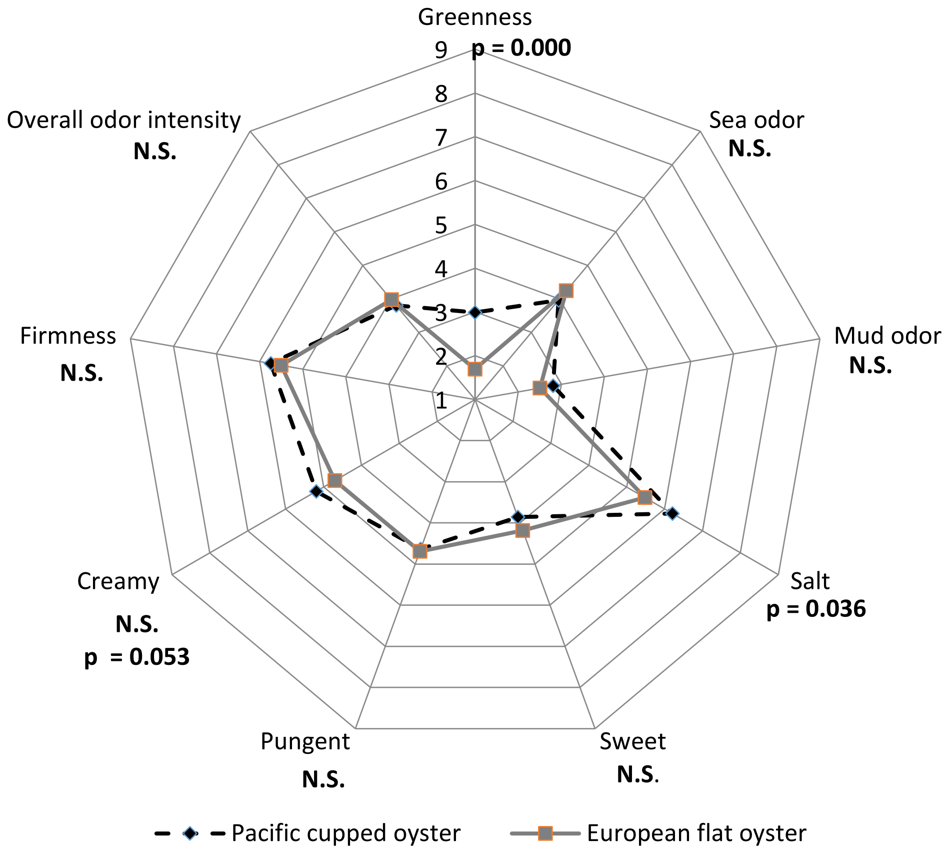

Figure 3.1 Sensory profile (n=78) of Pacific cupped oyster (*Crassostrea gigas*) and European flat oyster (*Ostrea edulis*) as determined by Dutch naïve consumers. N.S. stands for not significant.

Although in our study significant differences between the Pacific cupped oyster and the European flat oyster were found for greenness and saltiness and while creaminess tended to be different (p=0.053, not significant), these differences do not lead to a difference in preference for one of the oyster species.

It should be noted that the consumers did not suggest any significant difference for any of the other flavor attributes between the two species. This is in contrast with the opinion expressed by experts from the oyster supply chain that the flavor of the European flat oyster is distinctly different. The consumer panel gave a slightly, but not significantly higher score for all flavor attributes (except mud odor) (approx. 5%) for the European flat oyster in comparison with the Pacific cupped oyster. Although speculative, this might indicate that there is a difference. Besides the use of consumer panels for evaluation of sensory properties of oysters, it might be interesting in future research to also include the evaluation of a trained expert panel which might be more able to detect even those small differences at a significant level.

In this study, both different oyster species were derived from the same production area in the Netherlands (Lake Grevelingen). It has been shown by Pennarun et al. (2003) and by Cochet et al. (2013) that different diets and different locations of production could have an effect on the sensory properties of oysters of the same species. It can therefore not be excluded that a comparison of the sensorial properties of European flat oysters and Pacific cupped oysters from different production areas or cultivated with different feed could lead to oysters with a more distinct flavor detectable by consumers.

3.4 Conclusion

From this study, it can be concluded that consumers in the Netherlands have no preference for either the Pacific cupped oyster or the European flat oyster. No difference in preference is observed between the oyster species when consumers evaluate the oysters with or without seeing the oyster. The sensory profile evaluated by consumers revealed that greenness and saltiness differed significantly between the oyster species. Greenness was evaluated visually suggesting that the appearance of the oysters is different. Saltiness was the only taste attribute that consumers perceived as significantly different between the two oyster species although the difference in salt content between the species was small. We conclude that only a few sensory properties of the European flat oyster (*Ostrea edulis*) and the Pacific cupped oyster (*Crassostrea gigas*) are perceived by untrained, naïve consumers as being different. In contrast to experts from the oyster supply chain, Dutch consumers have no preference for European flat oysters or Pacific cupped oysters.

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Chapter 4: Comparison of dynamic sensory perception of the Pacific cupped oyster (*Crassostrea gigas*) and European flat oyster (*Ostrea edulis*)

"The man who doesn't like oysters, the woman who cannot abide sardines. We know the type."

Harold Nicolson, 'Food' (1942)

Jasper van Houcke, Jozef Linssen & Joop Luten

Abstract

Temporal Dominance of Sensations (TDS) is a sensory analysis method aimed at identifying the temporal sensory properties of products. The TDS methodology has never been used to describe dynamic sensory perception of oysters. The aim of this study was to identify temporal differences in the sensorial properties of the Pacific cupped oyster (*Crassostrea gigas*) and the European flat oyster (*Ostrea edulis*).

Untrained consumers (n=31) were familiarized with the TDS method before fresh Pacific cupped and European flat oysters were evaluated in duplicate using the attributes salt, sweet, creamy, earthy, pungent and firm.

Results show that differences between the two species of oyster are most pronounced by the attributes creamy, sweet and pungent. The dynamic sensory perception of the Pacific cupped oyster evolves from salty at the beginning of consumption to creamy at the middle of consumption proceeding to sweet at the end of consumption. The dynamic sensory perception of the European flat oyster evolves from salt at the beginning of consumption to pungent at the middle of consumption and sweet at the end of consumption.

4.1 Introduction

In France, Belgium and the Netherlands, raw fresh oysters are considered a delicacy. In the Netherlands two oyster species are cultivated. The native European flat oyster (*Ostrea edulis*) and the Pacific cupped oyster (*Crassostrea gigas*). Van Houcke, Altintzoglou, Stieger, Linssen and Luten (2016) compared the sensorial quality of these two species using consumers in a sensory evaluation. A hedonic scale was used for the attributes greenness, sea odor, mud odor, salt, sweet, pungent, creamy, firmness and overall odor intensity. Significant higher intensities were only reported for the attributes greenness and saltiness in the Pacific cupped oyster.

In other studies (Nell, 2002; Pennarun, Prost, Haure and Demaimay, 2003; Aaraas et al., 2004; Buzin, Baudon, Cardinal, Barillé and Haure, 2011; Cochet, Brown, Kube, Elliott and Delahunty, 2013) regarding the sensorial properties of oysters static sensory methodologies were employed without considering temporal effects of perception. Measuring temporal effects could however provide extra insight in the sensory perception of oysters over time. For instance, durations of perception were not evaluated in static methods such as qualitative descriptive analysis. Food and beverages undergo a series of physical and chemical reactions during mastication, salivation and so on, moreover the perception of aroma, taste, flavor and texture changes too. Thus, conventional static sensory methods, which require judges to average their dynamic sensations and give only a single point evaluation, are bound to miss some significant product information. To overcome this drawback, different dynamic sensory methods have been developed and refined (Ng et al., 2012; Di Monaco, Su, Masi and Cavella, 2014). Temporal dominance of sensations (TDS) is a relatively new method focused on assessing temporal differences in the sensory evaluation of food products during consumption (Pineau, Cordelle and Schlich, 2003).

In TDS either trained or untrained panelists are continuously asked to indicate which attribute is perceived as the dominant sensation during consumption of the product. The dominant sensation is usually defined as the most striking perception at a given time. During consumption perceived dominance can change and panelists are free to select other attributes as the dominant sensation. The TDS procedure is stopped when the product is completely consumed and none of the attributes is perceived as being dominant. TDS does not give information on the perceived intensity of the attributes, but indicates the consensus of the panelists on the dominance of the attributes over time. This consensus of the panelists is called the dominance rate (Labbe, Schlich, Pineau, Gilbert and Martin, 2009; Pineau et al., 2009).

Pineau et al. (2012) showed that panelists are only able to manage short lists of attributes. They recommend a maximum of 10 attributes in order to prevent panelists from forming their own subset of preferential subsets of attributes. Furthermore, the authors advise balancing the order of the attributes over the participating panelists. This is in order to prevent bias caused by the first mentioned attributes being chosen most often.

Since the introduction of TDS the method has been used to determine dynamic sensory perception of dairy products (Pineau et al., 2003), wine (Pessina, Boivin, Moio and Schlich, 2005; Meillon, Urbano and Schlich, 2009), hot beverages (Le Révérend, Hidrio, Fernandes and Aubry, 2008), flavored gels (Labbe et al., 2009) and breakfast cereals (Lenfant, Loret, Pineau, Hartmann and Martin, 2009). Recently the TDS-method was used for fish sticks (Albert, Salvador, Schlich and Fiszman, 2012). Three preparation methods for fish sticks (conventional oven, microwave oven and deep-fried) were compared by nine untrained panelists. Attributes were crunchiness, juiciness, oiliness, fried flavor, different textures, bolus and swallowing. The order in which the attributes were considered dominant did not differ between all fish sticks. However, the timing in which the attributes were considered dominant and the dominance rates did differ. For instance, a dominance rate of 65-70% for crunchiness was reported for both deep-fried fish sticks and fish sticks prepared in a conventional oven. For the microwave oven prepared fish sticks a dominance rate of 45-55% for crunchiness was reported. In all fish sticks crunchiness was considered the first dominant attribute. For deep-fried fish sticks a second dominance for crunchiness was reported after some mastication time. Furthermore, in the final stages of mastication bolus and swallowing were the least dominant attributes for all fish sticks. However, higher dominance rates in the microwave oven prepared fish sticks indicate an easier bolus formation and swallowing, most likely due to the less crunchy texture.

The TDS method seems to be an attractive method for its simplicity, easiness to understand for both trained and untrained panelists and could provide extra information on sensorial differences between products. The use of TDS in seafood research has so far been limited. The TDS methodology has, to the knowledge of the authors, not been used in the sensory evaluation of oysters. The aim of this study was to identify temporal differences in the sensorial properties of the Pacific cupped and the European flat oyster.

4.2 Material and methods

4.2.1 Oysters

Live Pacific cupped and European flat oysters cultivated in Lake Grevelingen (The Netherlands) were obtained from a Dutch shellfish company (Koninklijke Prins en Dingemanse, Yerseke, The Netherlands) at the end of April and beginning of May 2013. The weight of the individual oysters varied from 80 to 120 g. To ensure optimal condition and quality of the oysters, the oysters were obtained daily from the wet storage area of the shellfish company. The oyster samples were stored refrigerated for a maximum of four hours at 4-6° C up until sample preparation for the TDS evaluation. Oysters were opened by hand-shucking, the adductor muscles were cut on both sides and the oyster tissue was placed on the lower shell (half-shell product). The prepared samples were kept refrigerated (4-6 °C) for a maximum of one hour before the actual tests. Prior to serving the prepared samples the oysters were drained of remaining internal liquid. All samples were coded with a randomized three-digit code.

4.2.2 Gross biochemical composition

In order to determine the gross biochemical composition of the Pacific cupped and the European flat oyster the tissue of 10 specimens per species was homogenized for determination of protein, lipid, glycogen and salt content. Measurements were performed in triplicate. Dry matter content of the oysters was determined by drying 5 g of homogenate at 105 °C for 48 hours. The protein content of 20-25 mg of homogenized oyster tissue was measured using the Dumas method as described by Simonne, Simmone, Eitenmiller, Mills and Cresman (1997). The lipid content of the oysters was determined of approximately 2.5 g of homogenized oyster by extraction using a dichloromethane/methanol/water mixture (8:4:3) according to the method of Folch, Lees and Sloane-Stanley (1957). Whereas the glycogen content of 1.5 g of oyster tissue was determined using the method of Grodner, Lanc and Vidaurreta (1977), linked to the colometric method of Carroll, Longley and Roe (1956). Sodium content was determined by flame photometer (Jenway Flame Photometer, Pfp7) as described by Castanheira et al. (2009).

4.2.3 Consumer panel

Consumers were recruited in the Province of Zeeland, The Netherlands. Recruitment was done from a pool of consumers who had participated in a previous study with oysters (van Houcke et al., 2016). In total 31 consumers volunteered for participation in this study. Table 4.1 shows the socio-demographic characteristics of the consumer panel.

Table 4.1 Socio-demographic characterization of the consumer panel (n=31).

Gender (%)		Oyster eater (%)	
Female	16	Yes	100
Male	84	No	0
Age (years) (%)		Yearly frequency oyster consumption (%)	
< 25	6	Once a year	10
26 - 39	29	2 - 3	27
40 - 59	29	4 - 10	37
> 60	35	> 10	27
Highest educational level (%)*		Gross yearly income (%)	
Low	3	≤ €32 999	17
Middle	60	€33 000 - €49 999	27
High	37	≥ €50 000	57
Location of oyster consumption (%)		Weekly seafood consumption (%)	
At home	3	Less than once	16
Out of home	47	Once to twice	71
Both	50	More than twice	13

* Low educational level: primary school and secondary school; middle educational level: intermediate vocational education and bachelor's degree or equivalents and high educational level: master's degree and doctor of philosophy degree or equivalents.

The majority of the consumers were male (84%). Only 6% of the consumers were under 25 years of age, while 29% were between 26 and 39 years of age, 29% were between 40 and 59 years of age and 35% were over 60 years of age. About 10% of the consumers consumed oysters once a year while 27% consumed oysters two to three times a year, 37% consumed oysters four to ten times a year and 27% consumed oysters more than ten times a year. Consumers did not receive a participation fee. No information on the actual aim or the experimental design was disclosed beforehand.

4.2.4 TDS tests

The participating consumers were familiarized with the TDS method in two sessions using mussels (*Mytilus edulis*). In these sessions, prior to the actual TDS tests, researchers explained the TDS method. Furthermore, consumers had the opportunity to become familiar with the computer program. In the actual TDS tests consumers evaluated both the European flat oyster and the Pacific cupped oyster in duplicate. The test samples were served in a random order. In total 10 different sessions of approximately one hour each were organized during a period of 10 days (April – May 2013) at the HZ University of Applied Sciences in Vlissingen, The Netherlands. All session rooms were kept at room temperature. Consumers were seated in individual sensory booths. In between samples consumers were instructed to drink water and eat a cracker to clean the palate.

The attributes used in the TDS tests were salt, sweet, creamy, earthy, pungent and firmness. These attributes except pungent have been used in previous sensory studies of Pacific cupped oysters (Van Houcke et al., 2016; Cochet et al., 2013; Buzin et al., 2011;

Pennarun, Prost and Demaimay, 2002; Pennarun et al., 2003). The attribute pungent was used in sensory studies of the European flat oyster (van Houcke et al., 2016 and Aaraas et al., 2004). The dominant attribute in the TDS test was defined as being the attribute with the highest intensity at a certain time.

The computerized evaluation system showed the six attributes simultaneously with a start and a stop button on a computer screen. As the order of the attributes varied on the screen the panelists were asked to look at the order of the attributes before starting with the tests. Upon consumption of the oyster the panelists pressed the start button on the computer screen. During the consumption period the panelists were free to choose the attribute they perceived as being dominant and participants were free to switch between attributes whenever they felt necessary. It was also possible for participants to select the attributes multiple times or not at all during the total consumption period. When the panelists no longer perceived any attribute as being dominant the session was stopped by clicking on the stop button on the screen.

4.2.5 Statistics

The results of the gross biochemical composition are expressed as mean value ± standard deviation (SD). One-way analysis of variance (ANOVA) was used to compare the gross biochemical composition of both oyster species. Significance levels were set at $p<0.05$. Consumer response in the TDS tests was recorded using EyeQuestion software Version 3.9.5 from Logic8 BV. The TDS data, in the form of mean dominance rate, is expressed as a percentage. The dominance rate is calculated by dividing the number of citations of each attribute (over all assessors and replicates) by the total number of evaluations (number of assessors multiplied by the number of replicates) x 100. Since the mastication period could vary between consumers, the consumption time data was normalized for the TDS curves. TDS curves and the TDS difference curve were made using statistical package R.

4.3 Results and discussion

4.3.1 Gross Biochemical composition

The differences in the gross biochemical composition between the Pacific cupped oyster and the European flat oyster are shown in figure 4.1.

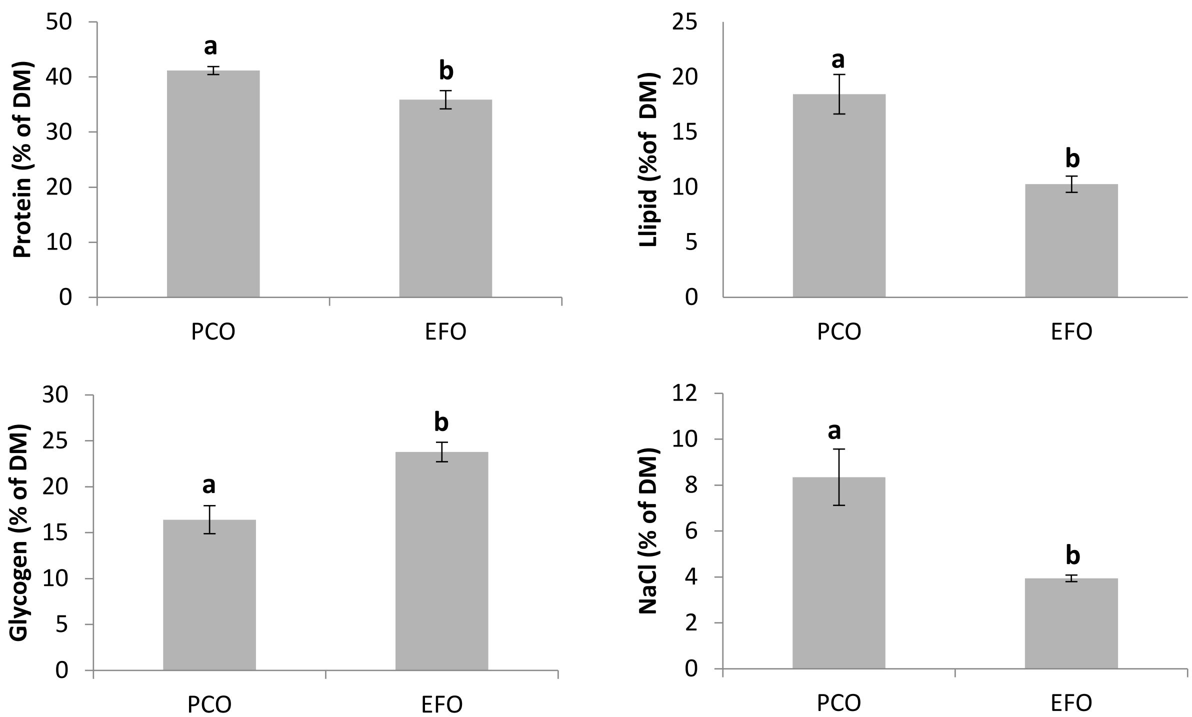

Figure 4.1 Biochemical composition of the Pacific cupped oyster (PCO) and the European flat oyster (EFO). Different superscripts indicate significant differences (P<0.05).

The protein content was higher in the Pacific cupped oyster (41.7±0.7% DW) in comparison with the European flat oyster (35.9±1.7% DW). Also, the lipid and NaCl content showed to be higher in the Pacific cupped oyster (18.4±1.8% and 8.3±1.2% DW) than in the European flat oyster (10.3± 0.7% and 3.9±0.1% DW). Conversely glycogen content proved to be higher in the European flat oyster (23.8±1.1% DW) in comparison to the Pacific cupped oyster (16.4±1.5% DW).

Compared to the data of Linehan, O'Connor and Burnell (1999) our results show the same range for protein content while lipid levels are relatively high and glycogen levels are relatively low in our study for Pacific cupped oysters. Similar lipid levels to our results were however shown in the study of Pazos, Ruíz, García-Martín, Abad and Sánchez (1996). The lipid levels found in the European flat oyster in our study are comparable with the results found by Abad, Ruíz, Martinez, Mosquera and Sánchez (1995). A higher NaCl content for Pacific cupped oysters in comparison with the European flat oyster was also found in the study of Van Houcke et al. (2016). Ash content, not measured in this study, is reported to range between approx. 10% DW (Van Houcke et al., 2016) and 30% DW (Pennarun et al., 2003).

4.3.2 TDS tests

Figure 4.2 shows the TDS graphs for the two oyster species used in this study (n=31).

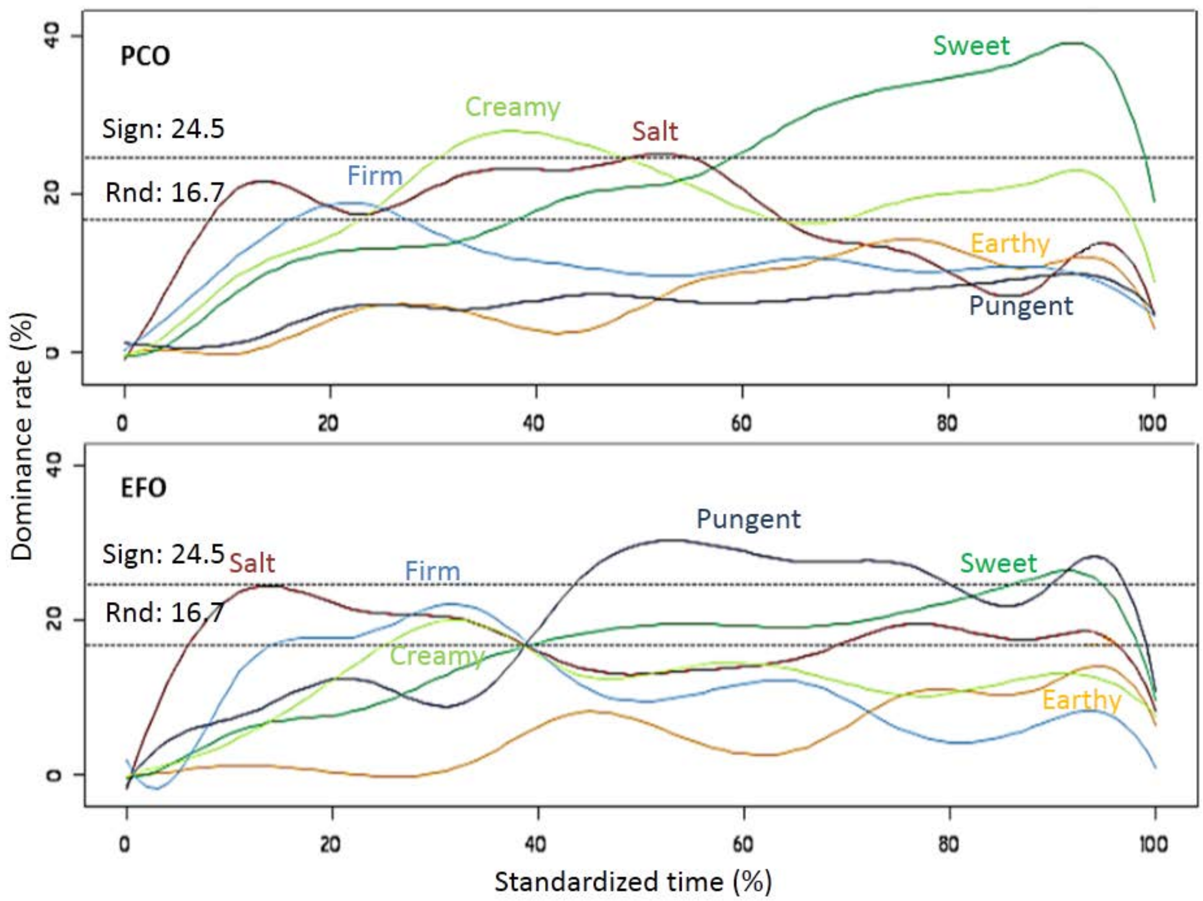

Figure 4.2 Normalized TDS curves of the Pacific cupped oyster (PCO) and the European flat oyster (EFO). Sign stands for significance level (24.5%) while Rnd stands for chance level (16.7%).

Each line represents the evolution of the dominance rate of an attribute over time. In addition, two horizontal lines are shown in the graph. The lower line represents the chance line which shows the chance of an attribute being chosen at the specific time. As six attributes were used in this study the chance of one being randomly chosen by the panelists was 16.7%. The upper line is the significance level and shows when the consensus of the panel is considered significant (in this case at 24.5%). As can been seen in figure 4.2 the first perceived attribute is salt for both the Pacific cupped oyster and the European flat oyster even though the dominance rate (21 and 24%) is not significant for the Pacific cupped oyster as well as the European flat oyster. The second and third dominant attribute in time are the attributes firm and creamy. For the Pacific cupped oyster, the dominance rate for the attribute creamy is considered significant between 31 and 47% of the normalized consumption time with a maximum dominance rate of 30%, while the attribute firm has a dominance rate of 19%. In the case for the European flat oyster the maximum dominance rates for creamy and firm were 23% and 20%, respectively. The Pacific cupped oyster than shows a second dominance for the attribute salt, being significant from 50 to 55% of the normalized consumption time with a maximum dominance rate of 25%. The attribute sweet is significantly dominant in the Pacific cupped oyster from 60% of the normalized consumption time onwards, with the highest dominance rate (39%) at 92% of the normalized consumption time. As for the European flat oyster the attribute pungent was considered to be significantly dominant between 43 and 80% and between 90 and 98% of the normalized consumption time with a maximum dominance rate of 31% at 53% of the normalized consumption time. The attribute sweet is also considered significantly dominant from 86 to 95% normalized consumption time with a maximum dominance rate of 27%.

In order to compare the TDS curves of the Pacific cupped oyster and the European flat oyster a TDS difference curve has been composed (figure 4.3). The TDS difference curve shows the significant differences in the attributes dominance rates between both oyster species.

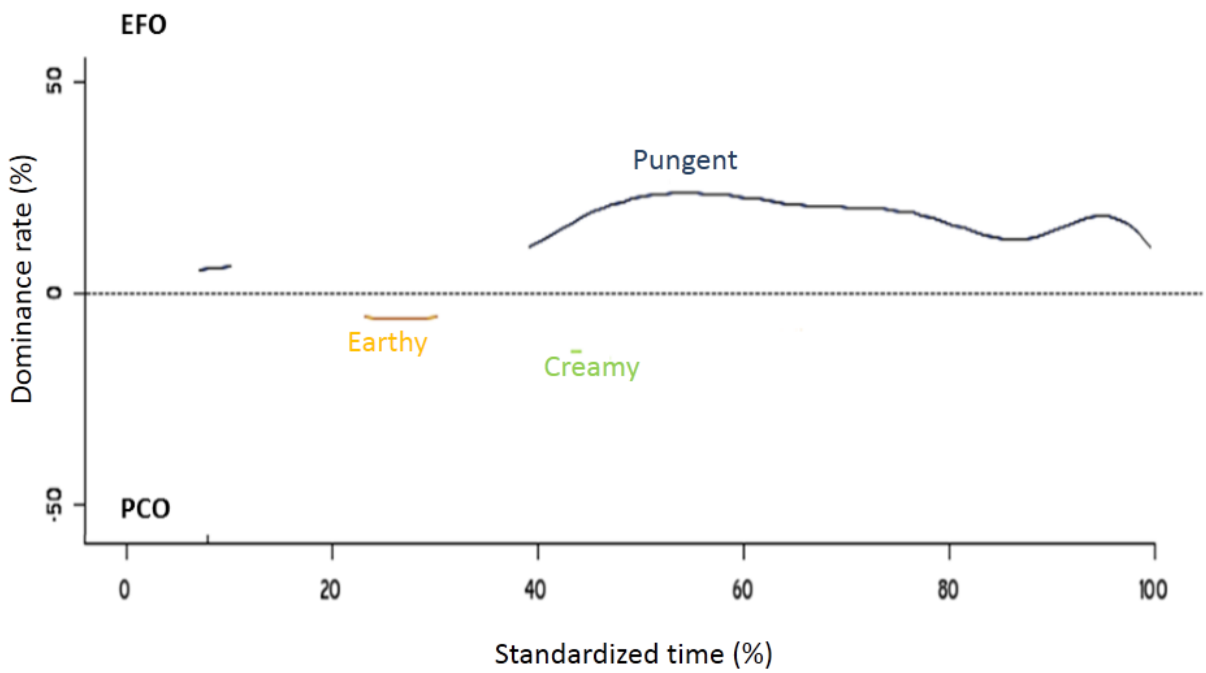

Figure 4.3 Normalized TDS difference curve of the Pacific cupped oyster (PCO) and the European flat oyster (EFO).

For the attributes earthy and creamy significant differences in dominance rate were found, indicating that the Pacific cupped oyster was perceived as being more earthy and creamy in comparison to the European flat oyster. The attribute earthy however, was never considered as being the dominant attribute at a given time by the panelists. The main difference between the two species is shown for the attribute pungent. The dominance rate of the attribute pungent was only considered to be significant in the TDS curves for the European flat oyster.

The attribute creamy, with a higher dominance rate in the evaluation of the Pacific cupped oyster, might be related to differences in biochemical composition of the oyster species. Glycogen and lipid levels may play a role in the creamy mouthfeel as suggested by Cochet et al. (2013). In their study it was suggested that high glycogen and lipid levels may lead to high creaminess perception. In our study the lipid levels for the Pacific cupped oyster (18.4±1.8% DW) were higher than in the European flat oyster (10.3±0.7% DW). However, the glycogen level was found to be higher for the European flat oyster (23.8±1.1% DW) than in the Pacific cupped oyster (16.4±1.5% DW).

The glycogen content of oysters has also been linked to the texture of oysters in the studies of Nell (2002) and Pennarun et al. (2003). In both studies higher glycogen levels led to a firmer texture. This might explain the differences found for the attribute firm in our TDS evaluations. The dominance rate for the attribute firm was slightly higher (23%) for the European flat oyster than for the Pacific cupped oyster (19%) as was the glycogen content (23.8±1.1 versus 16.4±1.5% DW).

Although higher NaCl levels were measured in the Pacific cupped oyster (8.3±1.2% DW) in comparison with the European flat oyster (3.9±0.1% DW) no apparent relation could be found in the TDS results on saltiness. Van Houcke et al. (2016) did show a relationship between the NaCl content of both species and the saltiness perception by naïve consumers.

The saltiness perception for the Pacific cupped oyster based upon sensory evaluation was higher than in the European flat oyster.

In addition to the sensory evaluation results of Van Houcke et al. (2016) on both the Pacific cupped and the European flat oyster the TDS evaluation presented in this chapter shows a significant difference for the attribute pungent. The results from the TDS indicate a lingering pungent taste for the European flat oyster. The attribute pungent was also used in the study of Van Houcke et al. (2016), but no significant difference was found between the oyster species.

4.4 Conclusion

It was shown that dynamic sensorial properties of the Pacific cupped oyster and the European flat oyster differ. The dynamic sensory perception of the Pacific cupped oyster evolves from salty at the beginning of consumption to creamy at the middle of consumption proceeding to sweet at the end of consumption. The dynamic sensory perception of the European flat oyster evolves from salty at the beginning of consumption to pungent in the middle of consumption and sweet at the end of consumption.

TDS curves for the Pacific cupped oyster showed significant dominance rates for the attributes creamy, salt and sweet, while for the European flat oyster significant dominance rates are observed for the attributes salt, pungent and sweet.

The TDS difference curve between the two species of oyster, showed significant differences for the attributes earthy, creamy (higher dominance rate in Pacific cupped oyster) and pungent (higher dominance rate in European flat oyster).

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Chapter 5: Biochemical and volatile organic compound profile of European flat oyster (*Ostrea edulis*) and Pacific cupped oyster (*Crassostrea gigas*) cultivated in the Eastern Scheldt and Lake Grevelingen, the Netherlands

"A good oyster cannot please the palate as acutely as a bad one can revolt it, and a good oyster cannot make him who eats it live for ever though a bad one can make him dead for ever."

Rebecca West

Jasper van Houcke, Isabel Medina, Jozef Linssen & Joop Luten

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Abstract

The aim of this study was to evaluate the effect of two important different geographical cultivation areas in the Netherlands (Eastern Scheldt and Lake Grevelingen) on the volatile organic compound (VOC) profile of European flat oyster (*Ostrea edulis*) and Pacific cupped oyster (*Crassostrea gigas*). Market size oysters were analyzed for biochemical composition (dry matter, ash, protein, lipid content and fatty acid profile) and VOCs from samples harvested in January and February. Significant differences in fatty acids and in VOC content were observed between the oyster species. The European flat oyster was found to have a higher level of unsaturated fatty acids in comparison with Pacific cupped oysters. The main VOC in the European flat oyster was found to be 3-cyclohexene-1-ethanol while 1,5-octadien-3-ol was the main VOC in the Pacific cupped oyster. Principle component analysis (PCA) not only showed separation between oyster species, but also between oysters originating from different cultivation areas as well as oysters harvested at different time intervals.

5.1 Introduction

Oysters are considered a delicacy in Europe. Usually they are eaten fresh and raw as a starter. The Netherlands is ranked as the fourth and fifth producing country within Europe for the European flat oyster (*Ostrea edulis*) and the Pacific cupped oyster (*Crassostrea gigas*), respectively (FAO, 2014). Cultivation takes place on approximately five hundred plots located in the Eastern Scheldt (only Pacific cupped oyster) and Lake Grevelingen (European flat oyster and Pacific cupped oyster). The two cultivation areas are situated in the southwestern part of the Netherlands and have different characteristics. Lake Grevelingen is a stagnant saline lake which suffers from stratification. The Eastern Scheldt is enclosed by a semi-permeable barrier limiting water flow. Furthermore the Eastern Scheldt is characterized by low primary production (van Wesenbeeck et al., 2014). The oyster species from the different areas are sold under different commercial product names. The price for the European flat oyster is approximately four times higher than the price of the Pacific cupped oyster (FAO, 2014). Experts in the oyster supply chain in the Netherlands have suggested that the European flat oyster has superior sensory qualities compared to the Pacific cupped oyster. However, no significant differences in preference between the oyster species were observed among Dutch consumers. They also did not perceive significant differences in odor properties (Van Houcke, Altintzoglou, Stieger, Linssen and Luten, 2016).

Geographical factors (water temperature, salinity and feed availability) have been shown to affect the biochemical composition and sensory properties of Pacific cupped oysters (Cochet, Brown, Kube, Elliott and Delahunty, 2013; Fratini et al., 2013; Hosoi, Kubota, Toyohara, Toyohara and Hayashi, 2003; Pennarun, Prost, Haure and Demaimay, 2003). An important factor for consumer acceptance of oysters is the odor, being composed of the volatile organic compounds (VOCs) present (Pennarun, Prost and Demaimay, 2002). VOCs are mainly derived via enzymatic degradation or auto-oxidative reactions of polyunsaturated fatty acids (PUFAs) present in oysters (Josephson, Lindsay and Stuiber, 1985). Up to 115 different VOCs have been identified by various analytical techniques (headspace purge-and-trap, vacuum steam distillation and solid phase micro extraction linked to GC-MS) in the European flat oyster (Fratini, Lois, Pazos and Medina, 2012) and Pacific cupped oysters (Fratini et al., 2013; Pennarun et al., 2003; Pennarun et al., 2002; Piveteau et al., 2000; Josephson et al., 1985). The main VOCs associated with fresh seafood odors are aldehydes, ketones and alcohols derived from PUFAs mainly by lipoxygenase activity (Josephson et al., 1985). Odor descriptors linked to VOCs in oysters are: mushroom, grass, moss, cucumber, citrus, melon, fatty, buttery, milky, white fish and almond (Pennarun et al., 2003; Pennarun et al., 2002; Piveteau et al., 2000; Josephson et al., 1985).

The aim of this current study was to evaluate the effect of the two important different geographical oyster cultivation areas in the Netherlands on the VOC profile of the European flat oyster and Pacific cupped oyster. In that context the biochemical composition and in particular the fatty acid composition were also measured and discussed.

This is the first study undertaken in the Netherlands with two different oyster species from the two most important production areas with a focus on the presence of VOCs and fatty acid profile. The results will form the basis for the design of an in-depth investigation on the effects of algae with different fatty acid profiles as feed for the innovation of on-land cultivation of oysters.

5.2 Materials and methods

5.2.1 Oysters

Live European flat and Pacific cupped oysters were harvested in January and February 2012 by the Dutch shellfish company (Koninklijke Prins en Dingemanse, Yerseke, The Netherlands). The oysters were cultivated in the Eastern Scheldt (Pacific cupped oyster) and Lake Grevelingen up to two and four years for the Pacific cupped oyster and European flat oyster, respectively. Both cultivation areas are located approximately 15 km away from each other in the Southwest of the Netherlands with similar meteorological and climatological circumstances. The average oxygen levels in the Eastern Scheldt were 12.6 and 12.5 mg l^{-1} while 12.4 and 12.5 mg l^{-1} was recorded in Lake Grevelingen in the experimental period (January and February, respectively). The salinity of the Eastern Scheldt showed an average value of 32.3 g l^{-1} in January and 32.2 g l^{-1} in February. In Lake Grevelingen the salinity recorded was 30.5 and 30.0 g l^{-1} in January and February, respectively. Chlorophyll levels of 0.68 and 0.90 μg l^{-1} were measured in the Eastern Scheldt while levels of 0.78 and 1.05 μg l^{-1} were measured in Lake Grevelingen in January and February, respectively. It is evident that the oxygen levels were similar in both areas. The salinity and chlorophyll levels in Eastern Scheldt are slightly higher respectively lower in comparison with the levels in Lake Grevelingen (RWS, 2016). Oysters used in this study were market size (average weight 91.0±11.2 g). Oysters were opened by hand-shucking and the adductor muscles were cut with a knife on both sides. Internal liquid was drained from the oysters which were then washed using demineralized water to remove excess salt.

5.2.2 Gross biochemical composition

The gross biochemical composition (dry matter, ash, protein and lipid content) was determined from three samples each consisting of 10 individual oysters per experimental group. Samples were homogenized using a Ultra Turrax T25 homogenizer (IKA Werke GmbH, Staufen, Germany) prior to the analysis. Dry matter content was determined by drying 5 g of homogenate at 105°C for 48 hours. The ash content was determined by incinerating 1 g of homogenate in a muffle oven at 550°C for 16 hours. The protein content was determined in 20-25 mg of homogenate using the Dumas method (Dumas, 1831) by N2 detection on a thermal conductivity detector as described by Simonne, Simonne, Eitenmiller, Mills and Cresman (1997). The lipid content of the oysters was determined in 2.5 g of homogenate by extraction with 50 ml dichloromethane/methanol/water mixture (8:4:3) according to the method of Folch, Lees and Stanley (1957).

5.2.3 Fatty acid composition

The lipid fraction obtained in the biochemical analysis was also used for the fatty acid composition determination. The fatty acid composition of the lipids was determined using the method described by Ichihara, Shibahara, Yamamoto and Nakayama (1996). After base catalyzed transesterification the resulting fatty acid methyl esters (FAMEs) were separated by gas chromatography (Focus GC, Thermo scientific, Breda, The Netherlands) equipped with an auto sampler (Triplus, Thermo scientific, Breda, The Netherlands) and a flame ionization detector (FID). The capillary column was 30 m in length, 0.25 mm ID and 0.25 µm film thickness (FameWax, Restek, Bellefonte, USA). The samples were analyzed by split injection (split- flow 50 ml min^{-1}; ratio 5.0) and helium was used as the carrier gas. The air flow was set to 350 ml min^{-1} and the injection volume of the sample was 1.0 μL. The temperatures of the injector and the detector were set at 250°C. The oven temperature was increased from 195°C (0 min) to 240°C (1 min) at a rate of 5°C min^{-1}. Sample fatty acids were identified by comparing the relative retention times of FAME peaks from samples with those of standards (PUFA No. 3 from Menhaden Oil, and F.A.M.E. Mix RM3 (Supelco 07256-1AMP)).

5.2.4 Volatile organic compounds

VOCs were extracted from five individual oysters per experimental group using headspace solid phase microextraction (HS-SPME) and analyzed by gas chromatography-mass spectrometry (GC-MS) following the procedures described by Fratini et al. (2012). For every individual oyster 10 ml of NaCl saturated ultrapure water was added to 5 g oyster tissue prior to homogenization. Homogenization was done for one minute using a T25 Ultra Turrax homogenizer (IKA Werke GmbH, Staufen, Germany) ensuring that the sample remained in ice thus preventing excess heat. The supernatant was obtained after centrifugation at 3750 rpm at 4°C for 20 min. The absorption of VOCs in the headspace of the supernatant was performed using CAR-PDMS fibers (75 μm carboxen/polydimethylsiloxane) for 30 min at 80 °C. The VOCs were thermally desorbed by inserting the fiber into the GC injector for 10 minutes at 260°C in split mode in the headspace unit of a Trace GC Ultra (Thermo Scientific, Breda, The Netherlands) coupled with a trace quadrupole mass detector (DSQII, Thermo Scientific, Breda, The

Netherlands). Absorption and desorption of VOCs was automated using a Triplus (Thermo scientific, Breda, The Netherlands) auto sampler. Determination of the VOCs was performed using the internal standard method (3-methyl-3-buten-1-ol) which was added to the supernatant prior to absorption of the VOCs.

Mass spectra of the VOCs were compared with mass spectra of the NIST and Mainlib libraries for identification of the VOCs.

5.2.5 Statistical data analysis

One way ANOVA followed by Post-hoc Tukey analysis when significant was used on the biochemical analysis, the fatty acids and VOC data.

Correlation between fatty acids and VOCs was tested using the concentration data of all experimental oyster groups using Spearman's rank correlation. Only significant correlations are shown and discussed in this article.

Principal Component Analysis (PCA) was performed using all measurements of the fatty acids and VOCs.

Data are expressed as mean ± standard deviation. The significance level was set at $p < 0.05$.

5.3 Results and Discussion

5.3.1 Gross biochemical composition

The gross biochemical composition of the European flat oysters and Pacific cupped oysters from the cultivation areas harvested in January and February 2012 are shown in Table 5.1.

Table 5.1 Gross biochemical composition of different experimental groups of European flat oysters (EFO) and Pacific cupped oysters (PCO). Data are either expressed as a percentage of the total wet weight (WW) or as a percentage of the total dry weight (DW). Different superscripts in the same row indicate significant differences between experimental groups.

	Jan EFO Gre			Jan PCO Gre			Jan PCO Eas			Feb EFO Gre			Feb PCO Gre			Feb PCO Eas		
	Ave		Stdev	Ave		Stdev	Ave		Stdev	Ave		Stdev	Ave		Stdev	Ave		Stdev
Dry matter (% WW)	20,34	a	0,56	20,64	a	0,47	20,71	a	0,86	21,06	a	0,18	19,75	ab	0,11	18,53	b	0,32
Ash content (% DW)	10,64	abc	1,75	10,42	ab	1,22	13,62	c	1,10	8,55	a	0,33	11,40	bc	0,93	11,88	bc	0,24
Protein (% DW)	49,81	ab	2,33	51,26	b	1,13	44,53	a	3,59	45,92	ab	1,55	49,19	ab	1,05	44,88	ab	3,48
Lipid (% DW)	5,38		0,83	5,73		0,61	6,40		0,65	4,71		0,21	4,72		0,05	4,97		0,59
Glycogen (% DW)	16,57	a	2,63	3,99	bc	0,68	0,93	d	0,07	12,30	a	2,43	5,65	b	0,53	1,92	cd	0,05

Jan Efo Gre, European flat oysters from Lake Grevelingen sampled in January; Jan PCO Gre, Pacific cupped oyster from Lake Grevelingen sampled in January; Jan PCO Eas, Pacific cupped oysters from the Eastern Scheldt sampled in January; Feb EFO Gre, European flat oysters from Lake Grevelingen sampled in February; Feb PCO Gre, Pacific cupped oysters from Lake Grevelingen sampled in February; Feb PCO Eas, Pacific cupped oysters from the Eastern Scheldt sampled in February.

The protein content of all investigated oyster samples varied from 44.5 to 51.2% DW. There were no significant differences between species, locations and harvesting time. Only the protein content of the January harvested Pacific cupped oyster from Lake Grevelingen was significantly higher in comparison with the January harvested Pacific cupped oyster from the Eastern Scheldt.

Significant higher levels of lipids were found in oysters harvested in January (5.4-6.4% DW) compared to oysters harvested in February (4.7-5.0% DW). No significant effects were found between oyster species and cultivation area. Pazos, Ruez, Garcia-Martin, Abad and Sanchez (1996) and Adab, Ruiz, Martinez, Mosquera and Sánchez (1995) reported higher lipid levels for European flat oysters and Pacific cupped oysters in February in comparison with January. Both studies (conducted in Galicia, Spain) correlate the lipid levels of the oysters to the sexual cycle and the chlorophyll a level in the water column. The differences obtained in our study could therefore most likely be explained by the lack of gametogenic development and decreasing feed abundance at both cultivation areas. The dry matter, ash, protein and lipid content are comparable with ranges found in Pacific cupped oyster monitored monthly in Cork Harbour, Ireland by Linehan, O'Conner and Burnell (1999).

5.3.2 Fatty acid composition

The percentages of total saturated fatty acids (SFA), mono saturated fatty acids (MUFA), poly unsaturated fatty acids (PUFA), PUFA n-3 and PUFA n-6 are presented in Figure 5.1.

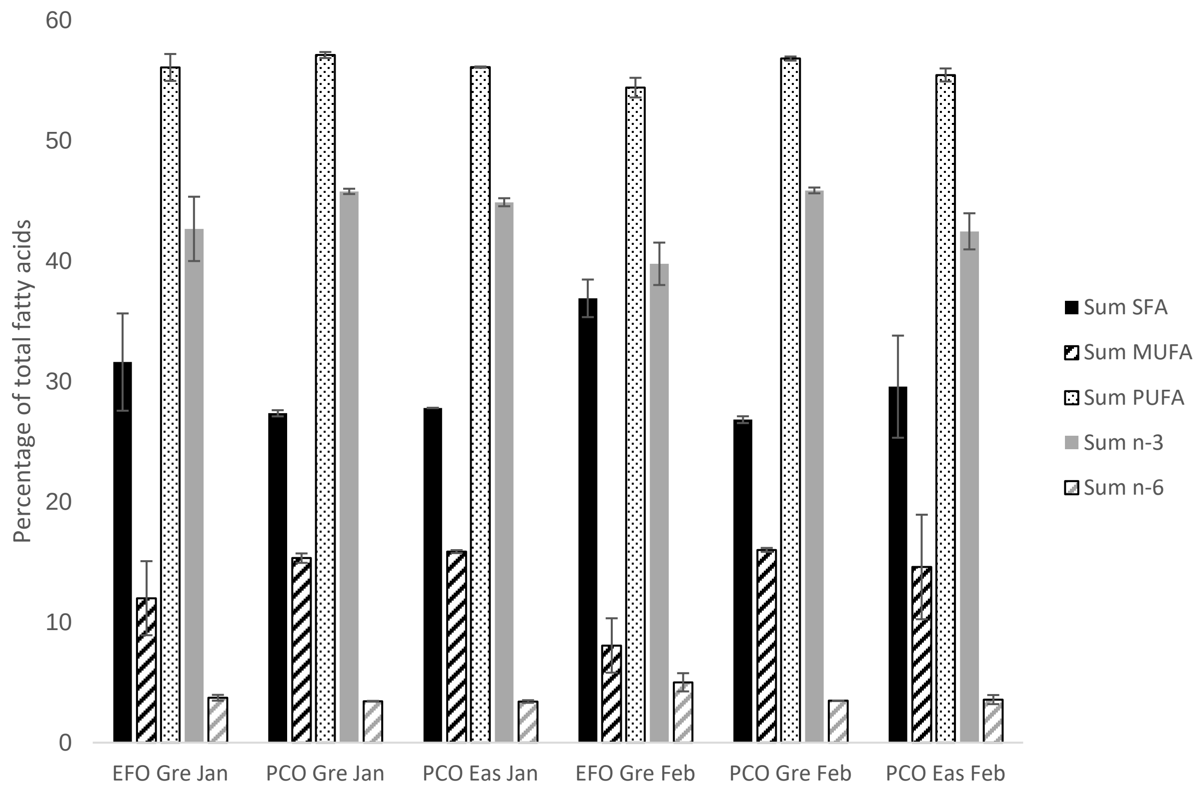


Figure 5.1 Total Saturated Fatty Acids (SFA), total Monounsaturated Fatty Acids (MUFA), total Poly Unsaturated Fatty Acids (PUFA), total PUFAn-3 and total PUFA n-6 of experimental groups of European flat oysters (EFO) and Pacific cupped oysters (PCO). Data are expressed as a percentage of total fatty acids. Jan Efo Gre, European flat oysters from Lake Grevelingen sampled in January; Jan PCO Gre, Pacific cupped oyster from Lake Grevelingen sampled in January; Jan PCO Eas, Pacific cupped oysters from the Eastern Scheldt sampled in January; Feb EFO Gre, European flat oysters from Lake Grevelingen sampled in February; Feb PCO Gre, Pacific cupped oysters from Lake Grevelingen sampled in February; Feb PCO Eas, Pacific cupped oysters from the Eastern Scheldt sampled in February.

The percentage of total SFA is significantly higher in the European flat oysters (31.6-36.9%) compared to the Pacific cupped oysters in January (27.4-27.8%) and February (26.8-29.6%). The percentage of total MUFA is significantly lower in European flat oyster groups (8.1-12.0%) compared to the Pacific cupped oyster groups in both sampling months (14.6-16.0%). Furthermore the percentage of total PUFA is slightly lower and not always significant in the European flat oyster groups (54.4-56.1%) in comparison with the samples of the Pacific cupped oysters (55.5-57.1%). European flat oysters show a lower total PUFA n-3 (39.8-42.7%) in comparison with the Pacific cupped oyster (42.5-45.9%). There are no significant differences between species, cultivation areas and harvesting time for total PUFA n-6. Only the European flat oyster from February from Lake Grevelingen was significantly higher (5.0%) in comparison with the other samples (3.4-3.7%). Fratini et al. (2013) and Fratini et al. (2012) reported slightly different values for total SFA (25.5 and 33.0%), MUFA (15.2 and 20.7%), PUFA (49.2 and 41.0%), n-3 PUFA (33.2 and 31.2%) and n-6 PUFA (6.9 and 6.8%) for European flat and Pacific cupped oysters from Italy, respectively.

The fatty acid composition of the different groups of oysters are shown in Table 5.2. The fatty acid composition of all experimental oyster groups is dominated by C16:0 (15.6-17.9%), C20:5n3 (21.2-30.6%) and C22:6n3 (8.8-14.8%). C16:0 has been reported as the main SFA (Pogoda, Buck, Saborowski and Hagen, 2013; Fratini et al., 2013; Fratini et al., 2012; Linehan et al., 1999; Pazos et al., 1996; Abad et al., 1995) in Pacific cupped oyster samples from Germany, Italy, Ireland and Spain and European Flat oyster samples from

Germany, Italy and Spain. The C16:0 percentages in those samples varied between 12.0-24.6% in Pacific cupped oysters and from 13.1 to 22.5% in European flat oyster.

Table 5.2 Fatty acid composition of different experimental groups of European flat oysters (EFO) and Pacific cupped oysters (PCO) expressed as percentage of total fatty acid content. Different superscripts in the same row indicate significant differences between experimental groups.

	Jan Flat			Jan Jap Grev			Jan Jap Oos			Feb Flat			Feb Jap Grev			Feb Jap Oos		
	Ave		Stdev	Ave		Stdev	Ave		Stdev	Ave		Stdev	Ave		Stdev	Ave		Stdev
C14:0	4,56		0,21	3,59		0,02	3,79		0,03	3,90		1,10	3,79		0,13	3,03		0,02
C15:0	0,80	a	0,02	0,54	bc	0,01	0,49	c	0,01	0,79	a	0,08	0,62	b	0,01	0,61	b	0,03
C16:0	17,93		0,68	16,76		0,27	15,58		0,24	15,56		2,58	16,37		0,17	15,80		0,76
C16:1n7	3,63		0,09	2,84		0,09	2,63		0,11	2,68		1,09	3,10		0,07	3,01		0,28
C16:2n4	1,01	a	0,08	0,72	c	0,01	0,92	ab	0,04	0,94	ab	0,10	0,82	bc	0,01	0,89	ab	0,09
C16:3n4	0,78	a	0,12	0,79	a	0,04	0,58	ab	0,06	0,38	b	0,17	0,63	ab	0,08	0,73	a	0,10
C17:0	0,37	ab	0,05	0,23	ab	0,00	0,19	b	0,06	0,61	a	0,34	0,34	ab	0,02	0,36	ab	0,03
C18:0	4,74	a	0,04	3,23	a	0,08	4,12	a	0,08	9,35	b	3,17	3,18	a	0,03	3,84	a	0,04
C18:1	0,04	a	0,02	1,27	b	0,03	1,12	b	0,02	0,04	a	0,04	1,29	b	0,02	1,19	b	0,06
C18:1n9	2,57	ab	0,11	3,70	c	0,04	1,84	a	0,20	2,30	a	0,64	3,27	bc	0,09	3,73	c	0,26
C18:1n7	3,58	a	0,18	6,30	b	0,16	8,36	c	0,23	2,77	a	0,70	6,93	bc	0,07	7,62	bc	0,52
C18:2	1,27	a	0,01	1,92	c	0,06	0,81	b	0,08	1,01	ab	0,32	1,24	a	0,02	0,95	ab	0,01
C18:2n6	0,06	a	0,00	0,15	bc	0,00	0,20	c	0,01	0,08	ab	0,06	0,15	bc	0,00	0,17	c	0,01
C18:3n3	1,06	ab	0,05	1,74	c	0,03	1,18	abc	0,03	0,77	a	0,42	1,42	bc	0,04	1,65	bc	0,02
C18:4n3	1,80	a	0,06	3,36	b	0,06	2,68	b	0,10	1,29	a	0,46	2,70	b	0,02	2,94	b	0,03
C20:0	5,74	a	0,21	2,88	bc	0,02	3,55	cd	0,02	5,71	a	0,75	2,71	b	0,05	3,68	d	0,11
C20:1n9	0,04	a	0,01	1,34	b	0,14	1,55	b	0,01	0,18	a	0,15	1,37	b	0,05	1,44	b	0,04
C20:4n6	3,07	a	0,06	2,54	b	0,01	2,37	b	0,04	4,00	c	0,38	2,65	b	0,01	2,51	b	0,02
C20:4n3	0,72		0,16	0,86		0,01	0,77		0,04	0,80		0,71	0,81		0,01	0,75		0,01
C20:5n3	24,81	ab	0,30	26,41	bc	0,02	30,59	c	0,57	21,24	a	3,11	27,06	bc	0,06	25,53	b	0,48
C22:0	0,13		0,02	0,09		0,01	0,09		0,00	0,35		0,29	0,07		0,03	0,09		0,00
C22:1	0,16		0,06	0,07		0,01	0,07		0,01	0,11		0,04	0,06		0,05	0,11		0,00
C22:2i	6,53	a	0,49	3,41	b	0,11	4,09	ab	0,19	6,60	a	2,35	3,69	b	0,14	4,92	ab	0,03

	Jan Flat			Jan Jap Grev			Jan Jap Oos			Feb Flat			Feb Jap Grev			Feb Jap Oos		
	Ave		Stdev	Ave		Stdev	Ave		Stdev	Ave		Stdev	Ave		Stdev	Ave		Stdev
C22:2j	0,98	ab	0,03	1,09	ab	0,04	1,45	b	0,03	0,69	a	0,32	1,09	ab	0,03	1,27	b	0,03
C22:4n6	0,53		0,05	0,61		0,02	0,68		0,04	0,44		0,19	0,52		0,03	0,50		0,02
C22:5n6	0,24	ab	0,01	0,15	b	0,01	0,18	b	0,01	0,50	a	0,22	0,17	b	0,01	0,18	b	0,01
C22:5n3	1,10	abc	0,02	1,01	ab	0,05	1,22	bc	0,03	0,92	a	0,19	0,94	a	0,02	1,33	c	0,04
C22:6n3	11,55	a	0,09	12,32	ab	0,01	8,82	c	0,05	14,75	b	1,99	12,95	ab	0,10	11,08	a	0,14
C24:0	0,20	a	0,03	0,08	a	0,01	0,08	a	0,01	1,24	b	0,95	0,10	a	0,02	0,08	a	0,00

Jan Efo Gre, European flat oysters from Lake Grevelingen sampled in January; Jan PCO Gre, Pacific cupped oyster from Lake Grevelingen sampled in January; Jan PCO Eas, Pacific cupped oysters from the Eastern Scheldt sampled in January; Feb EFO Gre, European flat oysters from Lake Grevelingen sampled in February; Feb PCO Gre, Pacific cupped oysters from Lake Grevelingen sampled in February; Feb PCO Eas, Pacific cupped oysters from the Eastern Scheldt sampled in February.

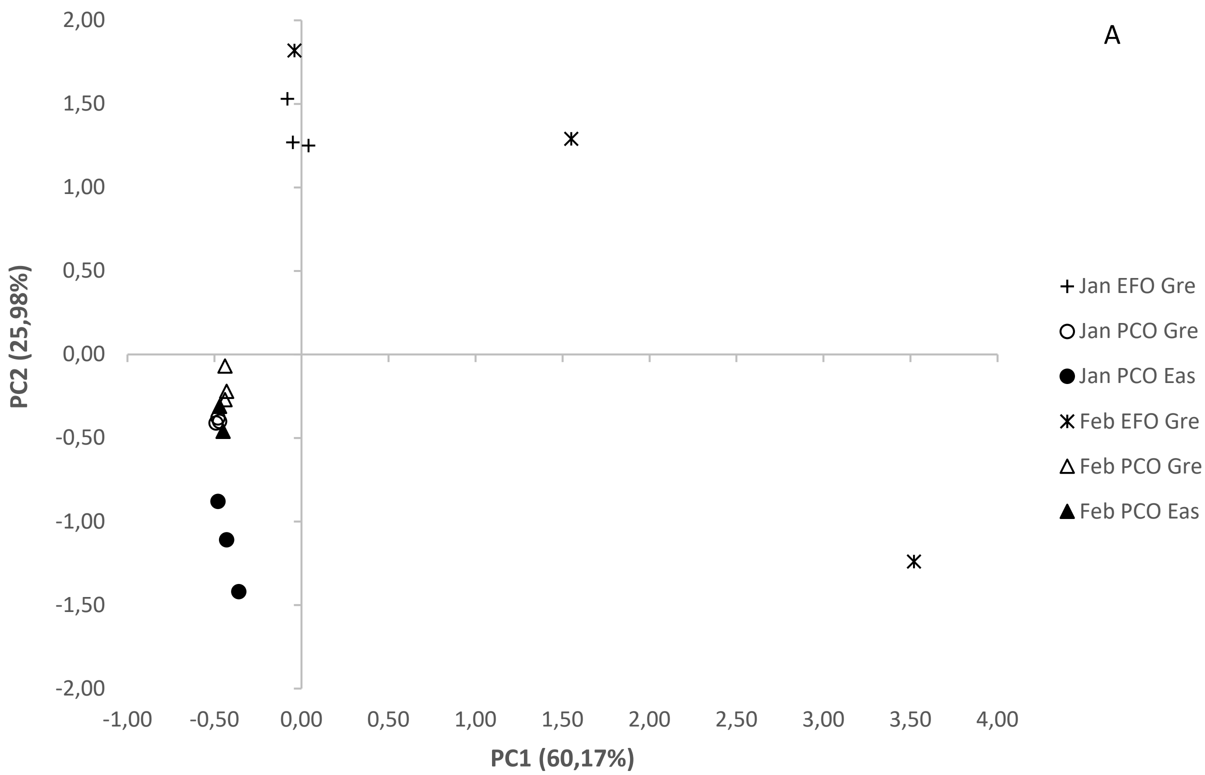
C20:5n3 levels tended to be lowest for the European flat oysters (21.2-24.8%) followed by Pacific cupped oysters from Lake Grevelingen (26.4-27.1%). Pacific cupped oysters from the Eastern Scheldt showed the highest levels of C20:5n3 (25.5-30.6%). C22:6n3 levels tended to be slightly higher (11.6-14.8%) in the European flat oysters when compared with the Pacific cupped oysters (8.8-13.0%). The levels of these essential fatty acids are comparable with the ranges found in the literature. In samples from Germany and Italy (Pogoda et al., 2013; Fratini et al., 2013; Fratini et al., 2012) the C20:5n3 levels varied between 12.5-19.9% in European flat oysters and from 7.5 to 22.5% in Pacific cupped oysters. C22:6n3 levels varied from 14.8 to 23.7% in the European flat oysters and from 13.4 to 21.3% in the Pacific cupped oysters.

Pacific cupped oysters cultivated in the Eastern Scheldt show higher levels of C18:1n7 (7.6-8.4%) in comparison with Pacific cupped oysters from Lake Grevelingen (6.3-6.9%). Possibly this is due to a difference in diatom availability between the cultivation areas. The literature states that the levels of specific fatty acids might indicate the main food source for the experimental oyster groups (Dalsgaard, John, Kattner, Muller-Navarra and Hagen, 2003). High levels of C16:1n7, C18:1n7 and C20:5n3 are found in diatoms (Sargent, Parkes, Mueller-Harvey and Henderson, 1987). Also studies by Pennarun et al. (2003) and Piveteau et al. (2000) showed elevated levels of C16:1n7, C18:1n7, C20:5n3 for Pacific cupped oysters fed with the diatom *Skeletonema costatum* for a period of six weeks.

The two unknown fatty acids are most likely to be two dienoic acids (C22:2), however positive identification was not possible. C22:2 has been reported (Pogoda et al., 2013;

Dridi, Romdhane and Elcafsi, 2007; Pazos et al., 1996; Abad et al., 1995) in similar concentrations to the unknowns in this study.

PCA (Figure 5.2A and 5.2B) was applied to the percentages of all individual fatty acids in order to facilitate the interpretation and elucidate the relative importance of the oyster species, harvest period and cultivation area. In figure 5.2A European flat oysters from Lake Grevelingen both in January and February are clearly separated from the Pacific cupped oyster groups indicating that most differences found are due to species. This separation is mainly caused by the fatty acids C15:0, C18:1, C18:1n7, C18:4n3, C20:0, C20:1n9, C20:4n6, unknown2 and C22:5n6. Higher percentages of C15:0, C20:0, C20:4n6, unknown2 and C22:5n6 were present in the European flat oysters in comparison with the Pacific cupped oyster while the percentages of the fatty acids C18:1, C18:1n7, C18:4n3 and C20:1n9 were lower in the European flat oysters. Figure 5.2B shows a PCA model of the fatty acid composition excluding the European flat oysters samples. Full separation occurs between both Pacific cupped oysters from different cultivation areas and different harvest periods based on the fatty acid profile of these oysters.


A
PC2 (25,98%)
PC1 (60,17%)
Jan EFO Gre
Jan PCO Gre
Jan PCO Eas
Feb EFO Gre
Feb PCO Gre
Feb PCO Eas

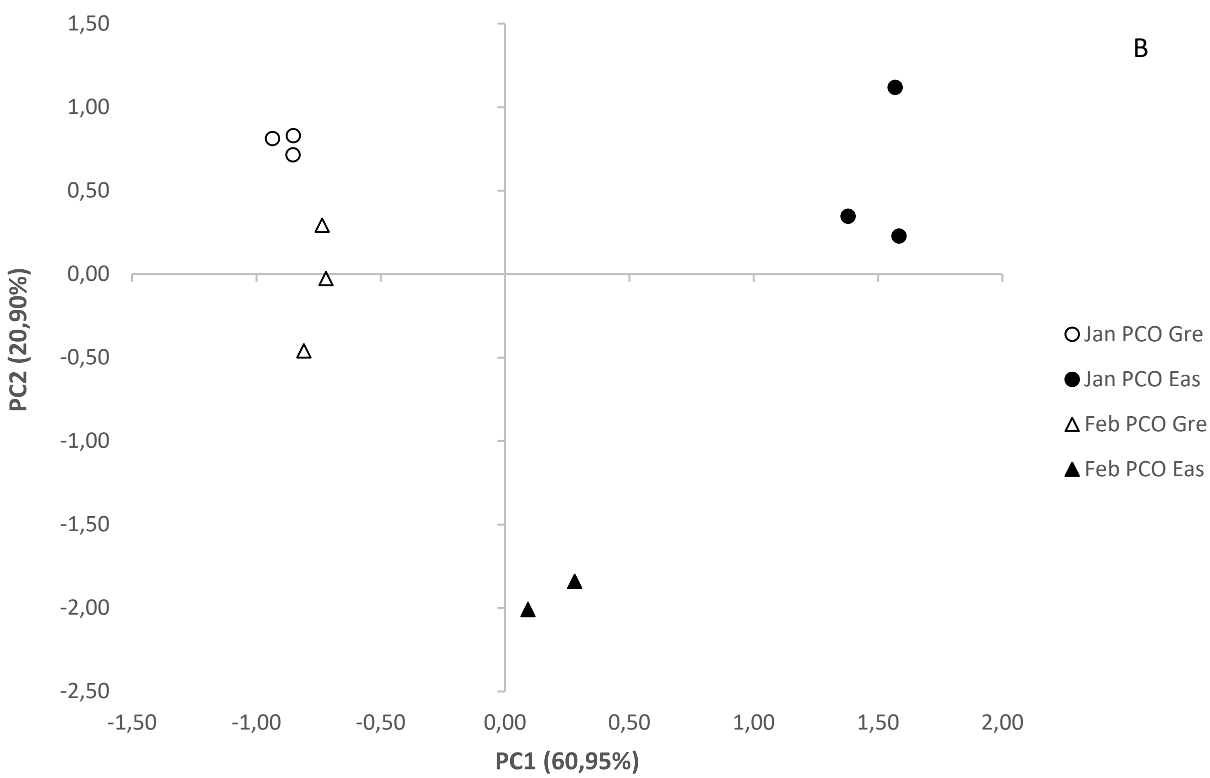

B
PC2 (20,90%)
PC1 (60,95%)
Jan PCO Gre
Jan PCO Eas
Feb PCO Gre
Feb PCO Eas

Figure 5.2 Principal component analysis for the fatty acid profile characteristics of different : experimental groups of European flat oysters (EFO) and Pacific cupped oysters (PCO) (A) and excluding European flat oyster groups (B). Jan Efo Gre, European flat oysters from Lake Grevelingen sampled in January; Jan PCO Gre, Pacific cupped oyster from Lake Grevelingen sampled in January; Jan PCO Eas, Pacific cupped oysters from the Eastern Scheldt sampled in January; Feb EFO Gre, European flat oysters from Lake Grevelingen sampled in February; Feb PCO Gre, Pacific cupped oysters from Lake Grevelingen sampled in February; Feb PCO Eas, Pacific cupped oysters from the Eastern Scheldt sampled in February.

5.3.3 Volatile organic compounds

In all experimental groups 24 VOCs were identified (Table 5.3). The VOCs found are mainly aldehydes (13), alcohols (5) and ketones (4). Only one alkane (hexane) and one furan (2-ethylfuran) was detected. The most abundant VOCs in the oysters samples were the alcohols ranging from 8.3-21.0% followed by the aldehydes (10.1-17.5%) and ketones (1.9-4.8%). Aldehydes were less abundant in the Pacific cupped oysters harvested in February (10.1%) while most abundant in the February harvested European flat oysters (17.5%). Alcohols were found to be significantly less abundant in February harvested Pacific cupped oysters from Lake Grevelingen (8.3%) compared with all other samples (15.6-21.0%).

Table 5.3 Volatile compounds composition of different experimental groups of European flat oysters (EFO) and Pacific cupped oysters (PCO). Data are either expressed as a peak area/I.S. area. Different superscripts in the same row indicate significant differences between experimental groups.

	EFO Grev Jan			PCO Grev Jan			PCO Eas Jan			EFO Grev Feb			PCO Grev Feb			PCO Eas Feb		
	Ave		Stdev	Ave		Stdev	Ave		Stdev	Ave		Stdev	Ave		Stdev	Ave		Stdev
Aldehydes	*12,47*	*ab*	*1,85*	*15,11*	*ab*	*3,10*	*13,96*	*ab*	*2,36*	*17,46*	*b*	*2,32*	*10,27*	*a*	*1,38*	*10,11*	*a*	*3,53*
Pentanal	0,37	bc	0,08	0,37	b	0,08	0,31	ab	0,05	0,50	c	0,07	0,34	ab	0,07	0,24	a	0,02
(E)-2-Pentenal	1,16	ab	0,21	1,69	b	0,42	1,71	b	0,40	1,67	b	0,28	0,84	a	0,17	0,95	a	0,43
Hexanal	0,98	b	0,15	0,78	ab	0,10	0,65	ab	0,14	1,37	c	0,24	0,47	a	0,10	0,58	a	0,30
(E)-2-Hexenal	0,47	abc	0,08	0,60	bc	0,12	0,66	c	0,06	0,58	abc	0,08	0,35	a	0,05	0,39	ab	0,21
Heptanal	0,77	a	0,11	0,66	a	0,18	0,77	ab	0,11	1,30	b	0,29	0,43	a	0,06	0,43	a	0,17
(Z)-4-Heptenal	0,29		0,08	0,28		0,06	0,33		0,06	0,33		0,07	0,44		0,07	0,43		0,17
(E,E)-2,4-Heptadienal(1)	0,55	ab	0,13	0,87	b	0,30	0,71	ab	0,19	0,84	b	0,11	0,54	ab	0,09	0,38	a	0,16
(E,E)-2,4-Heptadienal(2)	2,48	ab	0,59	3,46	b	1,15	3,25	ab	0,76	3,27	ab	0,81	2,23	ab	0,45	1,97	a	0,42
Octanal	0,65	b	0,06	0,26	a	0,05	0,35	a	0,11	0,67	b	0,12	0,59	b	0,12	0,21	a	0,09
(E)-2-Octenal	1,00	b	0,19	0,99	b	0,25	0,27	a	0,04	0,99	b	0,14	0,17	a	0,03	0,21	a	0,09
(E,Z)-2,6-Nonadienal	1,90		0,50	3,32		0,32	3,20		0,85	3,19		0,52	2,62		0,75	3,03		1,44
Benzaldehyde	0,75	b	0,18	0,75	b	0,20	0,70	b	0,10	1,13	c	0,23	0,34	a	0,03	0,33	a	0,13
Lilac aldehyde	1,11	a	0,22	1,10	a	0,31	1,03	a	0,21	1,63	b	0,38	0,90	a	0,07	0,96	a	0,22
Alcohols	*18,06*	*b*	*3,31*	*16,83*	*b*	*3,65*	*17,42*	*b*	*2,90*	*21,01*	*b*	*2,97*	*8,25*	*a*	*1,14*	*15,64*	*b*	*5,38*
1-Penten-3-ol	2,89	b	0,32	3,78	bc	0,84	4,04	c	0,66	3,10	bc	0,30	1,57	a	0,10	1,32	a	0,30
(E)-2-Penten-1-ol	0,93	ab	0,12	0,74	a	0,10	1,71	c	0,40	1,38	bc	0,36	0,69	a	0,06	0,73	a	0,25
1,5-Octadien-3-ol	5,03	a	1,06	8,78	ab	2,27	10,50	b	2,19	4,47	a	0,78	4,37	a	1,00	10,65	b	4,10
(Z)-2-Octen-1-ol	1,03		0,21	0,75		0,18	0,81		0,11	1,01		0,14	0,77		0,15	1,12		0,66
3-Cyclohexene-1-ethanol	8,18	b	2,56	2,78	a	1,67	0,36	a	0,05	11,05	b	2,55	0,86	a	0,31	1,82	a	0,97
Ketones	*2,07*	*a*	*0,12*	*3,23*	*b*	*0,92*	*2,84*	*ab*	*0,47*	*4,80*	*c*	*0,75*	*1,90*	*a*	*0,16*	*1,86*	*a*	*0,38*

	EFO Grev Jan			PCO Grev Jan			PCO Eas Jan			EFO Grev Feb			PCO Grev Feb			PCO Eas Feb		
	Ave		Stdev	Ave		Stdev	Ave		Stdev	Ave		Stdev	Ave		Stdev	Ave		Stdev
1-Penten-3-one	0,68	ab	0,10	0,79	ab	0,29	1,06	b	0,30	1,51	c	0,20	0,56	a	0,10	0,57	a	0,25
2,3-Pentadione	0,49	a	0,11	1,27	b	0,26	0,85	ab	0,09	1,91	c	0,30	0,60	a	0,07	0,70	a	0,14
1-Octen-3-one	0,65	bc	0,06	0,53	abc	0,20	0,43	ab	0,14	0,71	c	0,14	0,42	ab	0,09	0,37	a	0,06
(E,E)-3,5-Octadien-2-one	0,26	a	0,03	0,64	c	0,20	0,51	bc	0,06	0,67	c	0,15	0,32	ab	0,04	0,22	a	0,07
Alkanes	*0,52*	*a*	*0,08*	*0,35*	*ab*	*0,09*	*0,48*	*b*	*0,24*	*0,22*	*a*	*0,05*	*0,16*	*a*	*0,05*	*0,18*	*a*	*0,04*
Hexane	0,52	b	0,08	0,35	ab	0,09	0,48	b	0,24	0,22	a	0,05	0,16	a	0,05	0,18	a	0,04
Furans	*1,29*	*c*	*0,26*	*0,80*	*ab*	*0,24*	*1,04*	*bc*	*0,29*	*2,28*	*d*	*0,04*	*0,66*	*ab*	*0,09*	*0,44*	*a*	*0,21*
2-Ethylfuran	1,29	c	0,26	0,80	ab	0,24	1,04	bc	0,29	2,28	d	0,04	0,66	ab	0,09	0,44	a	0,21

Jan Efo Gre, European flat oysters from Lake Grevelingen sampled in January; Jan PCO Gre, Pacific cupped oyster from Lake Grevelingen sampled in January; Jan PCO Eas, Pacific cupped oysters from the Eastern Scheldt sampled in January; Feb EFO Gre, European flat oysters from Lake Grevelingen sampled in February; Feb PCO Gre, Pacific cupped oysters from Lake Grevelingen sampled in February; Feb PCO Eas, Pacific cupped oysters from the Eastern Scheldt sampled in February.

Alcohols were the most abundant due to the presence of 1,5-octadien-3-ol, 3-cyclohexene-1-ethanol and 1-penten-3-ol. The main aldehydes found were (E,E)-2,4-heptadienal, (E)-2-pentenal, (E,Z)-2,6-nonadienal and lilac aldehyde while 1-penten-3-one and 2,3-pentanedione were the main ketones.

Twenty one and 23 VOCs in the European flat oyster and the Pacific cupped oyster samples in our study were also identified by Fratini et al. (2013) and Fratini et al. (2012) in oyster samples from Italy. For the European flat and Pacific cupped oysters these VOCs were pentanal, (E)-2-pentenal, hexanal, (E)-2-hexenal, heptanal, (Z)-4-heptenal, (E,E)-2,4-heptadienal, (E)-2-octenal, (E,Z)-2,6-nonadienal, benzaldehyde, lilac aldehyde, 1-penten-3-ol, 1,5-octadien-3-ol, (Z)-2-octen-1-ol, 3-cyclohexene-1-ethanol, 1-penten-3-one, 2,3-pentanedione, 1-octen-3-one, (E,E)-3,5-octadien-2-one, hexane and 2-ethylfuran. The VOC (E)-2-penten-1-ol was also found in the trans-isomer form by Fratini et al. (2012) in the European flat oyster. Furthermore in the Pacific cupped oysters in our study two peaks were identified as being (E,E)-2,4-heptadienal. The alcohol 1-5-octadien-3-ol shows high abundance in all experimental groups in our study (4.4-10.7%). The other main alcohol 3-cyclohexene-1-ethanol was most abundant in the European flat oysters (8.2-11.1%) in comparison with the Pacific cupped oysters (0.4-2.8%) which is in agreement with the results found by Fratini et al. (2013) and Fratini et al. (2012).

The VOC octanal was also detected in Pacific cupped oysters in studies by Pennarun et al. (2003), Pennarun et al. (2002) and Piveteau et al. (2000) in their studies.

The odor description as well as the identified origin of 17 VOC's out of the 24 VOCs identified in the oysters samples from our study are presented in Table 5.4.

Table 5.4 Origin and odor description of volatile organic compounds found in European flat oysters and Pacific cupped oysters.

Compound	Odor description	Reference
n-3 PUFA oxidation		
(E)-2-Penten-1-ol	mushroom	Pennarun et al., 2002, 2003
(E)-2-Pentenal	grass	Pennarun et al., 2002, 2003
(E,E)-2,4-Heptadienal	mushroom, moss, green	Piveteau et al., 2000, Pennarun et al., 2002, 2003
(E,E)-3,5-Octadien-2-one	fatty fruity	Kawai, 1996
(E,Z)-2,6-Nonadienal	fatty odor, green, cucumber, melon	Josephson et al., 1985, Piveteau et al., 2000, Pennarun et al., 2002, 2003
(Z)-4-Heptanal	White boiled fish, cooked white fish	Pennarun et al., 2002, 2003
1,5-Octadien-3-ol	fresh mushroom, moss	Kawai, 1996
1-Penten-3-one	green	Piveteau et al., 2000
n-6 PUFA oxidation		
(E)-2-Octen-1-ol	green	German, Zhang and Berger, 1991
(E)-2-Octenal	citrus, cucumber, almond	Piveteau et al., 2000, Pennarun et al., 2003
1-Octen-3-one	mushroom	Piveteau et al., 2000
Hexanal	oxidized fatty, green, grass, powerful, penetrating	Piveteau et al., 2000, Turchini et al., 2010
n-9 MUFA oxidation		
Octanal	citrus	Piveteau et al., 2000, 2003
Amino acid degradation		
Benzaldehyde	candy, sweet, almond	Acree and Arn, 2008, Turchini et al., 2010
Unknown origin		
Lylac aldehyde	floral lilac	Acree and Arn, 2008
3-cyclohexene-1-ethanol	fresh, mint	Tanaka, Myazawa and Ujiie, 2010
2,3-Pentanedione	buttery, milky	Piveteau et al., 2000

Josephson et al. (1985) reported on the precursors of the VOCs of Atlantic and Pacific cupped oysters for the first time and showed clear relations between the presence of PUFA and the occurrence of alcohols and carbonyls in oysters. Since then other studies have added to the understanding of the origin of VOCs in oysters. Both Piveteau et al. (2000) and Pennarun et al. (2003) listed the most likely origin of the VOCs found in their studies on the Pacific cupped oyster. In our study we aimed to correlate the fatty acid content with the VOC content in order to assess the most likely precursors of the VOCs found. The main VOC in European flat oysters, 1,5-octadien-3-ol has been reported to originate from 20:5n3 12-lipoxygenase (Durnford and Shahidi, 1998) and has a fresh mushroom, moss odor (Kawai, 1996). In our study a positive correlation was found with C22:5n3 ($r = 0.89$). The most dominant VOC in the European flat oysters is 3-cyclohexene-1-ethanol. This alcohol has been described as having a fresh, mint odor (Tanaka, Miyazawa and Ujiie, 2010) and is positively correlated with the presence of the fatty acid C17:0 ($r = 0.83$) in our study. Pentanal is reported to be a decomposition product of C18:2n6 13-hydroperoxide (Frankel, 1998) while a positive correlation was found with C20:4n6 ($r = 0.89$). Furthermore octanal having a citrus odor (Piveteau et al., 2000; Pennarun et al., 2002; Pennarun et al., 2003) has been reported to originate by means of C18:1n9 oxidative degradation. In our case however a positive correlation with C20:4n6 ($r = 0.83$) was found.

Other VOCs such as (E)-2-pentenal, hexanal, (E)-2-hexenal, heptanal, (Z)-4-heptenal, (E,E)-2,4-heptadienal, (E)-2-octenal, (E,Z)-2,6 nonadienal, 1-penten-3-ol, (E)-2-penten-1-ol, (Z)-2-octen-1-ol, 1-penten-3-one, 1-octen-3-one, (E,E)-3,5-octadien-2-one and 2-

ethylfuran did not show any correlations with the fatty acids in our study. Even though n-3 and n-6 fatty acids have been reported as the most likely origin of these volatile compounds (Pennarun et al., 2003; Piveteau et al., 2000). Furthermore an amino acid has been suggested by Piveteau et al. (2000) as the precursor for benzaldehyde while the origin of VOCs; 2,3-pentanedione, with a buttery, milky odor (Piveteau et al., 2000), lilac aldehyde, with a floral, lilac odor (Acree and Arn, 2008) and hexane remains unknown. Figure 5.3 shows separation between all experimental groups based upon PCA, indicating differences in the VOC profiles due to oyster species, harvesting time and cultivation area. The most marked difference is species related and is mainly caused by the VOCs 1,5-octadien-3-ol and 3-cyclohexene-1-ethanol. In all cases a clear separation between January and February harvested oysters is noticeable which is mainly caused by the VOCs 1-penten-3-ol, (E,Z)-2,6-nonadienal and heptanal. Furthermore, separation between cultivation areas seems to be related to the VOCs 1-penten-3-one, hexane and lilac aldehyde. Discrimination of the geographical origin of oysters based on their VOC profile has also been demonstrated by Ratel, Berge, Berdague, Cardinal and Engel (2008). Based on the mass spectra of the VOCs in the SPME-GC-MS data virtual fingerprints of oysters from different geographical origin and harvest months were created and after data correction for instrumental drift the model was able to classify samples 100% accurately by their geographical origin. Fratini et al. (2013) also showed marked differences in the VOC profile of Pacific cupped oysters from different cultivation areas (lagoon site in comparison with open sea cultivation) while Fratini et al. (2012) showed different VOC profiles for different shellfish species.

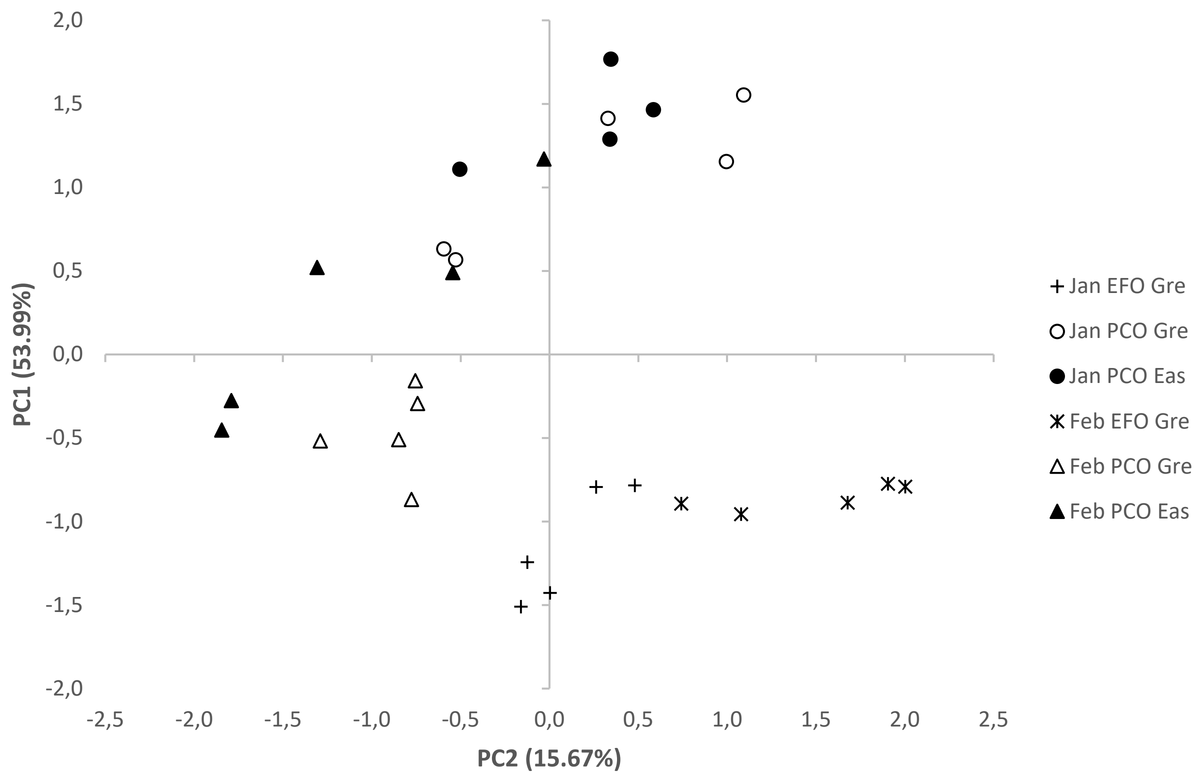


Figure 5.3 Principal component analysis for the volatile compound composition of different experimental groups of European flat oysters (EFO) and Pacific cupped oysters (PCO). Jan Efo Gre, European flat oysters from Lake Grevelingen sampled in January; Jan PCO Gre, Pacific cupped oyster from Lake Grevelingen sampled in January; Jan PCO Eas, Pacific cupped oysters from the Eastern Scheldt sampled in January; Feb EFO Gre, European flat oysters from Lake Grevelingen sampled in February; Feb PCO Gre, Pacific cupped oysters from Lake Grevelingen sampled in February; Feb PCO Eas, Pacific cupped oysters from the Eastern Scheldt sampled in February.

5.4 Conclusion

PCA analysis showed that differences in fatty acid profile and VOCs between experimental groups of oysters could be mostly attributed to being species (European flat oyster versus Pacific cupped oyster) related differences. However separation based on cultivation area and harvest period were also shown. Major differences in the fatty acid profile were due to higher abundance of C15:0, C20:0, C20:4n6, unknown2 and C22:5n6 and lower abundance C18:1, C18:1n7, C18:4n3 and C20:1n9 in European flat oysters in comparison with Pacific cupped oysters. While the major difference in the VOC profiles was due to the alcohol 3-cyclohexene-1-ethanol which was the most abundant VOC found in European flat oysters and 1,5-octadien-3-ol which was the most abundant VOC found in Pacific cupped oysters.

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Chapter 6: The effect of algae diets (*Skeletonema costatum* and *Rhodomonas baltica*) on the biochemical composition and sensory characteristics of Pacific cupped oysters (*Crassostrea gigas*) during land-based refinement

"An oyster, that marvel of delicacy, that concentration of sapid excellence, that mouthful before all other mouthfuls, who first had faith to believe it, and courage to execute? the exterior is not persuasive."

Henry Ward Beecher

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Abstract

Oyster refinement, a common practice in France, is aimed at increasing the weight of oyster tissue and influencing the taste properties of the refined oysters. Refinement usually takes place in land-based systems where the oysters are fed with relatively high concentrations of microalgae. In this study the impact of feeding *Skeletonema costatum* and *Rhodomonas baltica* on the biochemical composition and sensory characteristics of Pacific cupped oysters (*Crassostrea gigas*) from the Eastern Scheldt during land-based refinement was studied.

After a feeding period of four and seven weeks market-sized oysters were sampled for the analysis of fatty acids, free amino acids and volatile organic compounds and for a sensory evaluation by consumers and an expert panel.

The algae *Skeletonema costatum* showed a lower ∑PUFA, ∑n-3, ∑n-6, C18:2n6, C18:3n3, C18:4n3, C22:6n3 content as compared with *Rhodomonas baltica*. These differences were also reflected in the fatty acid profile of the oysters fed with the corresponding algae diets. Furthermore, general linear model and principal component analysis showed marked differences in free amino acids and volatile organic compound content between *Skeletonema*, *Rhodomonas* fed oysters and reference oysters. For example, threonine, glutamine, leucine, histidine, (E)-2-hexenal, (E)-2-octenal, (E)-2-octen-1-ol, (E,E)-2,4-octadien-1-ol, (E,Z)-3,6-nonadien-1-ol and (Z,E)-2,6-nonadienal contents were higher in *Skeletonema* fed oysters compared to *Rhodomonas* fed oysters. Sensory differences between the experimental oyster groups were shown. *Skeletonema* fed Pacific cupped oysters were characterized by a stronger seaweed flavor, higher perceived sweetness and a

firmer texture in comparison with *Rhodomonas* fed oysters. Naïve consumers were only able to differentiate between *Rhodomonas* fed oysters and reference oysters.

6.1 Introduction

Shellfish refinement also known as a fattening procedure is considered a common practice in France (Soletchnik et al., 2001; Piveteau, Gandemer, Baud and Demaimay, 1999; Robert, 1990). Market sized Pacific cupped oysters (*Crassostrea gigas*) are kept in basins and fed with naturally occurring algae in order to promote growth and to modify the taste of the oysters (Soletchnik et al., 2001; Piveteau et al., 1999). The oysters are kept in land-based systems where they are usually fed with relatively high concentrations of algae (approx. 45 mg l^{-1} DW algae per oyster) during a period varying from one to four months in order to increase the weight of oyster tissue. Shellfish refinement can lead to up to a 40% increase in the market value for Pacific cupped oysters (Barille, Bougrier, Geairon and Robert, 1994).

In many studies regarding the effects of refinement on oysters the diatom *Skeletonema costatum* has been used (Pennarun, Prost, Haure and Demaimay, 2003a; Pennarun, Prost, Haure and Demaimay, 2003b; Soletchnik et al., 2001; Méléder et al., 2001; Piveteau et al., 1999). *Skeletonema costatum* naturally occurs in the fattening ponds in the West of France. This diatom is easy to cultivate using the natural saline groundwater sources in the area (Baud, Haure and Garnier, 1990; Baud and Bacher, 1990). Soletchnik et al. (2001) and Piveteau et al. (1999) showed an increase in the weight of oyster tissue in their experiments showing the efficiency of using *Skeletonema costatum* as a refinement diet.

On the other hand, many other studies suggest that both the biochemical composition and therefore also the sensory characteristics of oysters can vary due to their respective diet. For instance Pennarun et al. (2003b) studied the effect of microalgal diets (*Skeletonema costatum* and *Isochrysis galbana*) on the VOC composition and the sensory properties of Pacific cupped oysters. Differences found in the VOC content of oysters were attributed to their algal diets and more specifically to the fatty acid content of both algae used. Also clear sensory distinctions were observed by a trained sensory expert panel between both algae fed oysters. Also Cochet, Brown, Kube, Elliott and Delahunty (2013) studied the effects of cultivation location on the sensory and biochemical characteristics of the Pacific cupped oyster. Clear distinction could be made between oysters cultivated in South Australia, New South Wales and Tasmania based upon their sensory characteristics. The authors hypothesized that some of these differences could be attributed to differences in the dietary composition between the different geographical locations.

The aim of our study was to investigate the effects of different algal diets (*Skeletonema costatum* and *Rhodomonas baltica*) with different fatty acid profiles on the biochemical composition (in particular fatty acids, FAAs and VOCs) and sensory characteristics of the Pacific cupped oyster (*Crassostrea gigas*) originating from the most important Dutch cultivation area (Eastern Scheldt). In addition changes in the gross composition and condition index were measured. Furthermore an expert sensory panel as well as an oyster consumer panel was used to evaluate sensory aspects of the oysters during the refinement. This in-depth investigation will contribute to the development of innovative land-based cultivation of oysters in the Netherlands. In addition new refinement varieties might lead to the development of new markets and improving the exploitation of the market potential of the Dutch oyster sector.

6.2 Materials and methods

6.2.1 Experimental design

Market size (80 to 120 g) Pacific cupped oysters cultivated in the Eastern Scheldt obtained from a Dutch shellfish company (Koninklijke Prins en Dingemanse, Yerseke, The Netherlands) in November 2014 were kept in small basins (1 m^3) in saline groundwater (30 g l^{-1}) for a period of seven weeks at the experimental facilities of the HZ University of Applied Sciences (Vlissingen, The Netherlands). The oysters were fed either *Skeletonema costatum* or *Rhodomans baltica* during the experimental period (November-December 2014). Water temperature in the basins was maintained at 13 ± 1 °C by means of a cooling and heating unit (TECO TC20, Italy). Salinity and oxygen content were measured daily. Salinity was measured using a conductivity meter (WTW, USA). Oxygen content was determined using a dissolved oxygen meter (WTW, USA). Aeration of the basins mixed the water column thus distributing the algae evenly in the water. Furthermore, aeration provided the oysters with the necessary oxygen. The complete water volume was replaced weekly with fresh saline groundwater. Oysters were sampled at the start of the experiment and after four and seven weeks of experimentation for analysis (gross biochemical composition, condition index, fatty acid composition, FAAs, VOCs) and sensory evaluation by an expert and a consumer panel. Oysters originating from the same cultivation location in the Eastern Scheldt, the Netherlands were used as reference samples.

6.2.2 Algal diets

The diatom *Skeletonema costatum* was grown in a semi-continuous culture in outdoor raceway systems (2.5 m^3). The temperature gradually decreased during the experiment from 14.4 ± 1 °C at the start to 12.3 ± 1 °C at the end of the experiment due to decreasing air temperature during the experimental period. The flagellate *Rhodomonas baltica* was cultivated indoors in 200 l plastic bag microalgae continuous cultivation reactors (SeaCAPS, UK) with continuous aeration. Ambient room temperature (20 ± 1 °C) was maintained for this culture, whilst 24 hours illumination was provided (150 μmol m^{-2} s^{-1}). Both algae culture systems were filled with filtered (1 μm) saline groundwater (30 g l^{-1}). Walne-medium (Walne, 1970) was used in all algal cultures. The algal cultures were kept in the exponential growth phase by daily harvesting. Cellular densities were determined daily by using a Bürker-Türk hemocytometer before feeding the oysters.
Feeding rates were set at 30 mg dry weight algae day^{-1} $oyster^{-1}$. Dry weight (DW) for the *Skeletonema costatum* was 45 pg algae $cell^{-1}$ while the DW of *Rhodomonas baltica* was 116 pg algae $cell^{-1}$. The oysters were fed once during the day at a fixed time in the morning.

6.2.3 Production

In order to assess oyster production the total wet weight and oyster tissue wet weight were recorded for 50 individual oysters in all experimental treatments. The condition index

(AFNOR, 1985) was measured in order to evaluate the amount of tissue in relation to the total weight of the oyster.

6.2.4 Biochemical composition

The biochemical composition (DW, ash, protein, carbohydrate, lipid, fatty acid profile and FAA content) was analyzed for both the algae diets (*Skeletonema costatum* and *Rhodomonas baltica*) and the reference and *Skeletonema* and *Rhodomonas* fed oysters at the start of the experiment and after four and seven weeks of feeding, respectively. The VOCs were only analyzed in the oyster samples.

Gross biochemical composition

In order to determine the gross biochemical composition (protein, lipid and carbohydrate content) of the algal diets three 50 ml samples of the algae culture were filtered over a Whatman GF/C filter paper. The biochemical composition (DW, ash, protein, lipid and carbohydrate content) of the oysters was determined for three pooled samples of 50 oysters The oysters were homogenized by using a Ultra Turrax T25 homogenizer (IKA Werke GmbH, Germany) prior to the analysis.

Dry matter content of the oysters was determined by drying 5 g of homogenate at 105 °C for 48 hours. The ash content of the oysters was determined by incinerating 1 g of homogenate in a muffle oven at 550 °C for 16 hours. Protein content for both algal diets and oysters was analyzed using the method described by Lowry, Rosebrough, Farr and Randall (1951). A commercial test kit (BIORAD, 500–0112) was used while Bovine serum albumin was used as a standard. Lipid fraction was extracted by the method of Bligh and Dyer (1959) using chloroform-methanol (1:2, v/v). The lipid content was measured spectrophotometrically according to Marsh and Weinstein (1966) after carbonization (200 °C for 15 min) with tripalmitin as a standard. Carbohydrate content was analyzed spectrophotometrically using the method of DuBois, Gilles, Hamilton, Rebers and Smith (1956). Glucose solutions were used as a standards in order to measure the carbohydrate concentration.

Fatty acids profile

The fatty acid profiles of both algal species were determined of three 6 ml samples taken from the algae cultivation systems as described by Caramujo, Boschker and Admiraal (2008). While the fatty acid profile of the oysters was determined for three pooled samples of 50 oysters. Briefly, the modified Bligh and Dyer method of Findlay, King and Watling (1989) was used for lipid extraction. Fatty acid methyl esters (FAME) were obtained from total lipid extracts using derivatization with 2.5% H_2SO_4 in methanol at 80 ˚C for 1.5 hours according to Guckert, Antworth, Nichols and White (1985). FAMEs were analyzed by capillary gas chromatography with flame ionization detection using a Varian 3400 gas chromatograph equipped with a Varian SPI injector, which was coupled via a Type II combustion interface to a Finnigan Delta S isotope ratio mass spectrometer. An a-polar analytical column (Hewlett-Packard Ultra-2 (50 m in length, 0.32 mm ID and 0.17 µm film thickness) was used. Helium was used as the carrier gas. The oven temperature increased from 80°C (1 min) to 130 °C at 40 °C min^{-1} and subsequently from 130 to 260 °C at 3 °C min^{-1}.

Identification of FAME was based on retention time data of known standards, later confirmed by mass spectrometry (Hewlett-Packard Mass Selective detector, U.S.A.).

Free amino acids

For the algal diets three samples (approx. 50 g filtrated fresh algae paste) were freeze-dried. For the oysters, 10 individual oysters (80 - 120 g) were freeze-dried (at -70 °C) for each experimental group.

Free amino acids were extracted according to Mierke-Klemeyer et al. (2008) with modifications described by Maehre, Malde, Eilertsen and Elvevoll (2014), by dissolving approximately 0.2 g freeze-dried material in a mixture of 9 ml distilled H_2O and 1 ml 20 mmol l^{-1} norleucine (internal standard), followed by homogenization for 15 seconds with an Ultra Turrax T25 homogenizer (IKA Werke GmbH, Germany). One ml of 35% sulfosalicylic acid was added for removal of proteins and large peptides, followed by homogenization for another 15 seconds and centrifugation at 4000 g for 10 minutes. Aliquots of 200 µl of the supernatants were diluted to a suitable concentration in lithium citrate buffer at pH 2.2 and submitted to analysis.

Analysis of FAAs was performed on a Biochrom 30 amino acid analyzer (Biochrom Co., UK). The amino acids were chromatographically separated on an ion exchange column, followed by post-column derivatization with ninhydrin and detection of UV signal at 440 nm and 570 nm (Spackman, Stein and Moore, 1958). UV-signals were analyzed by Chromeleon software (Dionex, USA) and compared with A9906 physiological amino acids standard (Sigma Chemicals Co., USA).

Volatile organic compounds

The VOCs were analyzed for five individual oyster (80-120 g) per experimental group. For every individual oyster 10 ml of NaCl saturated ultrapure water was added to 5 g oyster tissue prior to homogenization. Homogenization was performed for one minute using a T25 Ultra Turrax homogenizer (IKA Werke GmbH, Germany) ensuring that the sample remained in ice preventing excess heat. VOCs were extracted by HeadSpace Solid Phase Micro Extraction (HS-SPME) and analyzed by Gas Chromatography–Mass Spectrometry (GC-MS) according to Fratini, Lois, Pazos, Parisi and Medina (2012). GC–MS analysis was performed in a Thermo Finnigan ThermoQuest (USA) gas chromatograph equipped with a split/splitless injector and coupled to a trace quadrupole mass detector (Thermo Finnigan ThermoQuest, USA). Compounds were separated in a capillary column (30 m × 0.250 mm × 1 μm film thickness, fused silica DB-1701, Agilent Technologies, USA). All analyses were performed setting ionization energy at 70 eV, filament emission current at 150 μA and the electron multiplier voltage at 500 V (Fratini et al., 2012) and the spectra were acquired in full scan mode.

Identification of the components was based on computer matching with the reference mass spectra of the Wiley 6, Mainlib and Replib libraries and by comparison of mass spectra and retention times with commercial standards. Quantification of volatiles was performed by the method of internal standards using 3-methyl-3-buten-1-ol as described by Fratini et al. (2012).

The standards: 2-Ethylfuran, 2,3-pentanedione, (E)-2-pentenal, hexanal, (E)-2-hexenal, heptanal, (Z)-4-heptenal, (E,E)-2,4-heptadienal, (E)-2-octen-1-ol and 3-methyl-3-buten-1-ol (used as internal standard) were purchased from Sigma-Aldrich (Germany). 1-Penten-3-ol was obtained from Fluka (Switzerland) and 2,4-octadien-1-ol was purchased from Alfa Aesar (USA).

6.2.5 Sensory evaluation

Sensory evaluation of the reference oysters, the *Skeletonema* and the *Rhodomonas* fed oysters was conducted by both naïve consumers and trained panelists. In the consumer evaluation a three Alternative Forced Choice (3-AFC) method was used in order to investigate whether naïve consumers were able to discriminate between the experimental groups of oysters. The trained panelist's evaluation consisted of a Quantitative Descriptive Analysis (QDA).

Consumer evaluation

Up to 56 consumers were selected from a pool of consumers recruited in a previous study (Van Houcke, Altintzoglou, Stieger, Linssen and Luten, 2016a). Consumers in this pool were selected on the criteria that they were oyster consumers and had no allergies to shellfish or specifically, oysters. The majority of the consumers was male (approx. 75%) and above 55 years of age (approx. 55%). Consumers did not receive a financial reimbursement for participating in the study. No information on the actual aim or the experimental design was disclosed to the consumers.

Before sample preparation the oysters had a depuration period of 24 hours. Oysters were opened by hand-shucking, the adductor muscles were cut on both sides and the oyster tissue was replaced on the lower shell (half-shell product). All samples were coded with a randomized three-digit code.

In order to evaluate consumer discrimination between *Skeletonema* fed oysters, *Rhodomonas* fed oysters or reference oysters a full factorial design was implemented in this study, rendering into three possible combinations: two reference oyster vs. one

Skeletonema fed oyster, two reference oysters vs. one *Skeletonema* fed oyster and two *Skeletonema* fed oysters vs. one *Rhodomonas* fed oyster. The sample consisting of three half-shell oysters was presented on a plastic plate. Consumers were asked to indicate which of the oysters they considered different from the other oysters based upon outer appearance, smell and taste. Each consumer participated in two 3-AFC tests. The presentation order of the samples and 3-AFC combinations was completely randomized. The evaluation sessions were of approximately 40 minutes duration. Each 3-AFC test took 15 minutes with a break of 10 minutes in between in which the consumers could take a sip of water and a cracker.

Tests were carried out at room temperature in different classrooms at the HZ University of Applied Sciences in Vlissingen, The Netherlands. Consumers were seated approximately 1 m apart in the classrooms. Consumers were seated at random, received a participant number for anonymity and were instructed not to speak to each other during and between the tests.

Trained panelists evaluation

The Quantitative and Descriptive Analysis (ISO, 2003) tests were performed using 14 trained panelists (internal panel Ifremer, Nantes, France) experienced in seafood sensory evaluation and already involved in oyster assessment.

Prior to the QDA tests two sessions were organized for attribute generation, selection and consensus on the chosen attributes. The samples used in these sessions were reference oysters from the Eastern Scheldt and five weeks algae fed oysters (both with *Skeletonema costatum* and *Rhodomonas baltica*). The agreed attributes were: overall odor, marine odor, fruit odor, mud odor, darkness of the visceral mass, darkness of the gills, darkness of the mantle edge, fullness of the shell, crunchy texture, melting texture, chewiness, marine taste, algal taste, saltiness, sweetness, bitterness, metallic taste and astringency. In total three QDA tests were carried out with oysters derived from the start of the experiment and at the four and seven weeks' time feeding periods. All tests (including the two sessions on attribute generation and consensus on the attributes) took place in the sensory room at Ifremer in Nantes (France) at ambient temperature under daylight in isolated booths (ISO, 1988). Data were collected with a computerised system (Fizz, Biosystèmes, Dijon, France). Oysters were presented as half-shell products with the shell on the top to preserve the odor. The samples assessed consisted of two reference oysters, two *Skeletonema* fed oysters and two *Rhodomonas* fed oysters. Samples were assigned with three digit numbers and randomized for the order presentation within panelists (Latin square design). In the tests panelists were asked to score the perceived intensity of the 18 different attributes on a 16 cm unstructured line for each oyster presented. Anchors corresponded with no intensity and very strong intensity on the left hand anchor and the right-hand anchor, respectively. Water and crackers were available during the tests and panelists were free to use these at will.

6.2.6 Statistical data analysis

The data on biochemical composition, fatty acids, FAAs and VOCs of the oysters were processed by ANOVA using a two-way (diet, time) GLM model. Both the factors diet and time consisted of three levels (reference, *Skeletonema* and *Rhodomonas* and T0, T4 and T7, respectively). Furthermore standardized Principal Component Analysis (PCA) was performed on the fatty acids, FAAs and VOCs data.

Consumer evaluation data were tested for significant differences with the Chi-square test. While, trained panelist evaluation data were analyzed using both a two-way (diet, time) GLM model and PCA.

The data in the tables are expressed as mean ± standard deviation.

Three levels of significance are presented in this study: $P < 0.001$ (***), $P < 0.01$ (**) and $P < 0.05$ (*).

6.3 Results and Discussion

6.3.1 Production

AFNOR condition indexes showed a slight increase over time for both refinement diets although this was not significant. While reference oysters had a condition index of 16.0 *Rhodomonas* fed oysters showed a condition index of 16.4 and 16.9 after four and seven weeks of feeding respectively. *Skeletonema* fed oysters increased to a condition index of 16.7 after seven weeks even though there was a decrease in condition index after four weeks of feeding (15.9). Pacific cupped oyster fed with *Skeletonema costatum* in France showed increases in (AFNOR) condition indexes from 6 - 9 to 10 - 14 during six week periods (Pennarun et al., 2003a; Haure et al., 2003; Soletchnik et al., 2001; Baud, Brisset and Cardinal, 1995). A lack of significant increases in the condition indexes in our results could probably be explained by the relatively high condition indexes of the reference oysters at the start of the experiment.

6.3.2 Biochemical composition

Gross biochemical composition

Table 6.1 shows the gross biochemical composition of the algal diets, reference oysters and the oysters after four and seven weeks of feeding with *Skeletonema costatum* or *Rhodomonas baltica*. Significant diet and time effects are shown for the dry weight, protein and carbohydrate content of the oysters. Relatively low DW content (13.3% WW) was found in the reference oysters at the seven weeks sampling interval compared with the reference oysters at the beginning of the experiment and at the four weeks sampling interval (16.2 and 15.5% WW, respectively).

Table 6.1 Gross biochemical composition of algal diets, reference oysters (Ref) and oysters after four and seven weeks of feeding with *Skeletonema costatum* (Ske) or *Rhodomonas baltica* (Rho). Values for dry matter are expressed as % of wet weight (WW), while other values are expressed as % of dry weight (DW).

	Ske		Rho		T0 Ref		Ref T4		Ref T7		Ske T4		Ske T7		Rho T4		Rho T7		GLM analysis		
	Ave	Stdev	Ave	Stdev	Ave	Stdev	Ave	Stdev	Ave	Stdev	Ave	Stdev	Ave	Stdev	Ave	Stdev	Ave	Stdev	Diet (D)	Time (T)	D x T
Dry Matter (% WW)					16,2	2,5	15,5	2,3	13,3	2,1	17,7	2,7	16,6	2,3	17,7	2,6	17,7	2,7	***	***	**
Ash (% DW)					12,8	3,0	14,5	4,0	14,0	3,1	14,1	3,7	13,6	2,9	14,5	4,1	14,0	3,0	ns	ns	ns
Protein (% DW)	23,3	2,5	29,1	0,1	54,2	1,9	60,9	1,9	59,4	2,4	54,1	2,4	54,2	1,8	52,7	2,3	55,5	1,9	***	**	ns
Lipids (% DW)	16,2	0,6	12,1	0,5	8,8	0,6	7,8	0,7	9,1	1,0	8,5	0,9	8,2	0,9	9,0	0,6	8,7	0,9	ns	ns	ns
Carbohydrates (% DW)	26,4	0,7	11,7	0,8	23,8	1,4	17,3	2,0	19,2	2,3	25,5	2,3	23,8	1,1	25,3	1,4	23,5	2,3	***	**	ns

*** stand for P<0.001, ** stands for P<0.01, * stands for P<0.05.

Highest protein content was measured in the reference oysters at the four and seven week sampling intervals (60.9 and 59.4% DW, respectively) and lowest protein content in the *Rhodomonas* fed oysters after four weeks (52.7% DW). The carbohydrate content was highest in both the *Skeletonema* (25.5% DW) and *Rhodomonas* fed oysters (25.3% DW) after the four week sampling interval. The lowest content was found in the reference oysters at the four and seven week sampling intervals (17.3 and 19.2% DW, respectively). The lipid levels in the oysters remained relatively stable ranging from 7.8 to 9.1% DW.
The changes found in the biochemical composition in our study are in line with studies of Soletchnik et al. (2001) and Pennarun et al. (2003a). Soletchnik et al. (2001) reported significant increases in the carbohydrate content of Pacific cupped oysters supplemented with *Skeletonema costatum* in comparison with non-supplemented oysters kept in semi-closed land-based pond systems. The increase found in the carbohydrate content was mainly due to an increase in the glycogen content. No effect was found on the lipid content while the protein content showed a trend to lower levels for the *Skeletonema* fed oysters in the study of Soletchnik et al. (2001). Pennarun et al. (2003a) reported increasing carbohydrate (and glycogen) and decreasing protein content for *Skeletonema costatum* and *Isochrysis galbana* fed oysters. Pennarun et al. (2003a) showed a significant increase in the lipid content which is in contrast with our results. Furthermore Pennarun et al. (2003a) also showed a varying lipid content between oysters fed with the different algal diets. The carbohydrate, lipid and ash contents in our study are comparable with data from Pacific cupped oysters from Ireland (Linehan, O'Conner and Burnell, 1999), Germany (Pogoda, Buck, Saborowski and Hagen, 2013) and France (Pennarun et al., 2003a; Solechnik et al.,

2001). The protein content, however, seemed to be quite high in our study and is only comparable with protein content measured in Pacific cupped oysters from Ireland sampled in August (Linehan, et al., 1999).

Fatty acids

The fatty acid profile of the algal diets, the reference oysters and the oysters after four and seven weeks of feeding with *Skeletonema costatum* or *Rhodomonas baltica* are shown in table 6.2.

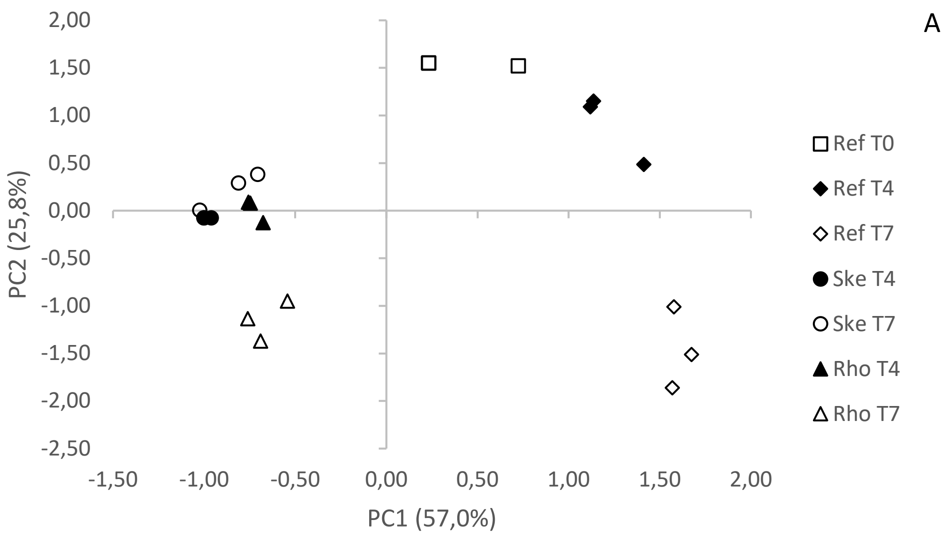
Table 6.2 Fatty acid profile of algal diets, reference oysters (Ref) and oysters after four and seven weeks of feeding with *Skeletonema costatum* (Ske) or *Rhodomonas baltica* (Rho). Values are expressed as % of total fatty acid content.

	Ske		Rho		T0 Ref		Ref T4		Ref T7		Ske T4		Ske T7		Rho T4		Rho T7		GLM analysis		
	Ave	Stdev	Ave	Stdev	Ave	Stdev	Ave	Stdev	Ave	Stdev	Ave	Stdev	Ave	Stdev	Ave	Stdev	Ave	Stdev	Diet (D)	Time (T)	D x T
∑SFA	17,3	1,1	13,3	0,4	29,9	0,3	31,2	0,5	31,5	0,3	29,4	0,2	29,3	0,1	29,3	0,5	28,4	0,4	***	**	ns
∑MUFA	21,1	1,1	7,6	0,2	16,9	0,4	18,0	0,3	18,0	0,4	21,0	0,2	19,4	0,3	17,8	0,2	18,7	0,1	***	ns	ns
∑PUFA	61,7	3,3	79,0	0,5	53,2	0,7	50,7	0,4	50,5	0,4	49,6	0,0	51,3	0,3	52,9	0,6	52,9	0,3	***	**	ns
∑n-3	22,1	1,2	71,8	0,8	46,8	0,6	46,3	0,4	46,0	0,6	44,4	0,1	45,6	0,6	46,8	0,9	46,7	0,3	***	*	*
∑n-6	4,6	0,7	5,7	0,3	6,5	0,7	4,4	0,1	4,5	0,1	5,2	0,1	5,6	0,5	6,1	0,1	6,2	0,0	***	***	ns
C14:0	9,6	0,5	6,7	0,2	3,9	0,1	4,4	0,1	3,5	0,8	4,2	0,0	4,2	0,2	4,0	0,1	3,5	0,6	ns	ns	ns
C16:0	6,4	0,3	6,3	0,0	20,8	0,4	21,0	0,6	20,5	0,6	18,9	0,2	19,1	0,4	19,2	0,4	18,7	0,1	***	ns	ns
C16:1n7	18,9	0,9	2,6	0,2	3,9	0,1	3,9	0,8	3,0	0,1	5,2	0,1	4,2	0,2	4,0	0,1	3,1	0,0	***	***	ns
C16:2n4	5,1	0,3	0,4	0,0	nd		nd		nd		nd		nd		nd		nd		-	-	-
C16:2n7	2,3	0,1	0,1	0,0	nd		nd		nd		nd		nd		nd		nd		-	-	-
C16:3n4	16,3	0,8	0,3	0,1	nd		nd		nd		nd		nd		nd		nd		-	-	-
C16:4n1	11,2	0,6	0,8	0,1	nd		nd		nd		nd		nd		nd		nd		-	-	-
C17:0	0,1	0,0	0,1	0,0	nd		nd		1,5	0,0	1,0	0,0	1,1	0,0	1,0	0,0	1,0	0,0	***	ns	ns
C18:0	1,1	0,1	0,3	0,0	5,2	0,1	5,9	0,1	6,0	0,1	5,2	0,1	4,9	0,5	5,1	0,1	5,2	0,0	***	***	ns
C18:1n9	1,0	0,0	0,6	0,0	2,6	0,0	2,9	0,0	4,5	0,1	3,1	0,0	3,2	0,1	3,0	0,1	3,1	0,0	***	***	***
C18:1n other	1,1	0,1	4,5	0,0	7,8	0,1	8,3	0,8	7,5	0,2	9,4	0,1	9,2	0,4	7,7	0,4	9,3	1,8	*	ns	*
C18:2n6	1,4	0,1	4,3	0,0	1,3	0,0	nd		nd		1,0	0,0	1,1	0,0	2,0	0,0	2,1	0,0	***	**	*
C18:3n3	0,8	0,1	28,0	0,5	1,3	0,0	nd		nd		1,4	0,6	1,1	0,0	2,7	0,6	3,1	0,0	***	ns	ns
C18:4n3	4,4	0,3	27,2	0,2	2,6	0,0	2,9	0,0	3,0	0,1	3,1	0,0	3,2	0,1	4,0	0,1	4,2	0,0	***	***	ns
C20:1n11	nd		nd		2,6	0,0	2,9	0,0	3,0	0,1	3,1	0,0	2,8	0,5	3,0	0,1	3,1	0,0	ns	ns	ns
C20:4n6	0,2	0,0	0,5	0,0	3,9	0,1	4,4	0,1	4,5	0,1	3,1	0,0	3,2	0,1	3,0	0,1	3,1	0,0	***	***	ns
C20:5n3	14,0	0,7	10,2	0,1	28,6	0,5	27,3	0,6	25,0	0,6	27,3	0,3	28,6	0,1	26,9	0,0	26,0	0,1	***	***	***
C22:2n6	2,8	0,7	0,4	0,2	nd		nd		nd		nd		nd		nd		nd		-	-	-
C22:4n6	0,2	0,0	0,5	0,0	1,3	0,0	nd		nd		1,0	0,0	1,4	0,6	1,0	0,0	1,0	0,0	ns	ns	ns
C22:5n3	0,1	0,0	0,2	0,1	2,6	0,0	2,9	0,0	1,5	0,0	2,1	0,0	2,1	0,1	2,0	0,0	2,1	0,0	***	***	***
C22:6n3	2,9	0,1	6,2	0,1	11,7	0,2	13,2	0,2	16,5	0,3	10,5	0,1	10,6	0,4	11,1	0,3	11,4	0,1	***	***	***

*** stand for $P<0.001$, ** stands for $P<0.01$, * stands for $P<0.05$.

The algal diets show differences between total saturated fatty acid (∑SFA), total mono-unsaturated fatty acid (∑MUFA) and total poly-unsaturated fatty acid (∑PUFA) content. The diatom *Skeletonema costatum* shows a higher ∑SFA and ∑MUFA content (17.3 and 21.1%, respectively) in comparison with the flagellate *Rhodomonas baltica* (13.3 and 7.6%, respectively). The largest difference between both algae species is however found in the ∑PUFA content (79.0% in *Rhodomonas baltica* and 61.7% in *Skeletonema costatum*). This difference in ∑PUFA content could mainly be attributed to differences in total n3 fatty acid (∑n-3) content of both algae species (22.1% for *Skeletonema costatum* and 71.8% for *Rhodomonas baltica*). *Rhodomonas baltica* shows the highest C18:3n3, C18:4n3 and C22:6n3 content (28.0, 27.2 and 6.2%, respectively) in comparison with *Skeletonema costatum* (0.8, 4.4 and 2.9%, respectively). These findings are in line with literature on the fatty acid profile of both *Rhodomonas baltica* (Kreibich, Saborowski, Hagen and Niehoff, 2008) and *Skeletonema costatum* (Pennarun et al., 2003a).

As table 6.2 shows differences in the fatty acid profiles of the Pacific cupped oysters are due to both the diet and the time interval. For instance, the ∑SFA content is slightly higher for the reference oysters in comparison with *Skeletonema* and *Rhodomonas* fed oysters. The ∑MUFA content seems to be lower for *Rhodomonas* fed oysters and higher for *Skeletonema* fed oysters in comparison with the reference oysters. While the ∑PUFA content is found to be higher for *Rhodomonas* fed oysters in comparison with either *Skeletonema* fed oysters or reference oysters. Furthermore the ∑SFA content shows a decreasing trend over the experimental period while the ∑PUFA content shows lower values at the four week interval. In addition, PCA based on the total fatty acid profile characteristics of both the reference oysters and the oysters fed with either *Skeletonema costatum* or *Rhodomomans baltica* (figure 6.1) shows a clear separation between experimental groups of oysters. The separation of the reference oysters from the algae fed oysters indicates that the fatty acid profiles of the reference oysters are most divergent and varied during the experiment. The separation of the seven weeks *Rhodomonas* fed oysters from the other algae fed oysters is mainly caused by the dietary and time effect found in the C14:0, C16:1n7, C18:2n6, C18:3n3, C18:4n3, C20:5n3 and C22:6n3 content.



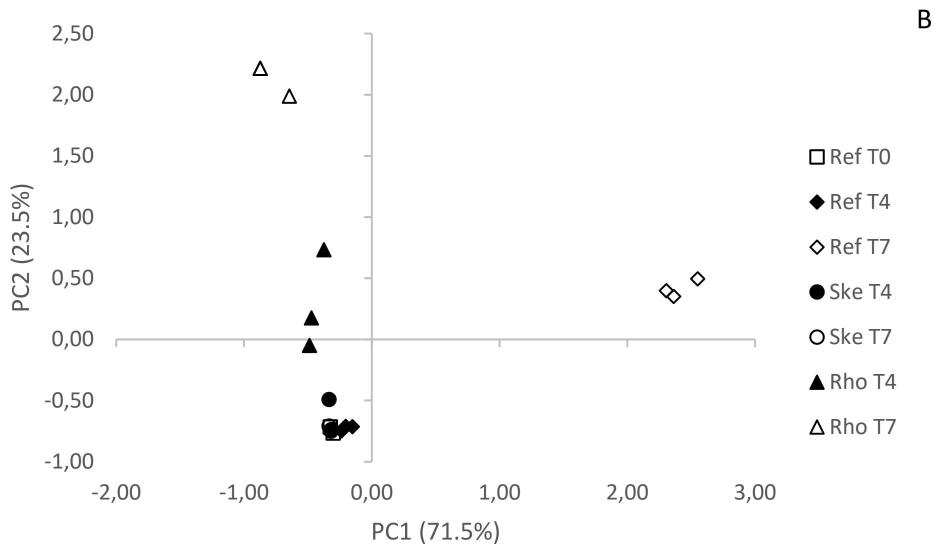


Figure 6.1 Principal component analysis for the fatty acid profiles (A) and the n-3 fatty acid profiles (B) of reference oysters (Ref) and oysters after four and seven weeks of feeding with *Skeletonema costatum* (Ske) or *Rhodomonas baltica* (Rho).

The main fatty acids in all experimental oysters are C16:0, C20:5n3 and C22:6n3. The highest C16:0 content was found in the reference oysters at the four week sampling interval

(21.0%) while the lowest content was found in *Rhodomonas* fed oysters after seven weeks of feeding (17.9%). Both C20:5n3 and C22:6n3 showed lowest content in the reference oysters at the seven week interval (25.0 and 16.5%, respectively). Highest content of C20:5n3 was found in the reference oysters at the start of the experiment and the *Skeletonema* fed oysters after seven weeks of feeding (28.6%). Highest content of C22:6n3 was found in *Skeletonema* fed oysters after seven weeks of feeding (16.5%). The levels of these essential fatty acids are comparable with the ranges found for Pacific cupped oysters in the literature (Van Houcke, Medina, Linssen and Luten, 2016b; Pogoda et al., 2013; Fratini et al., 2013; Linehan et al., 1999; Pazos, Ruez, Garcia-Martin, Abad and Sanchez, 1996).

From the literature it is known that the fatty acid profile of the algae diet can affect the fatty acid profile of the oysters. Pennarun et al. (2003a) and Piveteau et al. (1999) showed an increase in C18:1n9, C18:2n6, C18:3n3 content when Pacific cupped oysters were fed with *Isochrysis galbana*. When oysters were fed with *Skeletonema costatum* their C20:5n3 and C22:6n3 content increased. In our study higher contents of C18:1n9 and C20:5n3 were measured in the algae *Skeletonema costatum* and also a higher content of C22:6n3 in the algae *Rhodomonas baltica* which could explain the differences in the fatty acid profile of the oysters. The marked differences in the C18:3n3 and C18:4n3 content of both algae is also reflected in the oysters fed with the microalgae. Highest content for both C18:3n3 and C18:4n3 was measured in *Rhodomonas* fed oysters. Also the C16:1n7 and C18:2n6 content in the oysters show a clear dietary effect. It is known that diatom algae species such as *Skeletonema costatum* have a high content of C16:1n7 as compared to other algae (Sargent, Parkes, Mueller-Harvey and Henderson, 1987).

Free Amino Acids

Table 6.3 shows the FAAs of the algal diets, the reference oysters and the oysters after four and seven weeks of feeding with *Skeletonema costatum* or *Rhodomonas baltica*.

Table 6.3 Free amino acid profile of algal diets, reference oysters (Ref) and oysters after four and seven weeks of feeding with *Skeletonema costatum* (Ske) or *Rhodomonas baltica* (Rho). The values are expressed as mg g-1 DW.

	Ske		Rho		T0 ref		T4 ref		T7 ref		T4 Skel		T7 Skel		T4 Rho		T7 Rho		GLM analysis		
	Ave	Stdev	Ave	Stdev	Ave	Stdev	Ave	Stdev	Ave	Stdev	Ave	Stdev	Ave	Stdev	Ave	Stdev	Ave	Stdev	Diet (D)	Time (T)	D xT
Tau	n.d.		0,1	0,0	44,8	7,3	42,9	3,4	44,8	3,1	43,8	3,0	42,0	5,1	40,9	2,5	40,8	2,7	ns	ns	ns
Asp	1,8	0,0	0,6	0,1	3,8	0,7	3,6	0,9	3,8	0,6	4,5	0,6	4,9	0,4	5,0	0,6	5,2	0,6	***	ns	ns
Thr	0,4	0,0	0,1	0,1	1,0	1,0	0,5	0,1	0,3	0,2	0,7	0,4	0,9	0,6	0,5	0,2	0,5	0,2	*	**	ns
Ser	0,1	0,0	0,1	0,1	0,6	0,3	0,9	0,3	0,6	0,2	0,4	0,2	0,4	0,2	0,6	0,1	0,6	0,2	**	*	*
Asn	3,7	0,2	2,7	0,1	1,5	1,2	0,8	0,6	0,4	0,2	1,2	0,6	1,2	0,8	1,0	0,5	1,0	0,9	ns	ns	ns
Glu	3,0	0,2	3,6	0,5	6,0	1,4	5,8	0,7	5,4	0,7	5,8	1,0	5,4	1,2	5,2	0,8	5,0	0,7	ns	ns	ns
Gln	3,4	0,2	1,7	0,7	3,7	3,8	2,1	1,1	1,0	0,8	3,3	1,8	3,3	2,0	2,0	0,9	1,9	1,3	*	*	ns
Pro	nd		1,3	0,6	6,6	2,8	7,1	2,5	3,7	3,4	6,8	2,8	6,5	2,7	6,3	2,3	7,1	1,9	ns	ns	*
Gly	0,1	0,0	0,1	0,0	6,1	3,9	6,6	2,5	3,9	1,5	4,3	1,5	4,7	1,8	6,7	2,5	6,8	1,7	*	ns	ns
Ala	0,6	0,0	1,5	0,2	9,0	3,6	6,3	1,7	4,3	1,1	6,0	1,6	4,7	1,7	4,2	0,9	4,0	1,3	ns	***	ns
Val	0,4	0,0	0,1	0,0	nd		nd		nd		nd		nd		nd		nd		-	-	-
Ile	0,3	0,0	nd		nd		nd		nd		nd		nd		nd		nd		-	-	-
Leu	0,3	0,0	nd		0,2	0,1	0,2	0,1	0,2	0,0	0,4	0,2	0,4	0,3	0,3	0,1	0,3	0,1	***	ns	ns
Tyr	0,3	0,0	nd		nd		nd		nd		nd		nd		nd		nd		-	-	-
b-Ala	nd		nd		2,9	1,4	3,5	1,2	1,9	0,8	3,2	0,9	3,2	0,7	3,1	1,3	3,5	1,5	ns	ns	*
Phe	0,1	0,0	nd		nd		nd		nd		nd		nd		nd		nd		-	-	-
Orn	0,3	0,0	0,1	0,0	0,2	0,1	0,1	0,0	0,1	0,1	0,2	0,1	0,3	0,2	0,2	0,1	0,2	0,1	***	ns	ns
Lys	0,3	0,0	0,2	0,0	0,6	0,3	0,5	0,1	0,4	0,3	0,5	0,2	0,6	0,3	0,5	0,2	0,4	0,2	ns	ns	ns
His	0,2	0,0	nd		0,4	0,3	0,2	0,1	0,1	0,1	0,3	0,1	0,4	0,2	0,2	0,1	0,2	0,1	**	*	ns
Arg	0,8	0,1	1,0	0,3	2,0	1,0	2,0	0,4	1,1	0,5	1,6	0,2	1,7	0,4	1,7	0,3	1,9	0,4	ns	*	**

*** stand for P<0.001, ** stands for P<0.01, * stands for P<0.05.

In both algal diets the largest differences are found in the content of aspartic acid, asparagine , glutamine, proline and alanine. In *Skeletonema costatum* the aspartic acid (1.8 mg g^{-1} DW), asparagine (3.7 mg g^{-1} DW) and glutamine (3.4 mg g^{-1} DW) content is higher than in *Rhodomonas baltica* (0.6, 2.7 and 1.7 mg g^{-1} DW, respectively). Proline was only detected in *Rhodomonas baltica* (1.3 mg g^{-1} DW) while alanine content was higher in *Rhodomonas baltica* (1.5 mg g^{-1} DW) as compared to *Skeletonema costatum* (0.6 mg g^{-1} DW).

Threonine content was higher in *Skeletonema costatum* (0.4 mg l^{-1} DW) in comparison with *Rhodomonas baltica* (0.1 mg g^{-1} DW). Leucine and histidine were not detected within the algae *Rhodomonas baltica* while concentrations of 0.3 and 0.2 mg g^{-1} DW were found in the algae *Skeletonema costatum*, respectively.

The FAA composition of the oysters is dominated by taurine. The concentrations range from 40.9 to 44.8 mg g^{-1} DW. Other major FAAs are alanine, proline, glycine, and glutamate. These FAAs have also been reported as the main FAAs in Pacific cupped oysters from Australia (Cochet et al., 2013), from Japan (Sakaguchi and Murata, 1989; Murata and Sakaguchi, 1986) and China (Hong et al., 2002). Also taurine was the dominating FAA and the concentrations in these studies are comparable with our results.

Diet effects were shown for aspartic acid, threonine, serine, glutamine, glycine, leucine, ornithine and histidine. For aspartate and ornithine lowest content (ranging from 3.6 - 3.8 and 0.1 - 0.2 mg g^{-1} DW) was measured in reference oysters in comparison to *Skeletonema* fed oysters (ranging from 4.5 - 4.9 and 0.2 - 0.3 mg g^{-1} DW) and *Rhodomonas* fed oysters (ranging from 5.0 - 5.2 and 0.2 - 0.2 mg g^{-1} DW). However for serine the highest content was measured in reference oysters (ranging from 0.6 - 0.9 mg g^{-1} DW) when compared with *Skeletonema* (0.4 mg g^{-1} DW) and *Rhodomonas* fed oysters (0.6 mg g^{-1} DW).

The FAA content of the Pacific cupped oysters seems to be related to the FAA content of the algal diets. The glutamine, threonine, leucine and histidine contents were significantly higher for *Skeletonema* fed oysters (3.3, 0.8, 0.4 and 0.4 mg g^{-1} DW) in comparison with *Rhodomonas* fed oysters (2.0, 0.5, 0.3 and 0.2 mg g^{-1} DW).

For threonine, serine, glutamine, alanine, histidine and arginine content in the oysters significant time effects are also shown in table 6.3. In addition the PCA plot of the FAA profiles of the oysters (figure 6.2) shows that separation between the algae fed oysters are due to both diet and time effects. Cochet et al. (2013) hypothesized that the differences found in the FAA profile from Pacific cupped oysters from different cultivation locations in Australia could be attributed either to differences in the microalgae populations and concentrations at the different locations or timing differences in the reproductive cycles of the oysters.

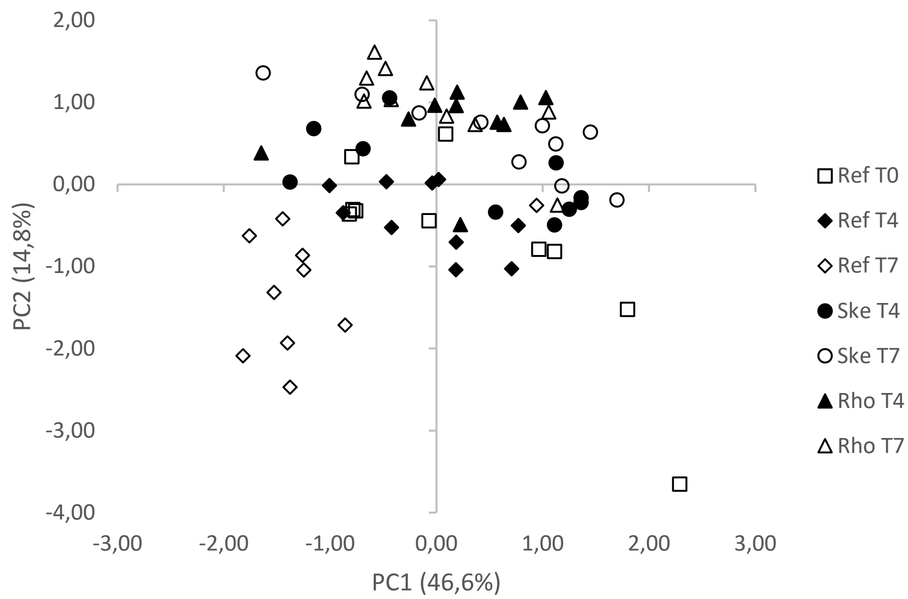


Figure 6.2 Principal component analysis of the free amino acid profiles of reference oysters (Ref) and oysters after four and seven weeks of feeding with *Skeletonema costatum* (Ske) or *Rhodomonas baltica* (Rho).

Volatile Organic Compounds

During the experimental period the total VOC content decreased from 9.2 mg kg^{-1} at the start of the experiment to 5.2 - 6.0 mg kg^{-1} at the seven week sampling interval (table 6.4). The VOCs found are alcohols (6), aldehydes (4), ketones (1) and furans (1). The main VOCs found were the alcohols 1,5-octadien-3-ol, (E,E)-2,4-octadien-1-ol and 1-penten-3-ol. All VOCs, except (E,E)-2,4-octadien-1-ol, were also identified in earlier studies with Pacific cupped oysters by Van Houcke et al. (2016b), Fratini et al. (2013), Pennarun et al. (2003b) and Pennarun, Prost and Demaimay (2002). (E,E)-2,4-Octadien-1-ol was however measured by Fratini et al. (2012) in the pullet carpet shells (*Venerupis pullastra*).

Table 6.4 Volatile organic compound profile of reference oysters and oysters (Ref) after four and seven weeks of feeding with *Skeletonema costatum* (Ske) or *Rhodomonas baltica* (Rho). The values are expressed as mg g-1 DW.

	T0 ref		T4 ref		T7 ref		T4 Skel		T7 Skel		T4 Rho		T7 Rho		GLM analysis		
	Ave	Stdev	Ave	Stdev	Ave	Stdev	Ave	Stdev	Ave	Stdev	Ave	Stdev	Ave	Stdev	Diet (D)	Time (T)	D x T
Aldehydes	*1,0*	*0,1*	*1,0*	*0,2*	*0,5*	*0,1*	*0,6*	*0,1*	*0,9*	*0,1*	*1,2*	*0,2*	*0,5*	*0,0*	*ns*	***	***
(E)-2-Pentenal	0,3	0,0	0,3	0,0	0,4	0,0	0,3	0,0	0,5	0,0	0,3	0,0	0,3	0,0	***	***	***
(E)-2-Hexenal	0,2	0,0	0,1	0,0	0,1	0,0	0,1	0,0	0,1	0,0	0,2	0,0	0,1	0,0	***	***	***
(E)-2-Octenal	0,1	0,0	0,1	0,0	0,0	0,0	0,0	0,0	0,1	0,0	0,1	0,0	0,0	0,0	***	***	***
(Z,E)-2,6-Nonadienal	0,4	0,1	0,5	0,1	0,1	0,0	0,2	0,0	0,2	0,0	0,5	0,1	0,1	0,0	ns	***	***
Alcohols	*8,1*	*0,9*	*7,8*	*0,5*	*4,5*	*0,7*	*3,7*	*0,2*	*4,4*	*0,4*	*5,4*	*0,7*	*5,4*	*0,9*	***	***	***
1-Penten-3-ol	0,5	0,0	0,5	0,0	0,7	0,0	0,7	0,1	0,6	0,0	0,7	0,0	0,9	0,1	***	*	***
(Z)-2-Penten-1-ol	0,1	0,0	0,2	0,0	0,2	0,0	0,2	0,0	0,2	0,0	0,2	0,0	0,3	0,0	***	***	ns
(5Z)-Octa-1,5-dien-3-ol	4,5	0,6	4,6	0,3	2,8	0,5	2,3	0,1	2,8	0,3	3,0	0,3	3,3	0,3	***	**	***
(E)-2-Octen-1-ol	0,2	0,0	0,2	0,0	0,1	0,0	0,0	0,0	0,0	0,0	0,1	0,0	0,1	0,0	***	***	***
(E,E)-2,4-Octadien-1-ol	2,0	0,2	1,6	0,2	0,7	0,2	0,5	0,1	0,7	0,1	1,0	0,2	0,8	0,0	***	***	***
(E,Z)-3,6-Nonadien-1-ol	0,7	0,1	0,7	0,1	0,0	0,0	0,1	0,0	0,0	0,0	0,3	0,1	0,1	0,0	***	***	***
Ketones	*0,1*	*0,0*	*0,1*	*0,0*	*0,1*	*0,0*	*0,1*	*0,0*	*0,1*	*0,0*	*0,1*	*0,0*	*0,1*	*0,0*	**	*ns*	*ns*
2,3-Pentanodione	0,1	0,0	0,1	0,0	0,1	0,0	0,1	0,0	0,1	0,0	0,1	0,0	0,1	0,0	**	ns	ns
Furans	*0,1*	*0,0*	*0,1*	*0,0*	*0,0*	*0,0*	*0,1*	*0,0*	*0,1*	*0,0*	*0,1*	*0,0*	*0,0*	*0,0*	***	*	***
2-Ethylfuran	0,1	0,0	0,1	0,0	0,0	0,0	0,1	0,0	0,1	0,0	0,1	0,0	0,0	0,0	***	*	***

*** stand for P<0.001, ** stands for P<0.01, * stands for P<0.05.

Almost all VOCs showed significant diet x time interactions as shown in table 6.4. The highest 1,5-octadien-3-ol content was measured in reference oysters at the start of the experiment and at the four week sampling interval (4.5 and 4.6 mg kg^{-1}, respectively). The lowest content was measured after four weeks of feeding with *Skeletonema costatum* (2.3 mg kg^{-1}). The (E,E)-2,4-octadien-1-ol content showed a decline in the reference oyster (from 2.0 mg kg^{-1} at the start of the experiment to 0.7 mg kg^{-1} at the seven week sampling interval). Levels in both *Skeletonema* and *Rhodomonas* fed oysters have already declined after four weeks of feeding (to 0.5 and 1.0 mg kg^{-1}, respectively). The 1-penten-3-ol content increases over time in the reference (from 0.5 at the start of the experiment to 0.7 mg kg^{-1} at the seven week sampling interval) and the *Rhodomonas* fed oysters (up to 0.9 mg kg^{-1} at the seven week sampling interval) while the (E)-2-pentenal content increases in the reference (from 0.3 at the start of the experiment to 0.4 mg kg^{-1} at the seven week sampling interval) and the *Skeletonema* fed oysters (up to 0.5 mg kg^{-1} at the seven week sampling interval). The higher 1-penten-3-ol content in *Rhodomonas* fed oysters is most likely related to the higher n-3 PUFA content in the algal diet. The higher (E)-2-pentenal, also known to originate from n-3 PUFA degradation, content in *Skeletonema* fed oysters might be related to the fatty acid C20:5n3, This n-3 PUFA is the only one measured with a higher content in *Skeletonema costatum* diet in comparison to the *Rhodomonas baltica* diet. C20:5n3 is, as mentioned earlier, found in relatively high concentrations in diatoms such as *Skeletonema costatum*. The other VOCs show similar trends with decreasing content over time for all treatments. The PCA plot of the VOCs (figure 6.3) also shows clear separation of the experimental groups due to diet and time effects.

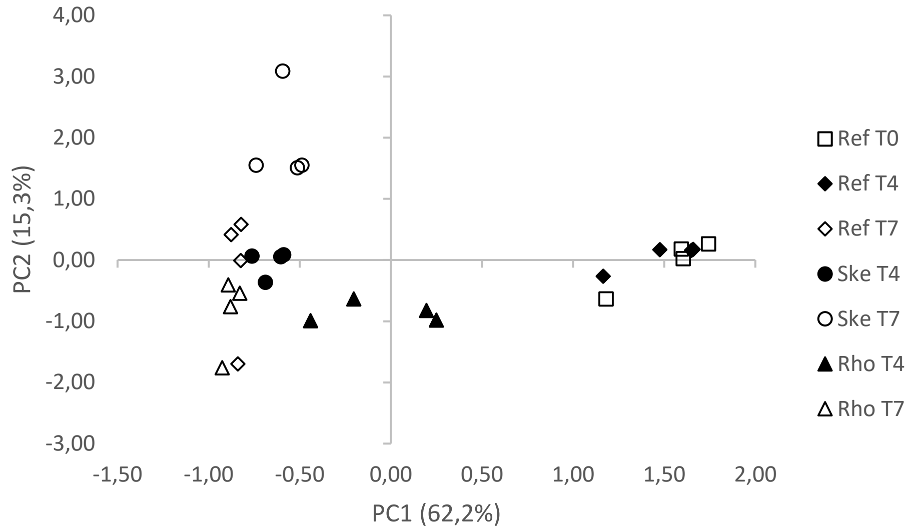


Figure 6.3 Principal component analysis of the volatile organic compound profiles of reference oysters (Ref) and oysters after four and seven weeks of feeding with *Skeletonema costatum* (Ske) or *Rhodomonas baltica* (Rho).

6.3.3 Sensory evaluation

Consumer evaluation

In the 3-AFC test the chance level was 33.3% for consumers to choose the divergent oyster from the three oysters presented. Results show that consumers were only able to discriminate between reference and *Rhodomonas* fed oysters. After the four week sampling interval 52.5% of the consumers chose *Rhodomonas* fed oysters to be divergent from reference oysters (k = 21, n = 40). At the seven week sampling interval 50.0% of the consumers chose *Rhodomonas* fed oysters to be divergent from the reference oysters (k = 20, n = 40).

The 3-AFC test with reference and *Skeletonema* fed oysters showed no significant difference. In total only 37.5% (k = 15, n = 40) and 39.5% (k = 15, n = 38) of the consumers were able to discriminate between both oysters at the four and seven week sampling intervals, respectively. In the 3-AFC composed with oysters fed with the different algal diets consumers were also not able to discriminate between oysters (40%, k = 16, n = 40 and 28.9%, k = 11, n = 38 after four and seven weeks of feeding).

Trained panelists evaluation

GLM analysis of the panelists evaluation data shows both dietary and time effects for the attributes overall odor and the fullness of shell (table 6.5). Overall odor intensity was highest in reference oysters at the start of the experiment (score of 5.4) and was lowest in *Rhodomonas* fed oysters after seven weeks of feeding (score of 2.9). A decline of overall odor intensity was found in all dietary treatments. Similar declines were observed for the marine odor and fruit odor intensity. Reference oysters at the start of the experiment scored highest (score of 5.0 and 1.4 respectively) while either *Rhodomonas* fed oysters after seven weeks of feeding and *Skeletonema* fed oysters after four weeks of feeding scored lowest (score of 2.2 and 0.4 respectively). Fullness of the shell decreased over time in the reference oysters from a score of 5.4 at the start of the experiment to 3.5 at the seven week sampling interval. In the algae fed oysters the score for the fullness of the shell increased to 5.8 and 6.1 for *Skeletonema* and *Rhodomonas* fed oysters respectively. Standardized PCA of the QDA data based on average panel scores (figure 6.4) shows separation of the experimental groups. Separation was mainly due to the characterization of reference oysters as having a high overall odor intensity, high saltiness and a strong marine flavor in comparison with the algae fed oysters. Reference oysters sampled at the different time intervals also show separation in the PCA plot. Darkness of the visceral mass, darkness of the gills and the blackness on the mantle edge seem to play an important role in the separation of the reference oysters.

Table 6.5 Quantitative descriptive analysis scores of reference oysters (Ref) and oysters after four and seven weeks of feeding with *Skeletonema costatum* (Ske) or *Rhodomonas baltica* (Rho).

	T0 Ref		Ref T4		Ref T7		Ske T4		Ske T7		Rho T4		Rho T7		GLM analysis		
	Ave	Stdev	Ave	Stdev	Ave	Stdev	Ave	Stdev	Ave	Stdev	Ave	Stdev	Ave	Stdev	Diet (D)	Time (T)	D x T
Overall odor	5,4	1,4	5,0	2,0	4,1	1,4	4,2	2,1	3,0	1,9	3,3	1,6	2,9	1,6	**	*	ns
Marine odor	5,0	1,8	4,0	2,2	3,5	1,8	3,7	2,2	2,6	2,4	3,4	1,9	2,2	1,5	ns	*	ns
Fruit odor	1,4	1,0	0,6	0,9	0,5	0,6	0,4	0,6	0,5	0,7	0,7	1,0	0,6	0,8	ns	**	ns
Mud odor	1,1	1,0	1,6	2,2	0,7	1,8	0,8	1,3	0,4	0,6	0,2	0,2	0,8	1,4	ns	ns	ns
Darkness of visceral mass	3,4	1,9	3,3	2,4	4,7	1,9	3,6	1,1	2,7	1,5	3,3	1,2	3,2	1,6	ns	ns	*
Darkness of gills	4,8	1,7	3,8	1,7	5,2	1,4	4,2	1,3	4,0	1,4	3,7	1,7	3,9	2,0	ns	ns	ns
Darkness of mantle edge	5,9	1,2	5,1	1,1	6,9	1,2	5,6	1,1	4,7	1,9	5,1	1,7	5,4	1,7	ns	ns	**
Fullness of shell	5,4	1,5	4,0	1,0	3,5	1,9	5,4	1,2	5,8	1,3	5,6	1,3	6,1	1,8	***	**	ns
Crunchy texture	5,2	1,9	4,8	1,8	4,5	2,0	5,1	1,4	5,4	2,1	4,7	1,3	5,4	2,0	ns	ns	ns
Melting texture	3,9	2,1	3,3	2,3	4,3	2,2	3,7	1,8	5,2	2,0	4,0	2,2	4,1	1,6	ns	ns	ns
Chewiness	4,3	1,4	3,9	2,2	2,9	1,5	3,3	1,6	3,8	1,7	3,0	1,6	3,9	1,8	ns	ns	ns
Marine taste	5,8	1,7	5,0	2,0	5,7	1,8	5,5	1,8	4,8	1,8	5,3	2,2	4,0	1,6	ns	ns	ns
Algal taste	1,7	1,8	1,7	1,4	2,4	1,2	2,3	1,6	2,1	2,2	1,3	1,4	2,3	1,6	ns	ns	ns
Saltiness	4,6	1,5	3,9	1,9	4,6	2,0	3,9	1,5	3,2	1,6	3,4	1,8	3,7	2,2	ns	ns	ns
Sweetness	2,5	1,6	1,9	1,1	2,0	1,4	1,7	1,1	3,0	2,2	2,3	1,6	2,0	1,9	ns	ns	ns
Bitterness	1,8	1,6	1,8	1,7	1,5	1,4	2,1	2,1	1,3	1,7	1,8	2,1	2,0	1,6	ns	ns	ns
Metallic taste	2,4	1,8	1,8	1,8	1,6	1,4	1,9	2,2	1,7	1,8	2,0	2,2	2,0	1,8	ns	ns	ns
Astringency	1,9	1,5	2,4	2,3	2,1	1,8	2,8	2,4	1,9	2,5	1,8	1,7	2,5	1,9	ns	ns	ns

*** stand for $P<0.001$, ** stands for $P<0.01$, * stands for $P<0.05$.

Differences in the QDA scores between *Skeletonema* fed oysters and *Rhodomonas* fed oysters are small. However, a separation in the PCA between *Skeletonema* fed oysters and *Rhodomonas* fed oysters is also shown in figure 6.4. *Skeletonema* fed oysters are characterized by a stronger seaweed flavor, and are more sweet in comparison with *Rhodomonas* fed oysters. Pennarun et al. (2003b) also found that algae fed oysters, with the algae *Skeletonema costatum* and *Isochrysis galbana*, were described by a sweeter taste in comparison to the reference oysters in their study. Pennarun et al. (2003b) attributed this sweeter taste to an increased carbohydrate content.

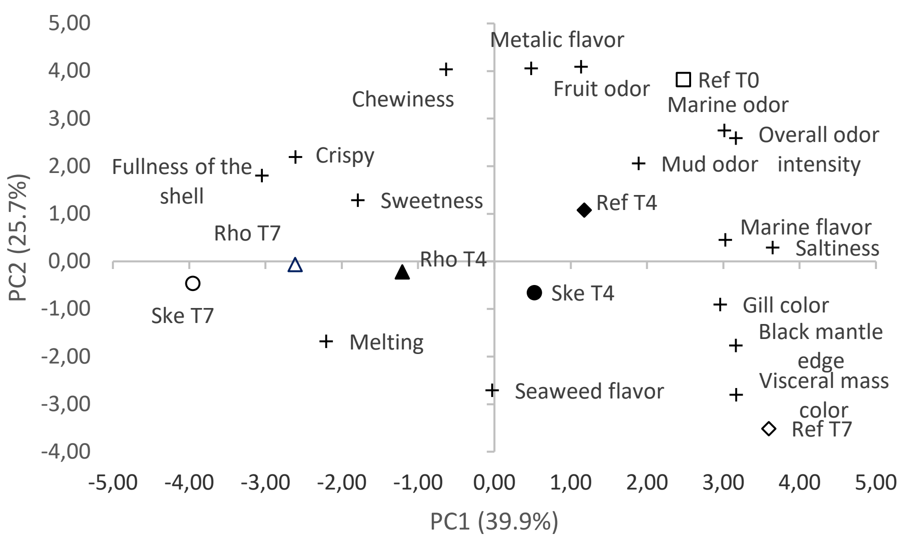


Figure 6.4 Principal component analysis of quantitative descriptive analysis data from reference oysters (Ref) and oysters after four and seven weeks of feeding with *Skeletonema costatum* (Ske) or *Rhodomonas baltica* (Rho).

A firmer texture for *Rhodomonas* fed oysters and a more melting texture for *Skeletonema* fed oysters were observed by the trained sensory panel. In the study of Pennarun et al. (2003b) *Skeletonema costatum* fed oysters were also described by a more melting texture as opposed to a firmer texture for *Isochrysis galbana* fed oysters. This difference was attributed to a higher glycogen and lipid content in *Skeletonema* fed oysters.

In our study both the *Skeletonema* and *Rhodomonas* fed oysters, from the four and seven week sample intervals, are mainly separated due to increasing fullness of the shell over time.

The role of carbohydrates in the texture and the fullness of the shell seem eminent in our results. Feeding oysters with both algal diets increases the carbohydrate level and therefore the oysters are considered to be have a higher chewiness and appear larger (higher fullness of the shell). The main reported reason for textural changes in oysters is the glycogen level (Pennarun et al., 2003b). Glycogen is the natural energy reserve of oysters and shows a seasonal variation with low levels just after spawning (Dridi, Romdhane and Elcafsi, 2007). Furthermore Dridi et al. (2007) also showed the relationship between the glycogen level and the condition index (a measurement of fullness of the shell), with higher condition indexes found for oysters with higher glycogen levels.

The observed differences in flavor properties could not be explained by the FAAs content in the different experimental oyster groups. Several FAAs are known to contribute to flavor development in seafood. For instance glutamate is known to decrease perceived sweetness in shellfish such as scallop (Wantanabe, Lan, Yamaguchi and Konosu, 1990) and Manilla clam (Fuke and Konosu, 1991). Glycine has been mentioned (Hong et al., 2002) as contributing to a fresh sweetness in Pacific cupped oysters. However, the glycine content in *Skeletonema* fed oysters in our study is lower in comparison with that in *Rhodomonas* fed oysters, while the highest perceived sweetness, in the QDA from the trained panelists, was scored for *Skeletonema* fed oysters. Conversely, alanine is also known to affect the perceived sweetness (Wantanabe et al., 1990). Even though no significant dietary effect was found for the alanine content in our study, higher alanine content was found for *Skeletonema* fed oysters (5.3 mg g^{-1} DW) in comparison with *Rhodomonas* fed oysters (4.1 mg g^{-1} DW).

The total VOC content decreases over time in all experimental oyster groups concomitantly with the perceived overall odor intensity as determined by the expert panel. However, the overall odor intensity for the reference oysters at the seven week sampling interval was considered stronger in comparison with both algae fed oysters while the total VOC content showed similar levels at the seven week sampling interval. Direct comparison between the odor scores in the QDA and the odor concentrations of the VOCs is difficult. The decrease in the marine odor (and possibly the marine taste) might be related to the decrease of (E,Z)-3,6-nonadien-1-ol which has been reported to have a marine, cucumber odor (Piveteau et al., 2000; Pennarun et al., 2003b).

Nowadays *Skeletonema costatum* is often used as the refinement diet (Soletchnik et al., 2001; Piveteau et al., 1999; Robert, 1990) while refinement using *Rhodomonas baltica* is not yet commercialized. However differences in both the biochemical composition and sensory characteristics of the oysters within this research are shown to occur between all experimental groups. Furthermore, naïve consumers are able to perceive differences between *Rhodomonas* refined oysters and reference oysters whilst this is not the case for *Skeletonema* refined oysters and reference oysters. *Rhodomonas* refined oysters could therefore be marketed as a 'new' oyster refinement variety. The high PUFA content of the *Rhodomonas* refined oysters might be used as a marketing tool for further commercialization.

6.4 Conclusion

In our study dietary effects were found for the gross biochemical, fatty acid, FAA and VOC composition of Pacific cupped oysters. *Skeletonema* fed oysters have a significant lower ∑PUFA, ∑n-3, ∑n-6, C18:2n6, C18:3n3, C18:4n3, C22:6n3, glycine, (E)-2-pentenal and (Z)-2-penten-1-ol content in comparison with *Rhodomonas* fed oysters. The content of ∑MUFA, C16:1n7, C20:5n3, threonine, glutamine, leucine, histidine, (E)-2-hexenal, (E)-2octenal, (E)-2-octen-1-ol, (E,E)-2,4-octadien-1-ol, (E,Z)-3,6-nonadien-1-ol and (Z,E)-2,6-nonadienal was found to be significantly higher in *Skeletonema* fed oysters in comparison with *Rhodomonas* fed oysters.

Refinement with different algal diets has an effect on the sensory characteristics of Pacific cupped oysters. PCA analysis of the QDA data showed separation between all experimental groups. Reference oysters are mainly characterized by a high overall odor intensity, high saltiness and a strong marine flavor. All algae fed Pacific cupped oysters are characterized by a high fullness of the shell. Furthermore *Skeletonema* fed Pacific cupped oysters are separated from *Rhodomonas* fed Pacific cupped oysters mainly by a stronger seaweed flavor, higher perceived sweetness and a more melting texture in *Skeletonema* fed oysters.

Naïve consumers only perceive differences between *Rhodomonas* fed oysters and reference oysters. Therefore refinement with *Rhodomonas baltica* seems more apparent. Differences in both biochemical composition and sensory characteristics of the oysters increased over time suggesting a minimum refinement time of seven weeks.

6.5 References

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Chapter 7: Quality perception, purchase intent and consumer evaluation of refined Pacific cupped oysters (*Crassostrea gigas*)

"No argument can persuade me to like oysters if I do not like them. In other words, the disturbing thing about matters of taste is that they are not communicable."

Hannah Arendt

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Abstract

Oyster refinement using land-based pond systems is a new activity in the Dutch oyster sector in order to increase the oyster tissue weight and change the sensorial properties of oysters. However, the response of Dutch consumers towards refined oysters is unknown. The research aim was to gain insight in the importance of oyster quality parameters, drivers for oyster consumption and acceptance of refined oysters by Dutch consumers taking into account the information given on the product and process.

Taste, texture and odor are the most important oyster quality characteristics for Dutch consumers. The outcome of questionnaires showed that willingness to buy and pay is influenced by factors such as country of origin, cultivation area and flavor profile. Refinement did not affect willingness to buy and pay. Furthermore Dutch consumers seem to have a preference for the flavor profile of refined oysters. Consumer evaluation showed that refined Pacific cupped oysters were perceived sweeter compared with non-refined oysters. When information on the cultivation process was disclosed overall appreciation of refined oysters by consumers increased.

New insights in the importance of oyster quality characteristics for Dutch consumers are generated which can be used in the development of refined Pacific cupped oysters.

7.1 Introduction

Oyster refinement or fattening of oysters using land-based pond systems is a new activity for the oyster sector in the Netherlands. In oyster refinement, market-sized Pacific cupped oysters (*Crassostrea gigas*, Thunberg) are kept in basins and fed with algae to increase the oyster tissue weight and to change the sensorial properties of the oysters (Piveteau, Gandemer, Baud and Demaimay, 1999; Soletchnik et al., 2001). Differences in odor, taste and appearance attributes between refined and non-refined oysters have been shown when trained panelists were used for the evaluation (Pennarun, Prost, Haure and Demaimay, 2003; Van Houcke et al., 2017). Refined Pacific cupped oysters have been characterized by a stronger grass odor, a sweeter, less salty and less bitter flavor, in comparison to non-refined oysters (Pennarun et al., 2003). Furthermore refined Pacific cupped oysters have also been reported to have a lower overall odor intensity and marine flavor (Van Houcke et al., 2017). In appearance, the tissue of the refined oysters seemed to be larger in comparison with non-refined oysters and the color of the visceral mass seems to be whiter. Furthermore, it was shown that naïve consumers were able to discriminate between refined and non-refined oysters in sensory evaluations (Van Houcke et al., 2017).

The profile of Dutch oyster consumers could be described as predominantly male, over 55 years of age with a relatively high educational level and gross yearly household income (Van Houcke, Altintzoglou, Stieger, Linssen and Luten, 2016 and Van Houcke et al., 2017). This consumer profile is very similar to that found in a French study (Debucquet, Cornet, Adam and Cardinal, 2012). Oyster consumers could be regarded as traditionalists in their choices and preferences regarding oyster products. Debucquet et al. (2012) showed that acceptance of new oyster products was influenced by the age of the consumers and whether the consumers were eating oysters on a regular basis. In their study different products containing oysters as an ingredient and with different processing levels were evaluated. The products used in their study were: cooked oysters in a half-shell, hot preparation for toast, potted oyster, oyster butter and oyster-based soup. When it was mentioned that the evaluated products included oysters as one of the ingredients, the opinion of the participating consumers changed towards a more negative evaluation. The authors attributed this change in evaluation to reasons of disgust from non-regular oyster consumers. On the other hand, the changes in evaluations by regular oyster consumers were attributed to a loss of naturalness of the product or downgrading of a delicacy. These findings stress the importance to understand consumers attitude before entering the market with new oyster products.

In many cases new products are launched into the market based upon intrinsic (e.g. appearance, taste, odor, texture) and extrinsic product characteristics (e.g. brand, packaging, nutritional and health claims). However, consumer food choice is more complex than that. Other factors like biological, psychological, situational and socio-cultural factors also play a role in consumer food choices (Köster, 2009). In particular in the case of refined oysters, which could be considered as a new product, neophobia, trust in food technology, cultural and economical influences might play a role in consumer acceptance. The acceptance of a new food technology, like refinement, is dependent on the perceived benefits, risks and naturalness of the process and product. Information on the benefits of the new technology and consumer trust have been reported as being essential for consumer acceptance (Siegrist, 2008). Product characteristics seem less important to consumers of luxury products as consumer satisfaction comes from the response of other people to the display of wealth and status of the luxury products (Mason, 1998; Mason, 2001).

Refined oysters are nowadays sold on the Dutch market as luxury products for a premium price. However little is known about the purchase drivers and acceptance of new oyster products by Dutch consumers. The aim of this study was to gain insight in the importance of oyster quality parameters, drivers for oyster consumption and acceptance of refined oysters by Dutch consumers.

7.2 Material and methods

Three independent studies with Dutch consumers were performed. In the first study the importance of a number of parameters relevant for the quality perception of oysters by Dutch consumers was evaluated using questionnaires. In the second study questionnaires were used in order to evaluate the effect of the information of the cultivation process (refinement versus no refinement of oysters) and other purchase intention drivers such as country of origin, cultivation area and flavor profile on Dutch consumers' willingness to buy and willingness to pay for new oyster products. In the third study, actual products, refined and non-refined oysters, were evaluated by Dutch consumers and also the effect of information was studied.

7.2.1 Consumer panel

In all three studies consumers were recruited through advertisements in local newspapers and from a pool of consumers used in previous studies (Van Houcke et al., 2016; Van Houcke et al., 2017). The main selection criteria for the consumers was that they were consumers of oysters. The number of participating consumers varied between 56, 72 and

85 participants in study 2, 3 and 1, respectively. Consumers were not paid for their participation and neither were they told the study's aim or experimental design. Table 7.1 shows the characteristics of the consumer panels.

Table 7.1 Sociodemographic characteristics of the consumer panels in different oyster evaluation studies. Study 1: Quality perception. Study 2: Purchase intent. Study 3: Consumer evaluation and impact information.

	Study 1 (n=85)	2 (n=56)	3 (n=72)
Gender (%)			
Female	35	23	42
Male	65	77	58
Age (years) (%)			
< 25	1	2	1
26 - 35	11	11	7
36 - 45	4	5	6
46 - 55	18	25	24
> 55	67	56	62
Weekly seafood consumption (%)			
Less than once	20	25	40
Once to twice	66	55	49
More than twice	14	20	12
Yearly frequency oyster consumption (%)			
Once a year	6	8	10
2 - 3	33	37	38
4 - 10	44	35	33
> 10	17	20	19
Highest educational level (%)*			
Low	7	4	7
Middle	69	65	65
High	24	31	28
Gross yearly income (%)			
≤ €32 999	35	27	31
€33 000 - €49 999	51	45	50
≥ €50 000	14	27	19

* Low educational level: primary school and secondary school; middle educational level: intermediate vocational education and bachelor's degree or equivalents and high educational level: master's degree and doctor of philosophy degree or equivalents.

In all studies consumers were seated at random approximately 1 m apart in classrooms. They were instructed not to speak to each other and received a participation number for anonymity.

7.2.2 Study 1: Quality perception

In two focus group sessions, scientists working in the field of oyster cultivation, oyster farmers, oyster traders and chefs (n=10) predefined oyster quality characteristics and sensorial properties. The agreed predefined oyster quality characteristics could be categorized into biometric (total weight, tissue weight, meat content, shell length, shell width, shell depth), phenotypical (shell shape, shell color, tissue color), sensorial (odor, taste, texture) and extrinsic (shelf life, country of origin, flavor profile, cultivation method, health claims, nutritional value, packaging) characteristics. The sensorial properties of oysters were further broken down into the following attributes: saltiness, pungency, sweetness, firmness and creaminess. The predefined quality characteristics and sensorial properties have also been used in previous studies (Ifremer, 1997; House, Hanson and Sureshwaran, 2003).

The questionnaires regarding the oyster quality characteristics and sensorial properties were distributed among the participating consumers (n=85). The attribute definitions were explained to the consumers in order to ensure agreement in the understanding of the terminology used. Consumers were asked to score the importance of the predefined oyster quality characteristics and sensorial properties on a seven-point scale, anchored with ‘not at all important to me' on the left-hand side and 'very important to me' on the right-hand side. The participants in the focus group sessions did not participate in these tests.

7.2.3 Study 2: Purchase intention

Consumers' purchase intention in relation to country of origin, cultivation area, type of cultivation process and flavor profile was measured using questionnaires. A full factorial design was used to reveal how much these factors influence willingness to buy and willingness to pay for oysters. Each factor had two levels:

- Country of origin: domestic vs. imported oyster. Presented to the consumers as Dutch or Irish oysters.
- Cultivation area: natural vs. cultivation waters. Presented to the consumers as oysters cultivated in the Eastern Scheldt (nature reserve area) or Lake Grevelingen (most important Dutch oyster production area).
- Type of cultivation process: refinement vs. no refinement. Presented to the consumers as refined or non-refined oysters.
- Flavor profile: including and excluding the attribute 'sweet'. Presented to the consumers as saline, creamy, sweet or saline, creamy.

The attribute sweet was selected since the perceived sweetness is known to change in refined oysters in comparison with non-refined oysters (Pennarun et al., 2003; Van Houcke et al., 2017). These factors were combined in a virtual oyster label which was presented in a questionnaire to the consumers (n=56, see figure 7.1). Willingness to buy was assessed using a seven-point scale ranging from 'not at all' on the left-hand side to 'surely' on the right-hand side of the scale. Willingness to pay was assessed using a seven-point scale ranging from € 0.50 to € 2.00 per individual oyster. The range in price level is comparable with Dutch retail price levels. Low price levels (approx. € 0.50 per oyster) have been recorded for domestic oysters in discount supermarkets while the high prices (approx. € 2.00 per oyster) have been recorded for high quality imported French oysters in seafood specialty stores. Participating consumers were asked to mention reasons for the willingness to buy on the questionnaire forms.

Dutch oyster
Cultivated in the Eastern Scheldt
Two month refinement treatment
Flavor profile: saline, creamy sweet

Figure 7.1 Example of oyster label.

7.2.4 Study 3: Consumer evaluation and impact information

Actual products, being refined and non-refined oysters, were evaluated by naïve consumers (n=72) by rating the intensity of key attributes and acceptance. Alive refined and non-refined Pacific cupped oysters were obtained from a shellfish company (Renart Boulon, Kamperland, The Netherlands). Non-refined oysters originated from the cultivation area Lake Grevelingen (The Netherlands) and received no further treatment. Refined oysters also originated from the same cultivation area, but were fed with a monoculture of microalgae for one month in land-based pond systems. Oysters were obtained daily from the wet storage area of the shellfish company in order to ensure optimal quality. The oyster samples were stored refrigerated for a maximum of four hours at 4-6° C until preparation for the consumer test. Oysters were opened by hand-shucking and the adductor muscles were cut with a knife on both sides. Prior to serving the prepared samples, the remaining internal liquid was drained from the oysters. In the tests, oysters were served as being a half-shell product.

In total six oysters were served in three rounds in the consumer evaluation test. Each round lasted approximately 15 minutes. Between rounds, consumers received a five-minute break to take a sip of water or to eat a cracker to clean their palates. Samples assessed consisted of three refined and three non-refined oysters. Two oysters (one refined and one non-refined) were presented without any information on the cultivation process. The remaining four oysters were presented with information on the cultivation process (either refined or non-refined oysters) by means of an accompanying label. Two of the presented oysters were correctly labeled (one refined and one non-refined) and two were mislabeled (a refined oyster was labeled as being 'non-refined' and vice versa). The presentation order of the samples was randomized.

The attributes in the consumer evaluation test were selected from studies regarding the sensory profile of oysters (Pennarun et al., 2003; Aaraas et al., 2004; Buzin, Baudon, Cardinal, Barillé and Haure, 2011; Cochet, Brown, Kube, Elliott and Delahunty, 2013; Van

Houcke et al., 2016). The comprehensibility of the selected attributes was discussed in a preliminary session with ten consumers. The agreed attributes for the sensory profile test were: greenness, odor intensity, sea odor, mud odor, sweetness, saltiness, astringency, firmness, creaminess and overall liking. The ten consumers from the preliminary session did not participate in the actual tests. The selected attributes were scored on a nine-point scale ranging from 'very low' on the left-hand side to 'very high' on the right-hand side. The attribute definitions and scales where explained to the consumers in order to ensure agreement in the understanding of the terminology used and the intensity scores.

Using trained panels is the most common approach in sensory evaluations. However, in our study we wanted to compare the evaluations of the different oyster products by end users i.e. naïve consumers. Previous studies showed that the use of naïve consumer panels is a good alternative to using trained panels, even in complex products such as perfumes (Worch, Lê and Punter, 2010; Ares, Bruzzone and Giménez, 2010).

7.2.5 Statistical data analysis

In study 1, the quality perception data from the questionnaires were ranked using Kruskal-Wallis tests. Differences between quality characteristics were evaluated using Wilcoxon signed rank tests.

The data from study 2 were analyzed using one-way ANOVA for the weighted averages based on factor scores in order to evaluate effects of country of origin, cultivation area, cultivation process and flavor profile of oysters on consumers' willingness to buy and willingness to pay for the tested oyster labels. Reasons mentioned by consumers explaining their willingness to buy were categorized into themes (country of origin, cultivation area, type of cultivation process and flavor profile) (Fernqvist, Spendrup and Ekelund, 2015). Reasons were considered positive when willingness to buy was scored above four, on a 7-point scale. Likewise, reasons were considered negative when willingness to buy was scored below four. The mentioned categories are expressed as percentages in the result section.

In study 3, differences in the consumer evaluations of refined and non-refined oysters were analyzed using one-way ANOVA. Likewise, for both refined and non-refined oysters, one-way ANOVA was used in order to evaluate the effect of providing information about the cultivation process on the consumer evaluations. As for the latter, Post-hoc Tukey analysis was applied when significant effects were found.

Results are reported as means ± standard deviation. Where $p < 0.05$, differences were deemed statistically significant.

7.3 Results

7.3.1 Study 1: Quality perception

Figure 7.2 shows that Dutch consumers regard sensory aspects such as taste (6.7±0.5), texture (6.0±0.8) and odor (5.6±1.1) as important quality characteristics ($\chi^2 = 418.524$, $p < 0.001$).

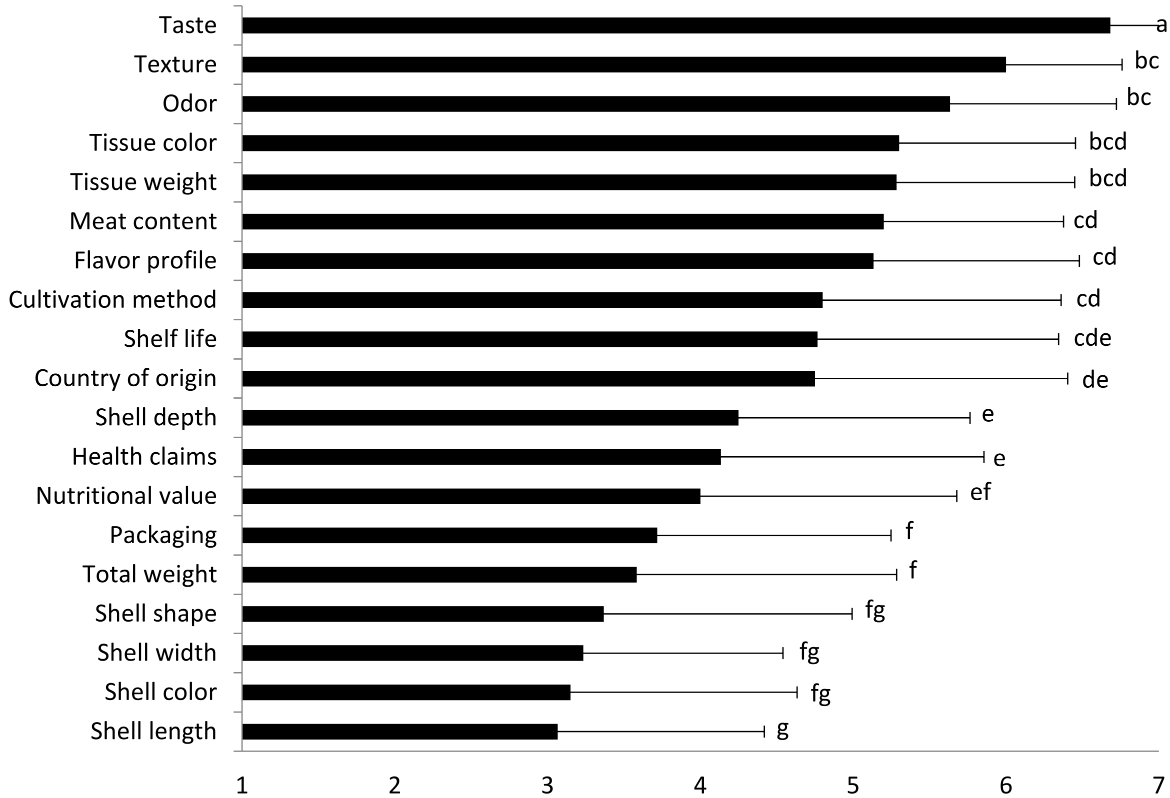

Figure 7.2 Mean (± standard deviation) importance of oyster quality characteristics according to Dutch consumers (n=85). 1 stands for 'not at all important', 7 stands for 'very important'. Different superscripts indicate significant differences (P<0.05).

Some of the phenotypical characteristics are also considered to be important as quality characteristics by the consumers. For instance, tissue color (5.3±1.2) and meat content (5.2±1.2) were considered important quality characteristics, while aspects like shell shape (3.4±1.6) and shell color (3.2±1.5) were considered to be less important.

Extrinsic characteristics such as cultivation method (4.8±1.6), shelf life (4.8±1.6), country of origin (4.8±1.7), health claims (4.1±1.7) and nutritional value (4.0±1.7) were considered less important than the phenotypical characteristics. Biometric characteristics such as total weight (3.6±1.7), shell width (3.4±1.3) and shell length (3.1±1.4) were considered the least important quality characteristics. Other biometric parameters like shell depth (4.3±1.5) and tissue weight (5.3±1.2) were considered to be more important as quality characteristics.

From the five pre-defined sensorial properties Dutch consumers rank sweetness and pungency as the most important (5.3±1.7 and 5.0±1.6, respectively) (figure 7.3). Firmness (4.1±1.4), creaminess (3.7±1.4) and saltiness (3.4±1.7) were considered less important ($\chi 2 = 57.875$, $p < 0.001$).

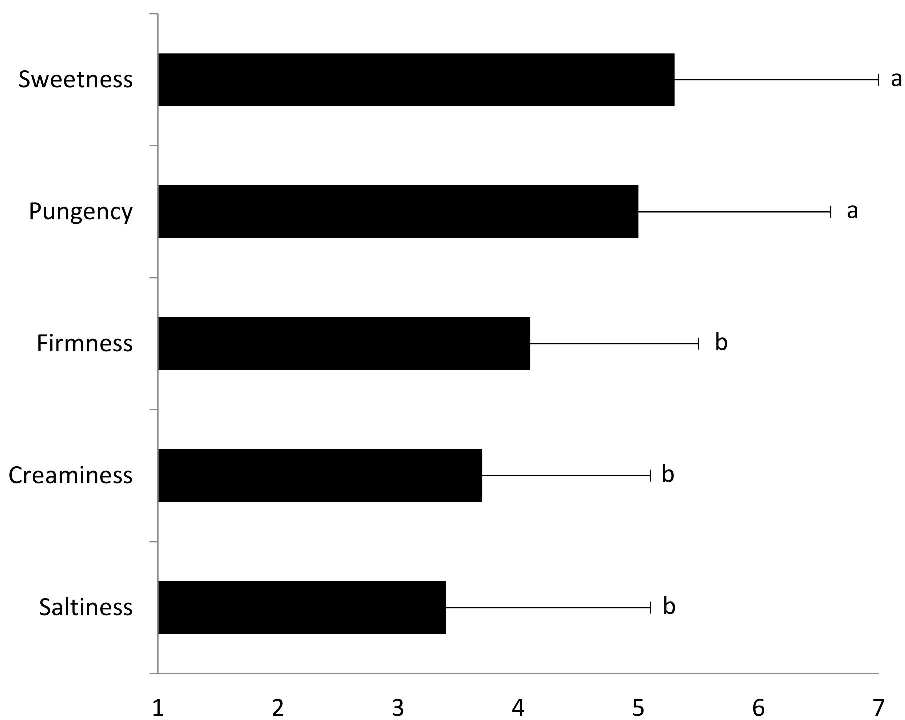

Figure 7.3 Mean (± standard deviation) importance of oyster sensorial properties according to Dutch consumers (n=85). 1 stands for 'not at all important', 7 stands for 'very important'. Different superscripts indicate significant differences (P<0.05).

7.3.2 Study 2: Purchase intention

Table 7.2 shows the effects of different purchase intention factors on Dutch consumers' willingness to buy and willingness to pay for oysters. Significant effects were found for the factors: country of origin, cultivation area and flavor profile. Consumers are more willing to buy domestic oysters in comparison with imported oysters ($F = 25,860$, $p < 0.001$). The average price consumers are willing to pay for domestic oysters is higher in comparison to the average price for imported oysters ($F = 9.382$, $p = 0.002$). Whether oysters originated from natural waters or specific cultivation waters did not affect consumers' willingness to buy oysters significantly ($F = 1.751$, $p = 0.187$). However, consumers were willing to pay more for oysters from natural waters than for oysters from cultivation areas ($F = 4.125$, $p = 0.043$).

Table 7.2 Effect of factors on oyster purchase intent (n=56): country of origin, cultivation area, cultivation process and flavor profile on consumer's willingness to buy (mean ± standard deviation), based on a 7-point scale from 1=not at all to 7=surely) and willingness to pay (€ per oyster).

	Country of origin Native				Non-native				Cultivation area Natural waters				Cultivation waters			
Willingness to buy	5,3	b	±	1,6	4,5	a	±	1,8	5,4		±	1,6	5,1		±	1,8
Willingness to pay	1,20	b	±	0,64	1,07	a	±	0,64	1,23	b	±	0,63	1,10	a	±	0,65
	Cultivation process Refined				Non-refined				Flavor profile Incl. sweet				Excl. Sweet			
Willingness to buy	5,1		±	1,8	4,9		±	1,7	5,3	b	±	1,7	4,5	a	±	1,8
Willingness to pay	1,14		±	0,65	1,15		±	0,64	1,18	b	±	0,64	1,08	a	±	0,64

Consumers were more willing to buy ($F = 21.092$, $p < 0.001$) and more willing to pay ($F = 5.755$, $p = 0.017$) for oysters which included the description 'sweet' in the flavor profile, when compared to oysters accompanied with a flavor profile excluding the description 'sweet'. No effect of treatment was found on the consumers' willingness to buy ($F = 1.292$, $p = 0.256$) and willingness to pay ($F = 0.088$, $p = 0.767$) for oysters.

The reasons consumers reported as influential for their willingness to buy could be grouped into the categories: country of origin, cultivation area, cultivation process and flavor profile. The flavor profile (51% of all reasons mentioned by consumers) and country of origin (27%) seemed to be the most important reasons for willingness to buy. Cultivation area (17%) and cultivation process (4%) scored lower. All categories were scored as reasons for low as well as high willingness to buy oysters. Low willingness to buy is mostly due to the country of origin (44%) followed by the flavor profile (34%), cultivation area (18%) and cultivation process (4%). High willingness to buy is mostly due to the flavor profile (69%) followed by cultivation area (17%), cultivation process (15%) and country of origin (10%).

7.3.3 Study 3: Consumer evaluation and impact of information

Dutch consumers' evaluation of Pacific cupped oysters showed significant differences between refined and non-refined oysters for the attribute sweetness (figure 7.4). Refined oysters were perceived as being sweeter compared with non-refined oysters (5.4 ± 2.0 and 4.7 ± 1.9, $F = 6.582$, $p = 0.011$ respectively). The refinement procedure did not lead to an increased overall appreciation by the consumers ($F = 0.336$, $p = 0.563$).

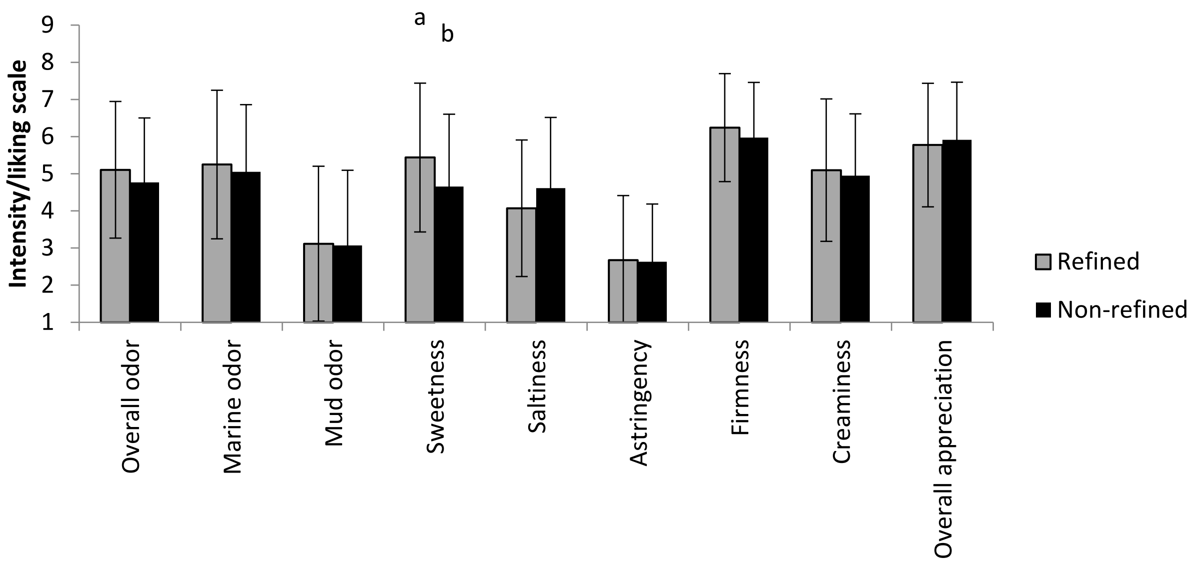

Figure 7.4 Mean (± standard deviation) consumer scores of sensorial properties (n=72) of refined and non-refined Pacific cupped oyster (*Crassostrea gigas*). 1 stands for 'very low', 7 stands for 'very high'. Different superscripts indicate significant differences (P<0.05).

Consumers' evaluations changed when information on the cultivation process was provided (figure 7.5 and 7.6). In the case of refined oysters, overall odor intensity was perceived as being less intense when information on the cultivation process was given. The score varied from 5.1±1.8 without information to 4.3±2.0 for the correctly labeled refined oysters and 4.3±1.9 for the mislabeled refined oysters ($F= 4.864$, $p = 0.009$). Furthermore, a significant difference ($F = 4.890$, $p = 0.008$) was found between the perceived sweetness of refined oysters without cultivation information (5.4±2.0) and refined oysters which were mislabeled as non-refined (4.5±1.8). For the attribute creaminess, a significant difference ($F = 3.417$, $p = 0.035$) was found for refined oysters which were either correctly (5.7±1.6) and mislabeled (4.9±1.7).

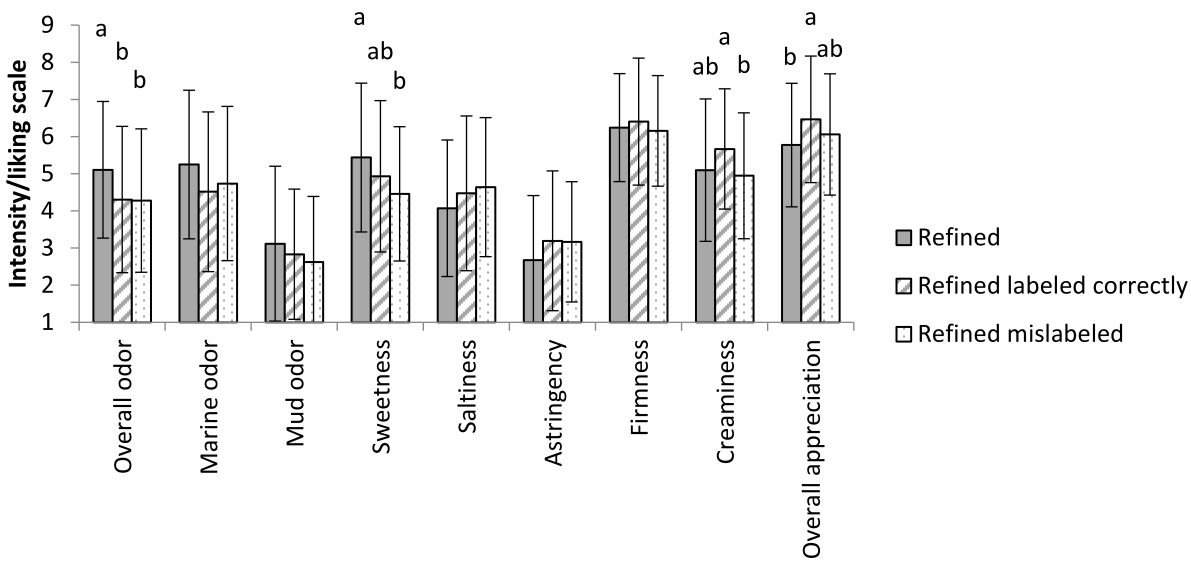

Figure 7.5 Mean (± standard deviation) consumer scores of sensorial properties (n=72) of refined Pacific cupped oyster (*Crassostrea gigas*) without information on the cultivation process and with correctly labeled or mislabeled information on the cultivation process. 1 stands for 'very low', 7 stands for 'very high'. Different superscripts indicate significant differences (P<0.05).

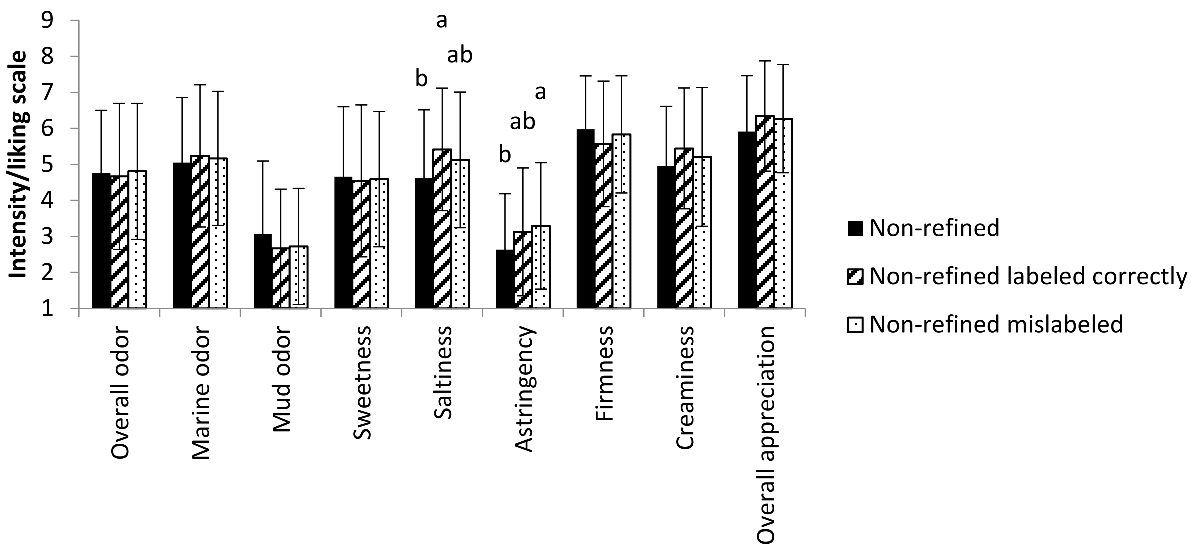

Figure 7.6 Mean (± standard deviation) consumer ratings of sensorial properties (n=72) of non-refined Pacific cupped oyster (*Crassostrea gigas*) without information on the cultivation process and with correctly labeled or mislabeled information of the cultivation process. 1 stands for 'very low', 7 stands for 'very high'. Different superscripts indicate significant differences (P<0.05).

The consumers' overall appreciation of the oysters increased by providing them with information on the refinement cultivation process. A significant difference (F = 3.265, p = 0.040) was found between refined oysters without information on the cultivation process (5.8±1.7) and refined oysters which were correctly labeled as being refined oysters (6.5±1.7). In the case of non-refined oysters, perceived saltiness and astringency increased, albeit not in all cases significant. When information on the cultivation process (no refinement) was provided to the consumers, significant differences (F = 3.899, p = 0.022) were found between perceived saltiness of non-refined oysters without information on the cultivation process (4.6±1.9) and non-refined oysters which were correctly labeled as being non-refined (5.4±1.7). Likewise, the difference between the perceived astringency of non-refined oysters without information on the cultivation process (2.6±1.5) and non-refined oysters which were mislabeled as being refined oysters (3.3±1.8) was also significant ($F = 3.253$, $p = 0.040$).

7.4 Discussion

Our aim was to gain insight in the importance of oyster quality parameters, drivers for oyster consumption of Dutch consumers and their acceptance for new oyster products such as refined oysters. Results show that consumers regard taste, texture, odor, tissue color and meat content as the most important quality characteristics for oysters. Biometric parameters such as total weight, shell shape, width and length were considered the least important characteristics. The importance of sensory aspects such as taste as a quality characteristic is not surprising. Several authors (Gempshaw, Bacon, Wessels and Manalo, 1995; Ifremer, 1997; Hanson, House, Sureshwaran, Posadas and Liu, 2002) found that taste, texture and odor are the main drivers for the consumption of oysters by US consumers. Texture was mentioned as one of the most important drivers for not consuming oysters (Hanson et al., 2002). Furthermore it was shown that French consumers do not pay attention to the shell shape of the oysters, whereas a high meat content is preferred by the majority of the consumers (Ifremer, 1997). The importance of meat content and the appearance of the oyster as quality characteristics were also emphasized by Ruello (2002).

Effects of purchase intention factors (land of origin, cultivation area and flavor profile) on consumers' willingness to buy and willingness to pay for oysters was clearly shown. As for country of origin, consumers showed a preference for domestic (Dutch) oysters in comparison to imported (Irish) oysters. Preference for domestic oysters was also seen in another questionnaire (Mueller Loose, Peschel and Grebitus, 2012). Loureiro and Umberger (2003) suggested that consumers associate land of origin with aspects such as food safety and freshness therefore explaining a preference for domestic products.

Results from the questionnaires in study 2 show that consumers prefer oysters cultivated in natural waters over oysters from known cultivation waters although no realistic samples were evaluated. Natural waters are likely associated with concepts such as, 'nature', 'pristine', or 'clean'. Siegrist (2008) suggested that concepts such as 'nature' and 'naturalness' related to food are positively valued by consumers. This positive association may explain the consumers' preferences in our study. In contrast, French consumer showed preference for oysters from renowned cultivation areas in a national questionnaire (Ifremer, 1997). This preference is most likely due to the greater familiarity of French consumers with the cultivation areas. In France, the cultivation area of the oysters is used as a distinctive marketing tool.

In our 2nd study described flavor profiles including the attribute sweet were given a higher score by consumers than flavor profiles without it. Furthermore, results show that Dutch consumers valued sweetness as the most important flavor characteristic, while saltiness was considered the least important. This suggests a preference of Dutch consumers for the flavor profile of refined oysters. Without actually tasting the refined or non-refined oysters, consumers' willingness to buy or willingness to pay for refined and non-refined oysters showed no significant differences. As oyster refinement is a new and not well-known cultivation method in the Netherlands, this might have affected the consumers' willingness to buy and willingness to pay for them. Trust towards new food technology or lack of consumer's knowledge thereon, is known to influence consumer perceptions (Behrens, Barcellos, Frewer, Nunes and Landgraf, 2009; Verbeke, Pérez-Cueto, Barcellos, Krystallis and Grunert, 2010; Mújica-Paz, Valdez-Fragoso, Samson, Welti-Chanes and Torres, 2011; Lee, Lusk, Mirosa and Oey, 2015). Verbeke et al. (2010) showed that new food technologies might evoke expressions of 'disgust', 'unnaturalness' or 'fear' and might lead to negative evaluations. Lee et al. (2015) showed that information on the food technology applied can lead to increased consumer trust towards the technology used. Our results show no lack of consumer trust towards refined oysters but it has to be remarked that no actual products were bought during this study. In reality both willingness to buy and willingness to pay might be different therefore the results should be used with caution. When consumers evaluated both the refined and non-refined oysters, they perceived refined oysters as being sweeter than non-refined oysters. This evaluation was done without providing information on the cultivation process. Providing information on the cultivation process of the oysters (being either refined or non-refined) affected the evaluation by consumers. Overall appreciation of refined oysters increased when consumers were aware of the refinement. Furthermore, odor intensity and marine odor perception decreased for refined oysters while creaminess perception increased. For non-refined oysters, information about the cultivation process led to an increase in saltiness perception by the consumers. Providing false information on the cultivation process (refined being labeled as non-refined and vice versa) only lowered the perceived creaminess of the refined oysters. Caporale and Monteleone (2004) suggested that information on food processing may influence how the taste of a product is evaluated. Moreover, information, or the lack thereof, has been shown to influence the willingness to buy and expected liking (Cardello, 2003; Deliza, Rosenthal and Silva, 2003; Deliza, Rosenthal, Abadio, Silva and Castello, 2005; Kole, Altintzoglou, Schelvis-Smit and Luten, 2009; Lee et al., 2015). Providing information could increase willingness to buy and expected liking of the product in question. In the case of mislabeling, it has been shown that consumer overall liking is significantly influenced by providing false information in mislabeled red wines (Meillon, Urbano, Guillot and Schlich, 2010). Prior to actual tasting, expectations did not show any effects of mislabeling as the expectation of the falsely labeled wines was equal to the correctly labeled wines.

Some of the limitations of this study include the lack of price aspect as a driver for consumer purchase intent. It has been shown that the aspect price is the strongest driver for consumer purchase intent of seafood in general (Olsen, 2004; Verbeke and Vackier, 2005; Brunsø, Verbeke, Olsen and Jeppesen, 2009) and oysters in particular (Ifremer, 1997; Mueller Loose et al., 2012). The aspect of price is not taken into account as an oyster quality characteristic in our study. Similarly to most consumer studies, we did not include a price variable as it might have reduced the variation of the rest of the attributes in the consumers evaluations. Furthermore no real money and products were involved as willingness to pay in our study was assessed using questionnaire data. The drawback of using questionnaire data is that it might lead to higher measured willingness to pay in comparison with real life settings involving actual purchase of the products (Neill, Cummings, Ganderton, Harrison and McGuckin, 1994; Wertenbroch and Skiera, 2002). In our study we were not interested in defining a realistic price for the oysters tested, but in differences in willingness to buy and willingness to pay between the products.

Our study gives new insight in the importance of quality and flavor characteristics for oyster consumers. These consumer insights can be used in product development of new oyster products. Furthermore, the results of our study show that Dutch consumers might prefer and buy refined oysters as the flavor profile of refined oysters is more in agreement to the preference of the Dutch consumers. No apparent negative attitude from the consumers towards the refinement process was observed in our studies. Providing the consumers with information on the production process leads to significant changes in the consumer evaluations of the oysters. Besides showing the importance of some intrinsic and extrinsic product characteristics to oyster consumers this study also provides an insight in some psychological factors affecting their choices and evaluations.

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Chapter 8: Discussion

"Oysters are very unsatisfactory food for the labouring men, but will do for the sedentary, and for a supper to sleep on."

Albert J. Bellows, 'The Philosophy of Eating' (1867)

The main focus of this thesis was to investigate the role of feed during refinement on the biochemical composition the quality of oysters. Quality was assessed by trained experts and by consumers. The aims of this thesis are 1. to study the impact of refinement (feed) on the biochemical composition, flavor profile and sensory characteristics during the cultivation of Pacific cupped oysters and 2. to gain insight into Dutch consumers' preferences, quality perception and factors influencing their evaluation of Pacific cupped oysters.

In the following paragraphs the main conclusions of this thesis are presented. In addition, the costs and benefits of refinement using microalgae are also discussed. Finally, future prospects for refinement and suggestions for research are presented.

8.1 Main findings and interpretations

A review on odor- and taste active compounds found in oysters (VOCs, FAA, '5 nucleotides and bromophenols) is presented in **Chapter 2.** It shows that VOCs, FAA and bromophenols find their origin in the feed of the oysters.

Many of the reported VOCs found in fresh oysters originate from n-3 PUFA, n-6 PUFA or n-9 MUFA oxidation. Other known VOCs in fresh oysters are formed by carotenoid, amino acid or polysaccharide degradation. The VOCs found can be categorized as aldehydes, alcohols, ketones, alkenes, cyclenes, alkanes, cyclanes, furans, acids and aromatic compounds. Most of the VOCs found in fresh oysters are aldehydes, alcohols or ketones. Main odor descriptors linked to VOCs from fresh oysters are mushroom, grass, green, cucumber, marine, citrus and fresh.

The main FAAs known to contribute to the overall taste of fresh oysters are glutamate, glycine and alanine.

In **chapter 3** the consumer preference for either the Pacific cupped oyster or the European flat oyster and the perceived differences in the sensory characteristics for both oyster species are presented. While Dutch experts in the oyster supply chain expressed their preference for the European flat oyster, no significant differences in Dutch consumer preference for either oyster species was shown. Presenting Dutch consumers with Pacific cupped oysters or European flat oysters with or without visual cues did not alter consumer preference. Sensory evaluation revealed that Dutch consumers perceived Pacific cupped oysters as saltier and more intense in greenness in comparison with European flat oysters. No significant differences were found in overall odor intensity, sea odor, mud odor, sweetness, pungency, firmness and creaminess. Salt content analysis of the oyster tissues confirmed a higher salt content (1.07% NaCl) in the Pacific cupped oyster compared to the European flat oyster (0.82% NaCl). The perceived differences in saltiness and greenness did not lead to a difference in consumer preference for one of the oyster species.

In **chapter 4** the dynamic sensory perception of the Pacific cupped oyster and European flat oyster were compared by consumers. Using TDS consumers perceived differences between the two species of oyster. Most pronounced differences were found for the attributes creamy, sweet and pungent. The dynamic sensory perception of the Pacific cupped oyster evolved from salty at the beginning of consumption to creamy in the middle of consumption proceeding to sweet at the end of consumption. The dynamic sensory perception of the European flat oyster evolved from salt at the beginning of consumption to pungent in the middle of consumption and sweet at the end of consumption.

In **Chapter 5** the biochemical and VOC profile of the European flat oyster and the Pacific cupped oyster from two important cultivation sites in the Netherlands (Lake Grevelingen and the Eastern Scheldt) were presented. Differences in biochemical composition and VOCs were detected between Pacific cupped and European flat oysters. The European flat oyster was characterized by a higher C15:0, C20:0, C20:4n6, C22:5n6 and an unknown fatty acid content and lower C18:1, C18:1n7, C184n3 and C20:1n9 content in comparison with the Pacific cupped oyster. In the European flat oyster the main VOC was 3-cyclohexene-1-ethanol while the main VOC in the Pacific cupped oyster was 1,5-octadien-3-ol. Furthermore, using PCA separation was shown for oyster species, cultivation areas and harvest month.

In **chapter 6** a higher ∑PUFA, ∑n-3, ∑n-6, C18:2n6, C18:3n3, C183:4n3 and C22:6n3 content in *Rhodomonas baltica* fed Pacific cupped oysters was shown in comparison with *Skeletonema costatum* fed oysters. The differences in the fatty acid profile of the oysters are coherent with the fatty acid profiles of the two microalgae. Other differences between *Rhodomonas* and *Skeletonema* refined oysters were in the FAA and VOC content.

The total VOC content decreased during refinement, with the lowest content found for the *Skeletonema* refined oysters. Threonine, glutamine, leucine, histidine, (E)-2-hexenal, (E)-2-octenal, (E)-2-octen-1-ol, (E,E)-2,4-octadien-1-ol, (E,Z)-3,6-nonadien-1-ol and (Z,E)-2,6-nonadienal content were found to be higher in *Skeletonema* fed oysters compared to *Rhodomonas* fed oysters. *Skeletonema* fed oysters were perceived as having a stronger seaweed flavor, higher perceived sweetness and a firmer texture in comparison with *Rhodomonas* fed oysters. Reference oysters were characterized by a higher overall odor intensity, higher saltiness and a stronger marine flavor in comparison with both refined oysters. In differentiation tests naïve Dutch consumers were only able to differentiate *Rhodomonas* fed oysters from reference oysters.

From a consumers perspective taste, texture and odor are the most important quality characteristics in oysters (**chapter 7**). Furthermore, consumers value sweetness as the most important taste characteristic while saltiness is considered the least important. Whether oysters were refined or not refined did not have any influence on the willingness to buy and willingness to pay by Dutch consumers. However, the flavor profile of the oysters did influence both willingness to buy and willingness to pay. Willingness to buy and willingness to pay by Dutch consumers were higher for oysters with flavor profiles including the description 'sweet' in comparison with oysters with flavor profiles excluding the description 'sweet'. Other factors such as country of origin and cultivation water of oysters also significantly affected willingness to buy and willingness to pay. When actual products, refined and non-refined oysters, were evaluated, Dutch consumers perceived refined oysters as sweeter in comparison with non-refined oysters. Providing information on the refinement process increased overall appreciation of the refined oysters by consumers.

Summarizing it can be concluded that the feed given during refinement alters the biochemical composition, volatile organic compounds, sensory and consumer evaluation of Pacific cupped oysters. The fatty acid profile and more specifically the polyunsaturated fatty acids of the microalgae given as feed play an important role. The fatty acid profile of the fed microalgae is reflected in the fatty acid profile of the oysters. Furthermore, the polyunsaturated fatty acids are known to be precursors of VOCs. The VOC profile of the Pacific cupped oysters is therefore also dependent on the feed of the oysters. Both expert and consumer panels were able to detect differences between reference oysters and refined oysters. In addition, the expert panel was able to point out subtle differences in the flavor profiles of oysters refined with different microalgae diets.

Dutch consumers showed no preference for either the Pacific cupped oyster or the European flat oyster. Dutch consumers rank sweetness as the most important taste characteristic of oysters. Consumers perceive refined oysters as being sweeter in comparison with reference oysters. Also, Dutch consumers indicated that they are willing to pay more for sweet oysters.

8.2 Practical considerations

8.2.1 Implementation of refinement of oysters in the Netherlands

Currently the majority of the oysters are cultivated on bottom plots in the Eastern Scheldt and Lake Grevelingen (Dijkema, 1997; Smaal and Lucas, 2000). Oyster spat is collected by the farmers on their designated plots by placing spat collectors on to which the oyster spat can settle. Settlement is due to the formation of shell material and the therefore increasing weight of the spat. During the growing process oysters are translocated to other plots (Dijkema, 1997). Translocation is done to match the requirements of the oysters in different life stages with the conditions present in the different plots and to control the shape of the oysters. In the final stage before harvest oysters are usually translocated to plots with high currents and high feed concentrations. After harvesting the oysters from the plots traders use the oysters to stock land based basins. The basins are usually fed with Eastern Scheldt water and follow the natural tides. Stocking the oysters has different goals 1. depuration of sand and silt 2. reducing stress and 3. strengthening the adductor muscle. During their stay in the land based basins the oysters are not fed. From the stocking basins they are ultimately processed, packed and transported (Doeksen and Symes, 2015).

In order to implement oyster refinement as a product innovation tool in the Dutch sector adaption to the current production chain is only required after harvesting the oysters from the cultivation plots. Oyster refinement in land based basins also takes place after harvesting oysters from their cultivation grounds. In France old salt pans or other ponds are sometimes used as basins (Soletchnik et al., 2001). The traditional Dutch stocking basins (figure 8.1) could be used for refinement after some adaptations as they are quite similar to the French refinement basins (figure 8.2).

Figure 8.1 Traditional oyster stocking basins in Yerseke, The Netherlands.

(https://www.zeelandnet.nl/uitinzeeland/kunst-en-cultuur/rondleiding-oesterputten).

Figure 8.2 Refinement basins at Marennes Oléron, France.

(https://www.huitresmarennesoleron.info/terroir_affinage.html).

Traditional stocking basins make use of the natural tide in the Eastern Scheldt in this way water in the basins is refreshed and oysters undergo a natural inundation time. Using sluices water can be retained in the stocking basins if required. Water temperature control in the refinement basins is important as the water temperature regulates the reproduction cycle of oysters. Baud, Mornet, Palvadeau and Haure (1998) studied the effect of water temperature on the reproductive cycle and growth of refined Pacific cupped oysters. After 35 days of refinement the approx. 60% of the oysters refined with water of a temperature of 18˚C were fully mature while 35% showed signs of maturation. Only approx. 5% of the oysters refined with a water temperature of 14˚C were fully mature while 65% showed signs of maturation and 30% showed no signs of maturation. No maturation was observed for oysters refined with a water temperature of 10˚C and 6˚C. Oysters refined at 6 and 10°C had a low tissue weight increase while oysters refined at 18°C mainly increased in shell weight. Baud et al. (1998) conclude that a water temperature of 14˚C is most suited for refinement due to resulting in the highest tissue weight increase.

The reproductive cycle has an effect on the biochemical composition and it is likely that maturation therefore also influences the sensorial properties of the oysters. Dridi, Romdhane and Elcafsi (2007) studied the biochemical composition of Pacific cupped oysters in relation to maturation of the oysters. In their study they showed high protein levels during oocyte maturation and decreasing levels during the spawning period. The glycogen content of oysters is typically high during gametogenesis and decreases during maturation and spawning. Conversely, lipid content increases during the maturation process.

8.2.2 Microalgae cultivation

During refinement oysters are fed with their natural diet: microalgae. In the French refinement basins naturally occurring microalgae are often used (Soletchnik et al., 2001). In certain cases the microalgae are cultivated in separate ponds and upon harvesting the algae they are fed to the oysters. The applicability of an algae species for oyster refinement depends on its nutritional value, which is determined by the algae's shape and size, digestibility, biochemical composition, toxins and the requirements of the oysters (Borowitzka, 1997; Becker, 2003; Hemaiswarya, Raja, Ravi Kumar, Ganesan and Anbazhagan, 2011). Certain algal species can be detrimental to bivalves as some produce toxic metabolites. These toxins can result in mortality, tumors or negatively affect the condition of shellfish. Other algae have metabolites which cause a bad smell or taste, making them unattractive for human consumption (Priyadarshani and Rath, 2012). Oysters are suspension feeders meaning they filter particles from the water column. The beating of cilia located on the ctenidial filaments creates a flow of water through the inhalant aperture or siphon to the gills where particles are caught in mucus and transported to the digestive tract (Ward and Shumway, 2004). Particles larger than approx. 5 μm are captured with an efficiency of 90% or higher. If the particles are smaller than 5 μm the uptake efficiency reduces proportionally with the size (Ward and Shumway, 2004). When particle concentration is high, the production of pseudofaeces increases and can even extend to a point where feeding is inhibited (Deslous-Paoli et al., 1992). The captured particles are transported to the mouth where they enter a short esophagus connected to the stomach. Oysters contain a crystalline style and a gastric shield in the stomach. The gastric shield provides protection for the stomach and a base for grinding particles with the rotating head of the crystalline style, necessary for breaking down rigid particles like diatoms such as *Skeletonema costatum* (Reid, 1965). The results presented in this dissertation indicate the importance of the fatty acid profile of the microalgae used for refinement.

In France the main microalgae species used in refinement are *Skeletonema costatum* and *Haslea ostrearia* (Pennarun, Prost, Haure and Demaimay, 2003a; Pennarun, Prost, Haure, and Demaimay, 2003b; Méléder et al., 2001; Piveteau, Gandemer, Baud and Demaimay, 1999; Soletchnik et al., 2001; Gastineau et al., 2012; Mejdandžić, Bosak and Ljubešić, 2017). *Skeletonema costatum* is easy to cultivate using natural saline groundwater sources and support good growth of the oysters (Baud, Haure and Garnier, 1990; Baud and Bacher, 1990). *Haslea ostrearia* is a diatom species known to produce a pigment which colors the gills of the oysters blue-green. This is also known as greening of oysters. Greening of oysters is mainly practiced on the Atlantic coast of France, in Marennes-Oléron Bay and Bourgneuf Bay. Green oysters have an approx. 20% higher market value in comparison with other refined oysters (Gastineau et al., 2012). Other microalgae pigments which might also have an effect on the color of oyster tissues have not been used in refinement processes. For example, astaxanthin is known for its use in the salmon industry in order to get the desirable pink color in farmed salmon. Microalgae species such as *Haematococcus* sp. can contain high astaxanthin levels.

In the Netherlands the low densities of naturally occurring microalgae in pond systems mean that microalgae should be cultivated for refinement purposes. Microalgae can be cultivated in semi-intensive systems like open ponds or intensive systems such as photo bioreactors, bubble columns and race ways (Borowitzka, 1997). In microalgae cultivation, operational costs are the most important factor determining total costs (Southgate, 2003). In photoautotrophic microalgae production systems, costs are determined by the energy costs for possible artificial lighting, operation of the systems, rate of mixing, photosynthetic efficiency and required quantities of medium and carbon dioxide (Guldhe et al., 2017). In addition, costs of land, water, harvesting, labor and transport add to the eventual price of the cultivated microalgae.

8.2.3 Costs and benefits

The additional cost of refining oysters is most likely mainly due to the high cost of microalgae production. Norsker, Barbosa, Vermuë and Wijffels (2011) calculated the production costs of microalgae cultivation in open ponds, horizontal photobioreactors and flat panel bioreactors at respectively 4.95, 4.15 and 5.96 € kg^{-1} DW in Dutch climate conditions. However, these values were calculated for farms up-scaled to 100ha from optimized small-scale conditions on the scale of 1 ha. Furthermore, costs for dewatering microalgae were also taken into account which is not required when feeding microalgae to shellfish. De Vree (2016) reported a biomass cost price of € 22,87 kg^{-1} DW algae for a

100 hectare production facility using outdoor vertically stacked photo bioreactors in the Netherlands. The reported cost price includes labor costs, energy consumption, raw materials, consumables, depreciation costs of the system and waste water treatment costs. This cost price seems more realistic as actual outdoor semi-commercial microalgae cultivation data was used in his calculations.

Taking into account the feeding rate of 30 mg DW microalgae day^{-1} oyster^{-1} and a total refinement period of 49 days, as reported in chapter 6 of this dissertation, 1.5 g DW microalgae oyster^{-1} is needed during the refinement process. With the most realistic cost price for microalgae of € 22,87 kg-1 DW algae the additional cost for each oyster would be € 0.03. Currently refined Pacific cupped oysters are sold in the Netherlands for a premium price of € 0.70 to 0.90 per oyster on a business-to-business basis (pers. comm. oyster trader). From retailers to consumers the prices for the Dutch refined oysters vary from € 1.60 up to 2.08 per oyster (http://www.versopjebord.nl and http://www.lekkerregionaal.nl). Reported cost prices for refined oysters in the Netherlands are approx. € 0.65 per oyster (pers. comm. oyster trader). Barille, Bougrier, Geairon and Robert (1994) reported an increase of 40% in the market value for refined Pacific cupped oysters in France compared to non-refined oysters. Refinement of Pacific cupped oysters in the Netherlands seems to be an economically feasible activity.

Research presented in this dissertation showed that consumers' willingness to pay for refined oysters was not higher than the willingness to pay for non-refined oysters. However, consumers were willing to pay more for sweet oysters as compared with non-sweet oysters. Furthermore, results in this dissertation indicate that refined oysters are evaluated as being sweeter according to a trained sensory panel. It might therefore be wise to use the sweet characteristic of refined oysters in marketing instead of using the refinement process as the main marketing message.

8.3 Future prospects and suggestions

The research presented in this dissertation showed that there is an impact of the algae diet on the biochemical composition, flavor profile and sensory characteristics of Pacific cupped oysters. Feeding different microalgae leads to different biochemical composition and sensory evaluations of both expert panels and consumers. The fatty acid profile of the microalgae play an important role in the development of VOCs and feeding different microalgae may lead to different compositions of Pacific cupped oysters. In this way 'new' oyster types or products could be produced and marketed as such. Several Dutch companies have already started pilot projects in order to produce refined oysters distinguished from the bulk volume of oysters produced in either Lake Grevelingen or the Eastern Scheldt. However, the concentrations of odor- and taste active compounds in the oysters (and therefore the flavor of the oysters) are dependent on many processes such as metabolism, osmosis regulation of the oyster and oxidative processes of the PUFA's originating from the microalgae fed (as mentioned in chapter 2). Therefore, it would be a challenge to control these processes and to tailor-make to a desired flavor.

In order to bridge the gap between desirable flavor from a consumer point of view and the flavor of the oyster itself new strategies should be developed in order to directly incorporate odor- and taste active compounds in the oyster tissue. Several promising methods from literature are:

1. **Microencapsulation**. Microencapsulation is a technique which is often used in disease treatment or prevention in fish cultivation (Rosas-Ledesma, León-Rubio, Alarcón, Moriñigo and Balebona, 2012; Borgogna, Bellich and Cesa´ro, 2011; Plant and LaPatra, 2011). Immunostimulants or immobilized pathogens are encapsulated using the polymers alginate, chitosan or polylactic-co-glycolic acid (PLGA). All above mentioned microcapsules are biodegradable, non-toxic and are permitted for use in food products. Recently Darmody et al. (2015) used alginate microcapsules in order to test the capsules as a delivery method for immunostimulants to European flat oysters. Darmody et al. (2015) showed ready ingestion of the alginate microcapsules and degradation in the gut of the oysters. From the three polymers PLGA seems the most likely candidate for delivery of either odor- or taste active compounds to oysters. Leaching of the odor- and taste active compounds from the alginate and chitosan capsules seems likely. PLGA on the other hand is suitable for encapsulation of small molecules (Danhier et al., 2012; Makadia and Siegel, 2011) and therefore seems a likely candidate for the delivery of odor- and/or taste active compounds to oysters. First steps in order to quantify ingestion and degradation of PLGA capsules by Pacific cupped oysters (*Crassostrea gigas*) are currently under investigation at the HZ University of Applied Sciences as a follow-up of this dissertation.

2. **Exogenous uptake of FAA**. Rice and Stevens (1987) showed an uptake of exogenously supplied FAA by the Pacific cupped oyster (*Crassostrea gigas*). In this study FAAs were actively pumped into the oyster shell with a flow rate of 2.5 l h^{-1}. Net influx rate (nmol g^{-1} h-1) was determined for several FAAs. Uptake of the FAAs occurred rapidly. The FAAs were rapidly transported to gill and mantle tissue of the oysters. Even though not discussed in the study by Rice and Stevens (1987) the exogenous supply of certain specific FAAs may prove a useful method to influence the flavor of cultivated oysters. First steps in applying this technique as a means of changing the flavor of oysters have been taken at the HZ University of Applied Sciences as a follow up of the work in this dissertation.

Consumer needs could be further met by 'tuning in' to the preferences of the consumers on aspects such as information, packaging, image, non-traditional products, etc. Involvement of consumers in the process of seafood or aquaculture product development has gained attention in the last decade. For instance, the concept of tailor-made seafood, launched in the SEAFOODplus project (EU 6th Framework Programme project 506359), is based on a consumer driven approach in new product development. A product can either be 'tailored' to fulfil the needs and wants of different consumers (Brunsø et al., 2008) or by taking away obstacles for consumption (Luten, 2008). Altintzoglou et al. (2010) used focus groups to develop new seafood product concepts based on the mentioned barriers to seafood consumption by young adults and the parents of young children in their study. Results from this study showed that consumers want attractive, healthy, palatable and convenient seafood products accompanied with advice on preparation method and materials. Recently Banović, Krystallis, Guerrero and Reinders (2016) actively involved seafood consumers in product development using different focus group sessions. In these focus group sessions participating consumers worked as a team to fulfill seven tasks: identifying new ideas, defining product personality, defining the product, modifying existing product ideas, profiling the consumer, creating new product ideas and finally selecting the best product ideas. This process led to different product ideas in the different countries involved in the studies. However fresh fish was mostly selected as being the best product idea. The ideas also describe presentation method, preferred packaging, preferred seasoning or sauce and even product messages. These approaches used by Altintzoglou et al. (2010) and Banović et al. (2016) could also be applied in the case of oysters and/or oyster products.

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Summary

Fresh oysters are considered a delicacy in Western Europe. In the Netherlands two species are cultivated; the Pacific cupped oyster (*Crassostrea gigas*) and the European flat oyster (*Ostrea edulis*). The Pacific cupped oyster is the main product of Dutch producers with a total production of 2300 tons in 2014. In order to increase the value of the Dutch Pacific cupped oyster product innovations, such as oyster refinement are necessary.
Oyster refinement is a relatively new process in the Dutch oyster sector. The refinement process usually consists of keeping live market-sized oysters in land-based systems for a period varying from one to four months in which the oysters are fed with (naturally occurring) microalgae.
The main aims of this thesis were to study the impact of feed on the biochemical composition, flavor profile and sensory characteristics during the cultivation and refinement of Pacific cupped oysters. Additionally, to gain insight into Dutch consumers' preferences, quality perception and factors influencing their evaluation of Pacific cupped oysters.
The presence of identified odor- and taste active compounds present in fresh oysters has been the subject of a review (**Chapter 2**). The main odor- and taste active compounds found in oysters are Volatile Organic Compounds (VOC's), Free Amino Acids (FAA), '5 nucleotides and bromophenols. Furthermore, the occurrence of many of these compounds has been found to be linked to the diet of the oysters. In this thesis the main focus is on VOC's and FAA. The VOCs found in oysters (aldehydes, alcohols, ketones, alkenes, cyclenes, alkanes, cyclanes, furans, acids and aromatic compounds) originate from n-3 PUFA, n-6 PUFA, n-9 MUFA oxidation or carotenoid, amino acid or

polysaccharide degradation. The VOCs are known to be odor active compounds and are linked to odor descriptors in oysters such as mushroom, grass, green, cucumber, marine, citrus and fresh. The FAA however, are known to be taste active compounds. The FAA known to have an impact on the taste profile of oysters are glutamate, glycine and alanine. Glutamte has been reported to increase both the umami taste and sweetness of oysters. Both glycine and alanine influence the perceived sweetness of the oysters.

The next step in this research was to evaluate Dutch consumer preference for either the Pacific cupped oyster (*Crassostrea gigas*) or the European flat oyster (*Ostrea edulis*) (**Chapter 3**). Consumer preference was evaluated using two-Alternative Forced Choice (2-AFC) tests while the sensory properties were assessed using a nine attribute consumer evaluation. In total 74 naïve Dutch consumers participated in this study. No significant differences in Dutch consumer preference for Pacific cupped or European flat oysters was shown. Furthermore Dutch consumer preference for Pacific cupped or European flat oysters was unchanged when presenting oysters without visual cues. In the consumer evaluation Pacific cupped oysters were perceived as being saltier and more intense in greenness in comparison with European flat oysters. No significant differences were found for the attributes overall odor intensity, sea odor, mud odor, sweetness, pungency, firmness and creaminess. A salt content analysis of the oyster tissues confirmed a higher salt content (1.07% NaCl) in the Pacific cupped oyster compared to the European flat oyster (0.82% NaCl).

In **Chapter 4** consumers used the temporal dominance of sensations method in order to compare the dynamic sensory perception of the Pacific cupped oyster and European flat oyster. This sensory method was used in order to assess flavor differences of oysters during consumption. The attributes salt, sweet, creamy, earthy, pungent and firm were used in this evaluation. Both Pacific cupped oysters and European flat oysters were evaluated by 31 naïve Dutch consumers. Most pronounced differences between the two oyster species were found for the attributes creamy, sweet and pungent. The dynamic sensory perception of the Pacific cupped oyster evolved from salty at the beginning of consumption to creamy at the middle of consumption proceeding to sweet at the end of consumption. The dynamic sensory perception of the European flat oyster evolved from salt at the beginning of consumption to pungent at the middle of consumption and sweet at the end of consumption.

In **Chapter 5** of this thesis the differences in biochemical composition and VOC content of the Pacific cupped oyster and the European flat oyster from the main Dutch cultivation grounds were investigated. Standard analytical methods were used in order to analyze the biochemical composition of the oysters. The VOC content was analyzed using headspace solid phase micro extraction. Marked differences were found between the two oyster species investigated. The fatty acid profile of the European flat oyster was found to contain higher C15:0, C20:0, C20:4n6, C22:5n6 and an unknown fatty acid content and lower C18:1, C18:1n7, C184n3 and C20:1n9 content as compared with Pacific cupped oysters. The main VOC found in the Pacific cupped oyster was 1,5-octadien-3-ol while the main VOC in the European flat oyster was 3-cyclohexene-1-ethanol.

The effects of refinement with different microalgae (*Rhodomonas baltica* and *Skeletonema costatum*) on the biochemical composition, volatile organic compounds, sensory and consumer evaluation of Pacific cupped oysters were studied in **Chapter 6**. Pacific cupped oysters were fed for a period of seven weeks with both microalgae diets while being kept under controlled conditions in land-based basins. The composition of the oysters was influenced by the microalgae diet fed. Higher ∑PUFA, ∑n-3, ∑n-6, C18:2n6, C18:3n3, C183:4n3 and C22:6n3 content was found in *Rhodomonas* fed Pacific cupped oysters in comparison with *Skeletonema* fed oysters which is coherent with the differences in the fatty acid profiles of the two microalgae. The total VOC content decreased during refinement, with lowest content found for the *Skeletonema* refined oysters. Furthermore, threonine, glutamine, leucine, histidine, (E)-2-hexenal, (E)-2-octenal, (E)-2-octen-1-ol, (E,E)-2,4-octadien-1-ol, (E,Z)-3,6-nonadien-1-ol and (Z,E)-2,6-nonadienal content were found to be higher in *Skeletonema* fed oysters compared to *Rhodomonas* fed oysters. The differences in the biochemical composition (incl. fatty acid, FAA and VOC content) led to perceived sensory differences in trained panelists evaluations. *Skeletonema* fed oyster were perceived as having a stronger seaweed flavor, higher perceived sweetness and a more melting texture in comparison with *Rhodomonas* fed oysters. Reference oysters from the Eastern Scheldt were characterized by a higher overall odor intensity, higher saltiness and a stronger marine flavor in comparison with both refined oysters. In the differentiation tests naïve Dutch consumers were only able to differentiate *Rhodomonas* fed oysters from reference oysters.

Dutch consumers' preferences, quality perception and factors influencing their evaluation of Pacific cupped oysters were investigated in **Chapter 7**. Using questionnaires the importance of oyster quality parameters to Dutch consumers and the willingness to buy-pay in relation to purchase intention drivers, such as refinement, were evaluated. Furthermore, the effect of product information on consumer evaluation of refined and non-refined oysters was studied. Consumers evaluated taste, texture and odor as the most important quality characteristics in oysters. Consumers also considered sweetness as the most important taste characteristic while saltiness was considered the least important. Whether oysters were refined or non-refined did not have any influence on the willingness to buy and willingness to pay by Dutch consumers. Other factors such as flavor profile, country of origin and cultivation area did influence both willingness to buy and willingness to pay by consumers. Consumers preferred to buy native oysters, oyster cultivated in natural waters and oysters with the attribute sweet included in the flavor profile. When actual products, refined and non-refined oysters, were evaluated Dutch consumers perceived refined oysters as being sweeter. Providing information on the refinement process increased overall appreciation of the refined oysters by consumers.

In **Chapter 8** practical considerations on the implementation of refinement for the Dutch oyster sector, options for microalgae cultivation and the costs and benefits of refinement are discussed. After some small changes the traditional oyster stocking basins in the Netherlands could be used as refinement basins. The additional costs of required microalgae as refinement diet could be as low as € 0.03 per oyster.

The research presented in this thesis shows that the feed given during refinement may alter the biochemical composition, volatile organic compounds, sensory and consumer evaluation of Pacific cupped oysters.

Furthermore, it was shown that Dutch consumers do not have a negative attitude towards refined oysters. Dutch consumers might even prefer refined oysters as refined oysters are characterized as being sweeter in comparison with non-refined oysters. Dutch consumers showed no preference for either the Pacific cupped oyster or the European flat oyster. Dutch consumers rank sweetness as the most important taste characteristic of oysters. Consumers perceive refined oysters as being sweeter in comparison with reference oysters. Also, Dutch consumers indicated that they are willing to pay more for sweet oysters.

Samenvatting

In West-Europa worden verse levende oesters als een delicatesse beschouwd. In Nederland worden twee soorten gekweekt. De Japanse oester (*Crassostrea gigas*), of wel de creuse genoemd, en de Europese platte oester (*Ostrea edulis*). De Japanse oester is de meest gekweekte oestersoort in Nederland. In 2014 werd 2300 ton geproduceerd. 'Affinage', een proces afkomstig uit de Franse oesterkweek is relatief nieuw voor de Nederlandse oestersector. Tijdens de affinage worden marktwaardige oesters gedurende een periode van een tot vier maanden gevoerd met van nature voorkomende microalgen in op land gesitueerde bassins. Nieuwe producten zoals Japanse oesters uit de affinage kunnen de waarde van de oesters verhogen.

De belangrijkste doelen van dit proefschrift zijn: 1.) het bestuderen van de invloed van voeding (algen) op de biochemische samenstelling, geur en smaakstoffen profiel en de sensorische kenmerken van de Japanse oester tijdens de teelt en de affinage en 2.) het inzicht krijgen in de voorkeur, kwaliteitsperceptie en factoren die de beoordeling van Japanse oesters door de Nederlandse consument beïnvloeden.

In **hoofdstuk 2** van dit proefschrift wordt een overzicht gegeven van de voorkomende en geïdentificeerde geur- en smaakstoffen van verse oesters. De voornaamste geur- en smaakstoffen zijn vluchtige organische verbindingen, vrije aminozuren, nucleotiden en broomfenolen. Veel van deze stoffen komen van nature voor in de voeding van oesters. In dit proefschrift ligt de nadruk op de vluchtige organische verbindingen en vrije aminozuren. De vluchtige organische verbindingen in oesters (aldehyden, alcoholen, ketonen, alkenen, cycloalkenen, alkanen, cycloalkanen, furanen, zuren en aromatische verbindingen) zijn veelal oxidatieproducten van omega-3, omega-6, omega-9 vetzuren.

Een andere bron van de vluchtige organische verbindingen zijn reactieproducten van bijvoorbeeld carotenoïden, aminozuren of polysachariden. Vluchtige organische verbindingen worden soms geassocieerd met geuren zoals paddenstoelen, gras, groen, komkommer, zee, citrus en fris. Daarnaast is het bekend dat vrije aminozuren zoals glutamine, glycine en alanine, een invloed hebben op het smaakprofiel van oesters. Van glutamine is bekend dat het zowel de umami smaak als de zoetheid van oesters verhoogt. Zowel glycine als alanine beïnvloeden de waargenomen zoetheid van de oesters.

In **hoofdstuk 3** wordt het onderzoek naar de voorkeur van de Nederlandse consumenten voor de Japanse oester dan wel de platte oester gepresenteerd. De voorkeur van consumenten werd onderzocht door een geforceerde keuzetest uit te voeren met beide oestersoorten. De sensorische eigenschappen van beide oestersoorten werden eveneens bestudeerd in een consumententest. In de consumententest, waaraan 74 Nederlandse consumenten deelnamen, werden negen sensorische kenmerken van de oesters beoordeeld. De resultaten tonen aan dat de Nederlandse consument geen uitgesproken voorkeur heeft voor één van de genoemde oestersoorten. Uit de consumententest bleek dat de consument de Japanse oester zouter en groener van kleur ervaarden in vergelijking met de platte oester. In de Japanse oester werd eveneens een hoger zoutgehalte (1.07% NaCl) gemeten in vergelijking met de platte oester (0.82% NaCl). Voor andere sensorische kenmerken zoals, totale geur intensiteit, zeegeur, moddergeur, zoetheid, pittigheid, stevigheid en romigheid werden geen significante verschillen waargenomen door de consumenten.

De resultaten uit een andere sensorische test, de zogenoemde temporal dominance of sensations, worden in **hoofdstuk 4** beschreven. Temperal dominance of sensations is een sensorische test gericht op het vaststellen van smaakverschillen tijdens de consumptie. Deze test werd uitgevoerd met de Japanse oester en de platte oester. Kenmerken zoals zout, zoet, romigheid, gronderigheid, pittigheid en stevigheid zijn in deze testen beoordeeld door 31 Nederlandse consumenten. De meest uitgesproken verschillen tussen de twee oestersoorten werden gevonden voor de kenmerken romigheid, zoet en pittigheid. Het smaakprofiel van de Japanse oester ontwikkelde zich tijdens de consumptie van zout naar romig en zoet. Het smaakprofiel van de platte oester ging tijdens de consumptie van zout naar pittig en zoet.

In **hoofdstuk 5** worden de verschillen in biochemische samenstelling en vluchtige organische verbindingen van de Japanse oester en de platte oester uit de belangrijkste Nederlandse kweekgebieden (Oosterschelde en Grevelingenmeer) beschreven. Voor de analyse van de biochemische samenstelling (droge stof-, as-, eiwit-, vet gehalte en vetzuursamenstelling) werden gestandaardiseerde methoden gebruikt. Het gehalte aan de verschillende vluchtige organische verbindingen werd gemeten door toepassing van een headspace solid phase micro extractie methode in combinatie met gaschromatografie en massaspectrometrie.

De vetzuursamenstelling van de twee oestersoorten waren duidelijk verschillend. Het gehalte aan de vetzuren C15: 0, C20: 0, C20: 4n6, C22: 5n6 en een onbekend vetzuur was hoger in de platte oester terwijl de gehalten aan de vetzuren C18: 1, C18: 1n7, C184n3 en C20: 1n9 juist lager waren in de platte oester in vergelijking met de Japanse oester.

Kenmerkend voor de Japanse oester was een hoog gehalte aan de vluchtige verbinding 1,5-octadien-3-ol. Een hoog gehalte aan 3-cyclohexeen-1-ethanol was juist kenmerkend voor de platte oester.

Het effect van verschillende microalgen (*Rhodomonas baltica* en *Skeletonema costatum*) als voeding gedurende de affinage op de biochemische samenstelling, vluchtige organische verbindingen, sensorische eigenschappen en de beoordeling door de consumenten staat beschreven in **hoofdstuk 6**. Japanse oesters kregen gedurende een periode van zeven weken onder gecontroleerde omstandigheden in op land gesitueerde bassin één van de soorten microalgen gevoerd. De samenstelling van de oesters veranderde afhankelijk van het algendieet. Hogere totaal gehalten aan meervoudige onverzadigde, omega-3 en omega-6 vetzuren en de individuele vetzuren C18:2n6, C18:3n3, C183:4n3 en C22:6n3 werden gevonden in oesters gevoerd met de alg *Rhodomonas*. De veranderingen in de vetzuurprofielen van de oesters was in overeenstemming met verschillen in de vetzuurprofielen van de algensoorten. Het totaal aan vluchtige organische verbindingen daalde gedurende de experimentele periode. Het laagste gehalte aan vluchtige organische verbindingen werd gemeten in de met *Skeletenoma* gevoerde oesters. Verbindingen zoals threonine, glutamine, leucine, histidine, (E)-2-hexenal, (E)-2-octenal, (E)-2-octen-1-ol, (E,E)-2,4-octadien-1-ol, (E,Z)-3,6-nonadien-1-ol en (Z,E)-2,6-nonadienal werden in hogere concentraties gevonden in de met *Skeletonema* gevoerde oesters. De gevonden verschillen in de biochemische samenstelling, vluchtige organische verbindingen en vrije aminozuren leidde tot verschillen in de sensorische beoordeling door een expert panel. Oesters met het *Skeletonema* dieet hadden een sterkere zeewiersmaak, waren zoeter en hadden een meer smeltende textuur in vergelijking met de *Rhodomonas* gevoerde oesters. Referentieoesters uit de Oosterschelde hadden een hogere geurintensiteit, waren zouter en hadden een sterkere zee smaak in vergelijking met de oesters na de affinage. In een driehoekstest konden Nederlandse consumenten alleen de met *Rhodomonas* gevoerde oesters onderscheiden van de referentieoesters.

De voorkeur en kwaliteitsperceptie van Nederlandse consumenten en de factoren van invloed op hun beoordeling van Japanse oesters worden beschreven in **hoofdstuk 7**. Op basis van enquêtes werden verschillende kwaliteitskenmerken van oesters op belangrijkheid gerangschikt door de consumenten. Factoren die de aankoop- en betalingsbereidheid van consumenten kunnen beïnvloeden werden onderzocht. Tevens werd de invloed van productinformatie over het productie proces (wel of geen affinage) op de beoordeling van kwaliteit door de consumenten bestudeerd. Consumenten gaven aan dat smaak, textuur en geur de belangrijkste kwaliteitskenmerken van oesters zijn. Zoet wordt als belangrijkste smaakkenmerk aangeduid, terwijl zout de minst belangrijke is. De aankoop- en betalingsbereidheid van de Nederlandse consumenten wordt niet beïnvloed door eventuele affinage van oesters. Andere factoren zoals informatie over smaakprofiel, land van herkomst en kweekgebied beïnvloedde de aankoop- en betalingsbereidheid van de consumenten wel. De consumenten gaven de voorkeur aan oesters afkomstig uit Nederlandse wateren die aangemerkt zijn als natuurgebied, en oesters met smaakprofielen waarin zoet genoemd werd. Uit de beoordeling bleek dat Nederlandse consumenten oesters na affinage als zoeter ervaarden. Informatie betreffende de affinage leidde tot een hogere appreciatie van de oesters door de Nederlandse consument.

In **hoofdstuk 8** worden de mogelijkheden voor de implementatie van de affinage in Nederland beschreven. De teelt van microalgen en de kosten en baten van de affinage worden in kaart gebracht. De traditionele oester verwaterbassins zoals in gebruik in Yerseke (Nederland), zouden met enkele kleine aanpassingen ingezet kunnen worden bij de affinage. De extra kosten voor de benodigde microalgen voor de affinage worden ingeschat op een minimaal bedrag van € 0.03 per oester.

De resultaten van het onderzoek, zoals gepresenteerd in dit proefschrift tonen aan dat de voeding van oesters gedurende de affinage van invloed is op de biochemische samenstelling, vluchtige organische verbindingen, sensorische eigenschappen en consumenten beoordeling van de Japanse oester. Daarnaast is aangetoond dat Nederlandse consumenten geen negatieve houding hebben ten aanzien van oesters die een affinage hebben ondergaan. Nederlandse consumenten zouden zelfs een voorkeur voor oesters na affinage kunnen hebben op basis van de zoetere smaak van de oesters. De Nederlandse consument heeft geen voorkeur voor de Japanse oester (de creuse) of de platte oester. Zoetheid van oesters wordt als belangrijkste smaakeigenschap gezien. Consumenten vinden oesters na affinage zoeter in vergelijking met oesters die geen affinage hebben ondergaan. De Nederlandse consument geeft daarnaast ook aan bereid te zijn om meer te betalen voor zoetere oesters.

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Speciaal voor de oesters: jullie zijn nog niet van mij af, work continues…

Overview of completed training activities

Discipline specific activities

Courses

Sensory perception and food preference (5th edition)	VLAG, Wageningen	2011
SPME GC-MS Training	CSIC-IMM, Vigo, Spain	2012
Advanced Food Analysis (4th edition)	VLAG, Wageningen	2013
Workshop Mild conservation techniques	WUR-FBR, Wageningen	2014

Conferences

Aquaculture Europe 2010, poster presentation	Porto, Portugal	2010
West European Fish Technologists Association (WEFTA), oral presentation	Gothenburg, Sweden	2011
Feed your Knowledge workshop, COST Action FA 0802, oral presentation	Barcelona, Spain	2012
Aqua 2012, poster presentation	Prague, Czech Republic	2012
Feed for Health final congress, COST Action FA 0802, oral presentation	Milan, Italy	2013
West European Fish Technologists Association (WEFTA), oral presentation	Tromsø, Norway	2013
West European Fish Technologists Association (WEFTA), oral presentation	Bilbao, Spain	2014
Shellfish conference, oral presentation	Neeltje Jans, The Netherlands	2015
Food Professional Day, oral presentation	Wageningen, The Netherlands	2015
Trans-Atlantic Fisheries Technology conference (TAFT), oral + poster presentation	Nantes, France	2015
Aquaculture Europe 2015, oral + poster presentation	Rotterdam, The Netherlands	2015
West European Fish Technologists Association (WEFTA), oral presentation	Split, Croatia	2016
West European Fish Technologists Association (WEFTA), oral presentation	Dublin, Ireland	2017
Shellfish conference, poster presentation	Neeltje Jans, The Netherlands	2017

General courses

Teach the teacher didactics conference	HZ/VHL/HR, Kamperland	2012
Customer Relation Management	HZ, Vlissingen	2013
Teach the teacher didactics conference	HZ/VHL/HR, Utrecht	2013
Statistics using R	HZ, Vlissingen	2014
Information Literacy including EndNote Introduction	WUR, Wageningen	2015
Techniques for Writing and Presenting a Scientific Paper	WUR, Wageningen	2015

Other activities

Preparation of research proposal	VLAG, Wageningen	2011
Delta Academy Days	HZ, Vlissingen	2010-2018
Activity commission NGvA	NGvA, Wageningen	2010-2013
Innovation Events Zeeland	HZ, Vlissingen	2011-2014

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