Effect of polyphosphates on the quality of frozen light salted cod (*Gadus morhua* L.) fillets

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A B S T R A C T

Frozen light salted fillets have been subject to discussion concerning their definition as foodstuff. EU has recently placed these products in the frozen category. The aim was to investigate how a commercial polyphosphate blend affected the quality of light salted fillets. Fresh and previously frozen fillets of cod were injected with a brine containing 0, 4.5, 9 or 18 g/L of Carnal 2110. Quality, chemical characteristics and phosphate residues were analyzed in fillets. Compared to previously frozen, light salted fillets from fresh raw materials had a whither and less yellow color while significantly lower yields and oxidation levels were registered. No differences were found in drip loss during thawing. Addition of polyphosphate contributed to increased whiteness of both frozen and thawed fillets from both raw material groups, while no effects on yields or drip loss were detected. Polyphosphate reduced oxidation only in the frozen raw material group. Total phosphate contents were higher in fillets from fresh than frozen raw materials and residues were mainly detected as monophosphate. The choice of raw material will affect the end quality and yields and results suggest that polyphosphate can improve light salted fillet quality and therefor may be defined as a food additive.

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1. Introduction

In this study, light salted cod fillets from fresh and previously frozen raw materials were treated with the commercial polyphosphate blend Carnal 2110 (Budenheim, Germany) before frozen storage for 3 months. This topic is important considering the limited amount of literature on how polyphosphate addition and raw material properties influence the end quality of these products. More knowledge is needed on how product quality parameters are affected by these additives and on the polyphosphate degradation after application. This will help optimize the implementation of polyphosphates in the seafood industry and improve their regulation by control authorities.

Light salted cod is a relatively new product. It was first introduced to the Southern European markets in the mid 1980's (Thorarinsdottir, Bjørkevoll, & Arason, 2010) and has steadily grown in popularity as an alternative to the more traditional heavy salted products (Thorarinsdottir, Gudmundsdottir, Arason, Thorkelsson & Kristbergsson, 2004). The market driving forces for

light salted fish is convenience (a ready-to-use product) and a relatively low price compared to traditional products (Lindkvist, Gallart-Jornet, & Stabell, 2008). The more expensive traditional heavy salted or dried salted cod products must undergo desalting for 2–3 days before consumption while light salted fillets are generally traded frozen, or as defrosted products packed in vacuum or modified atmosphere (Lindkvist et al., 2008; Fjørtoft, 2015).

Light salted fish can be defined as products with a water and NaCl content of approximately 82–85 g/100 g and 2 g/100 g, respectively (Thorarinsdottir, Bjørkevoll et al., 2010). The flavour is often described as milder and with softer texture than traditionally salted cod products. Specific import data concerning this product group is not available, since they are aggregated within the frozen fillets category (TARIC: 030471) in the international trade regulation (EEC, 1987). Industry data suggests that most of the Spanish frozen fillet import volume is likely in the form of light salted products, which have increased from 13.000 tonnes in 2004 to nearly 29.000 tonnes in 2015 (DATACOMEX, Spain). Market preferences have also shifted towards whiter and thicker fillets for heavy salted products, especially in Spain (Lindkvist, 2009). In this sense, the introduction of light salted fillets fulfils consumer preferences by often being both whiter and thicker than the traditional

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products. The more traditional dried salted cod products still dominate the Portuguese market, while in Brazil convenience products such as desalted cod have increased significantly the last decade. The increase is mainly supplied through import of these products from Portugal and China (Røst, 2012).

Light salted fillets have recently been categorized as frozen and not salted products, ending many years of dispute in the EU (EC, 2014) legalizing the use of polyphosphates as food additives. This is also the case for wet salted products (EC, 2013). Food additives, in contrary to technical aids, can both influence and be detected in the final product. To improve product and weight stability, up to 5 g/kg of polyphosphates (expressed as P_2O_5) can be added to fish fillets whenever they are further preserved as frozen products (EC, 2011a, 2011b). Detecting and monitoring polyphosphates can be challenging since they are often degraded to monophosphate during the shelf life of the product, as recently described for heavy salted cod (Bjørkevoll, Reboredo, & Fossen, 2014). It is unclear in what state polyphosphate additives are found in light salted products on the market today. The properties of polyphosphates in foodstuffs include reduction of drip loss during thawing and cooking (Ellinger, 1972; Kin et al., 2010; Lampila, 1990; Thorarinsdottir et al., 2004; Woyewoda & Bligh, 1986;), and prevention of muscle discoloration during storage by anti-oxidative properties (Dziezak, 1990; Masniyom, Benjakul, & Visessanguan, 2005; Nguyen et al., 2012).

In industrial production, fresh or previously frozen fish are filleted before light salting by brine injection or brine curing (brining) (Thorarinsdottir et al., 2004; Lindkvist et al., 2008). This process differs from the traditional method of laying fish and salt in alternating layers in a container for 1–2 days. Further industrial processing includes single freezing and glazing of fillets before packaging. An increasing percentage of light salted fillet production has shifted from fresh to onboard frozen raw material origin (Esaiassen et al., 2005). One explanation for this is that frozen raw materials can be exported from harvest areas to low cost developing countries for processing. The effects of phosphate treatment of fresh compared to previously frozen raw materials on light salted product quality have not been reported. In an industry perspective, describing processing protocols for polyphosphate addition to achieve optimal quality and yield has significant economic potential. There is also a potential to improve quality and yields by further tailoring and optimizing processing to the available raw materials. Information concerning phosphate additive degradation in these products, and in which form polyphosphate residues can be found, will be valuable for control authorities in order to optimize methods for analysis and control of polyphosphate additives in frozen and salted fish products.

In summary, the aim of this work is to study the effects of fresh and previously frozen raw materials and polyphosphate levels on the quality of frozen stored light salted cod fillets as described by surface color, yield, pH, proximate composition, oxidation, minerals and the quantity and type of added polyphosphates. Additionally, the study was carried out to achieve more knowledge on how phosphates should be implemented and categorized in seafood processing, and how these additives can be monitored by control authorities.

2. Materials and methods

2.1. Raw materials

Raw materials were handled and processed as shown in the flow diagram (Fig. 1). Cod were caught with gillnets (15 h fishing time) by a traditional coastal vessel off the coast of Northern Norway and bled for approximately 30 min in seawater holding 3–5 °C. After bleeding, whole fish were kept for approximately 6 h in containers with seawater (3–5 °C) until delivery to the fish processing plant. Further, the fish were headed and gutted (2.5–3.5 kg headed and gutted weight) before storage on ice at 1–2 °C for 3 days. The post-rigor cod were divided into two different sub-trials; one immediately hand filleted and light salted (fresh fish group), and another where cod were immediately single frozen at − 30 °C in sealed plastic bags and stored in larger black plastic bags with no light exposure for 10 weeks before thawing (frozen group). Thawing was carried out in fresh water (water temperature of 3 °C at the start) at chilled storage (1–2 °C) with addition of ice after approximately 20 h. After 36 h, the core temperature in the fish was 0.2–1.2 °C. After hand filleting, the average fillet weight was 953 ± 115 g and 905 ± 117 g, respectively for the fresh and previously frozen raw material group.

2.2. Sampling

Skin and bone-free muscle samples were analyzed chemically prior to light salting of fresh or previously frozen cod fillets. Sampling from whole fillets was carried out as shown in Fig. 2. Samples were taken from the most anterior part of the fillet for quantifying pyro and tri polyphosphates, total phosphate level (P_2O_5) and chemical characterization. A sample from the mid-section of the fillet was selected for the oxidation analyses. Water and NaCl content analyses were carried out on the most posterior section of the fillet. Five fillet pieces of 200–250 g were collected and analyzed individually for all five groups.

2.3. Light salting procedure

From both raw material groups, hand filleted post-rigor cod with skin were divided into four groups (n = 10 per group) and processed as shown in Fig. 1. All brines were made from sea salt (GC Rieber, Fredericia, Denmark) with a concentration of 180 g/L with the addition of Carnal 2110 (Budenheim Ltd., Germany), at levels of 0, 4.5, 9 or 18 g/L brine. Respective brine pH values were 7.1, 6.6, 6.9 and 6.9. This commercial blend is composed of sodium and potassium, pyro and tri polyphosphate, with P_2O_5 and Na_2O contents of 50.0–52.0 g/100 g and 12.0–16.0 g/100 g, respectively. The brine was continuously stirred during injection to avoid precipitation of phosphate in the bottom of the tanks.

The light salting procedure was carried out using a brine injector (Fomaco 16/64 F, Sandvadsvej, Denmark) with an injection pressure and conveyer belt velocity of 0.95 Bar and 30 injections per minute, respectively. After injection, fillets were placed on trays and stored for 24 h at - 30 °C. Frozen fillets were dipped in fresh water three times (each dip lasting 2–3 s) for glazing before being packed in plastic sheets inside cardboard boxes and stored at - 30 °C for three months. After the frozen storage period, four fillets from each group were taken out of the packaging and air thawed at 2–4 °C on a table for approximately 12 h prior to analysis.

2.4. Analytical methods

Calculation of yields for raw materials and light salted fillets (after three months of frozen storage) was performed by dividing fillet weight at each sampling point (after injection (n = 10), before (n = 4) and after thawing (n = 4)) by the fillet raw material weight prior to brine injection (after thawing for the frozen group) and calculated as percent yield. Fillet drip loss was measured (n = 4) as weight percentage lost during the 12-h thawing period. The surface color of thawed light salted fillets measured as L-, a- and b-values was determined inside a light box (Salmon color box) with a Minolta Croma Meter (Model CR-200, Minolta, Japan). Three measurements were carried out on each fillet (n = 4 per group).
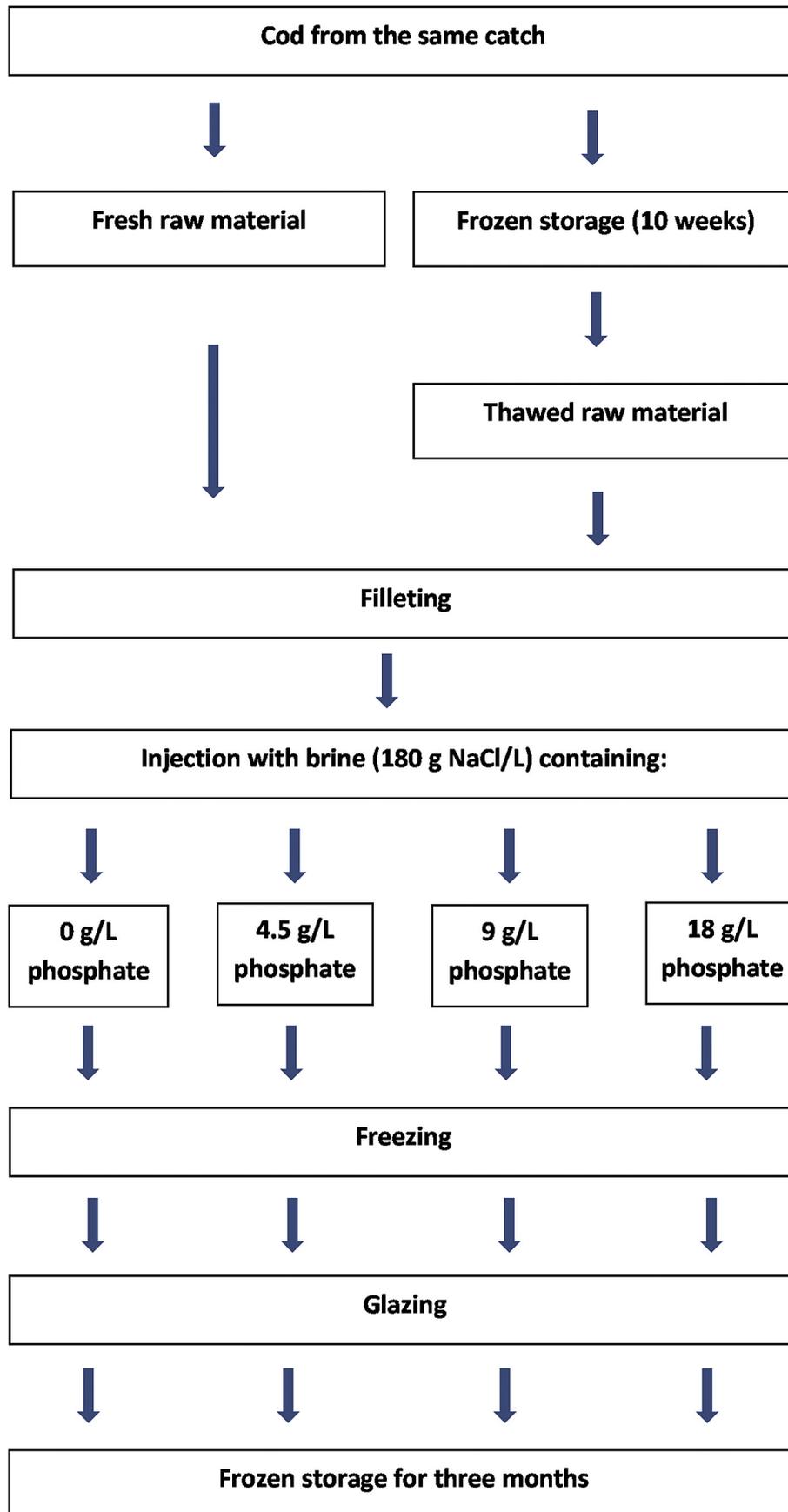
Fig. 1. Processing scheme for light salting of cod fillets using brine (180 g NaCl/L) added 0, 4.5, 9 or 18 g of the phosphate blend Carnal 2110/L. After freezing in overnight, the fillets were glazed prior to frozen storage at − 30 °C for three months before thawing and sampling.
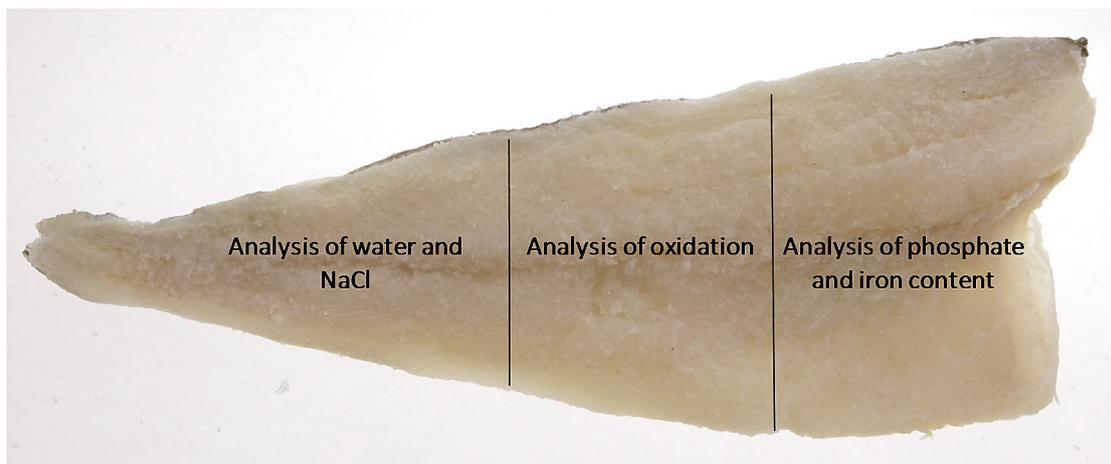
Fig. 2. Sampling diagram for raw materials and light salted fillets. Three pieces (200–250 g each) without skin and bone were made from whole fillet and analyzed for respectively phosphate and iron content, oxidation and water and NaCl contents.

Sensory analysis was performed by a panel of five scientists trained in fish quality assessment and conducted for the parameters white and yellow muscle color, blood spots and gaping for thawed, light salted cod ($n = 4$). For the color and gaping determinations, a score from 0 to 3 was used where higher scores indicated whiter, more yellow and more gaping of fillets. For blood spots, the score 0 or 1 was given for respectively none or presence of blood spots in the fillet.

The pH measurement was performed by inserting a pH sensor (WTW, pH 3310, Weilheim, Germany) directly into the loin part of the muscle. For raw materials and thawed light salted fillets, 10 and four fillets were measured, respectively. Water content was determined by AOAC method 950.46 B (1950). Three replicates were analyzed in each of the groups, except for the raw material where five replicates were characterized.

For chemical analysis, three light salted cod fillet samples from each group were individually analyzed. Determination of pyro and tri polyphosphates, total phosphate (P_2O_5), minerals, oxidation and water content were carried out in the same manner as for raw materials (Fig. 2). Primary and secondary lipid oxidation was determined using Peroxides and TBARS (Thiobarbituric Acid Reactive Substances) indexes. Peroxide quantification was based on an iodine titration method with a sodium thiosulfate solution, and starch as the indicator. Lipid extraction from fish tissue was carried out using chloroform in the presence of sodium sulfate and propyl gallate to further prevent oxidation of samples during preparation and analysis. Peroxide value (PV) was determined as the quantity of active oxygen (milliequivalents O_2) contained in 1 kg of lipid oxidizing potassium iodine (Cox & Pearson, 1962, chap. 14; AOAC 965.33, 1965). For TBARS analysis, the method proposed by Vyncke (1970), including modifications by Cervantes and Robles-Martinez (1984), was employed.

Determination of mineral content (NaCl, calcium and iron) in cod samples was carried out by complete digestion of the samples in pressurized containers with nitric acid and hydrogen peroxide as an oxidizing agent. Samples underwent 18 min of heating in a microwave-oven. After complete mineralization, samples were dissolved with Milli-Q water. All minerals were analyzed by ICP-OES (Varian Vista MPX). At least two emission lines were selected for each element. Sodium and potassium quantification included on-line addition of Internal Standard (Y) and Ionization Buffer (CsCl) (Robinson & Calderon, 2011).

Quantification of pyro and tri polyphosphate was carried out by

High Performance Thin Layer Chromatography (HPTLC) with an incorporation of standard ISO 5553-1980 used for detection of polyphosphates in meat products (Krzynowek & Panunzio, 1995; Marescot, Giorgio, & Maillard, 1998, pp. 85–87). The method consisted of the injection and separation of a trichloroacetic (TCA) fish extract on a cellulose layer, elution in a mobile phase (2-propanol/TCA/1.4-dioxane/NH₃) drying, and a two-step chromogenic derivatization (CAMAG Linomat 5 injector, Automatic Developing Chamber (ADC2)). Densitometry at 586 nm (CAMAG TLC Scanner 3, WINCATS software) was carried out for the phosphate detection and quantification. Due to the uncertainty of this method concerning quantitative measurements, the method was primarily used to detect residuals in a qualitative way.

2.5. Statistical methods

A selection of ANOVA models were utilized to analyze differences between treatments with regards to yield and quality parameters. ANCOVA models were used to isolate the effect of phosphate concentration and raw material. A Bonferroni pairwise hypothesis test (Systat Software, 2009) was used to compare groups.

3. Results

3.1. Yield calculations

Light salted fillets produced from previously frozen cod produced significantly higher yields than fresh raw materials ($F_{1, 31} = 190.5$, $p < 0.001$). Yields for the previously frozen cod product were in the range of 132–133% compared to 122–125% for products made from fresh raw materials. No significant effects of phosphate addition on yield were recorded ($F_{3, 31} = 6.0$, $p = 0.200$) (Fig. 3). For both groups, the glazing procedure contributed to a weight increase of approximately 10%. Drip loss during thawing of injected and glazed fillets was in the same range for all groups, and thus not affected by phosphate treatment.

3.2. Muscle surface color

Previously frozen raw materials had higher L-values (more white) (63.0–64.7) than fresh raw materials (54.9–57.0) prior to processing (Table 1). The average difference in L-values between
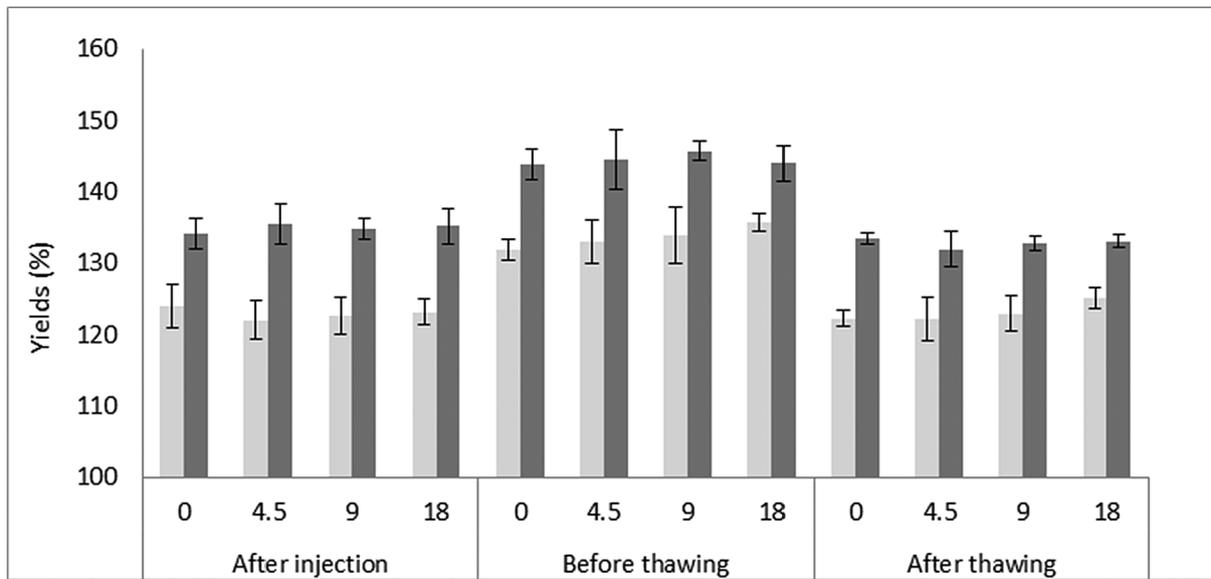
Fig. 3. Light salted fillet yields from fresh (light grey columns) or previously frozen (dark grey columns) cod raw materials (ave ± stdev shown). Fillets injected with brine of 180 g NaCl/L added 0, 4.5, 9 or 18 g Carnal 2110/L. Samples analyzed after injection ($n = 10$), before thawing ($n = 4$) and after thawing ($n = 4$).

Table 1
Instrumental L- (white), a- (red) and b-value (yellow) in raw materials ($n = 40$) and light salted fillets ($n = 4$) of cod processed from fresh or previously frozen raw materials (ave ± stdev shown). Light salting was carried out by injection of brines containing Carnal 2110 levels of 0, 4.5, 9 or 18 g/L.

Phosphate	Material	Fresh			Frozen		
		L-value	a-value	b-value	L-value	a-value	b-value
0 g/L	Raw material	56.1 (4.2)	−1.9 (0.4)	−4.7 (0.9)	63.0 (4.1)	2.0 (0.4)	1.5 (1.5)
	Light salted	54.0 (3.4)	−3.5 (0.6)	−10.3 (1.5)	47.5 (2.0)	−2.3 (0.2)	−8.7 (1.7)
4.5 g/L	Raw material	54.9 (3.4)	−1.7 (0.3)	−5.1 (1.3)	64.7 (4.7)	1.8 (0.5)	1.5 (1.5)
	Light salted	56.1 (2.4)	−3.7 (0.6)	−10.2 (1.3)	50.3 (2.3)	−2.5 (0.3)	−8.3 (1.3)
9 g/L	Raw material	56.5 (3.5)	−1.7 (0.4)	−5.3 (0.9)	64.3 (4.5)	2.1 (0.3)	1.6 (1.1)
	Light salted	58.3 (3.1)	−3.4 (0.5)	−9.7 (0.9)	52.1 (3.0)	−2.6 (0.3)	−8.6 (1.0)
18 g/L	Raw material	57.0 (4.1)	−1.9 (0.2)	−5.3 (1.0)	64.3 (5.0)	2.1 (0.3)	1.7 (1.2)
	Light salted	59.1 (2.4)	−3.2 (0.3)	−10.1 (1.0)	54.4 (1.8)	−2.7 (0.2)	−7.2 (1.7)

corresponding raw material groups was 5.8 points. For light salted samples, L-values in fillets from previously frozen raw materials were significantly lower compared to fillets from fresh raw materials ($F_{1, 95} = 118.5$, $p < 0.001$). In most cases, processed fillets from previously frozen raw materials had lower L-values (darker) than their corresponding raw materials, while the opposite was found for the fresh raw material groups. An ANCOVA model showed that L-values for light salted products increased in line with phosphate concentration ($F_{1, 96} = 23.94$, $p < 0.001$) when combining data from both material groups. This pattern was evident in both raw materials, although products made of fresh fish had higher L-values overall (Table 1).

Concerning a-values (red) and b-values (yellow), previously frozen raw materials scored on average 3.8 and 6.7 points higher (more red and more yellow) than fresh raw materials, respectively. These values were slightly lower in the final products when processed from fresh compared to frozen and thawed raw materials (Table 1). No significant effects of phosphate on a- or b-values were found ($F_{3, 95} = 0.591$, $p = 0.623$ and $F_{3, 95} = 1.824$, $p = 0.148$, respectively).

3.3. Sensory characterization

Sensory characterization of thawed light salted control samples of both raw materials (0 g phosphate/L) showed similar white color,

amount of blood spots and gaping degree, while yellow color was more prominent in the previously frozen group (Table 2).

Phosphate treatment did not contribute to abnormal quality defects in any of the samples examined. White color was more pronounced with increasing phosphate concentrations for fillets from both raw material groups ($F_{3, 47} = 7.77$, $p < 0.001$). This pattern was most evident for the previously frozen group (Table 2).

Phosphate levels were significantly correlated to variation in yellow color scores ($F_{3, 47} = 4.44$, $p = 0.008$) when data from both raw material groups were combined. Yellow color scores decreased as phosphate levels increased (Table 2) and light salted fillets from previously frozen raw materials had on average a higher score (more yellow) than the fresh group. No trends were observed with respect to gaping or the occurrence of blood spots for neither raw materials nor phosphate levels. However, the data indicates that fillet gaping was reduced with increased phosphate levels.

Upon ranking of the groups with respect to overall quality and appearance, the light salted fillets treated with 18 g phosphate/L were given the highest score (highest quality) for both raw material groups. For samples produced from previously frozen raw materials, the increasing addition of phosphate led to higher quality scores (18 g/L > 9 g/L > 4.5 g/L > 0 g/L). However, a similar systematic ranking order (18 g/L > 0 g/L > 9 g/L > 4.5 g/L) was not found for fresh fillet group.

Table 2
Sensorial characterization of light salted fillets produced from fresh or previously frozen raw materials ($n = 4$) after frozen storage and thawing. For parameters white color, yellow color and gaping a score from 0 to 3 (highest intensity) was given. For blood spots the score 0 (blood spots not present) or 1 (blood spots present) was given (ave $\pm$ stdev shown).

Phosphate	Light salted fillets	White color	Yellow color	Blood spots	Gaping
0 g/L	Fresh	1.3 (0.5)	0.3 (0.5)	0.3 (0.5)	1.7 (1.2)
	Frozen	1.6 (0.4)	1.5 (1.2)	0.3 (0.4)	1.9 (0.4)
4.5 g/L	Fresh	0.8 (0.5)	0.3 (0.5)	0.3 (0.5)	1.3 (1.3)
	Frozen	1.7 (0.7)	0.4 (0.4)	0.3 (0.5)	1.4 (0.4)
9 g/L	Fresh	1.0 (0.8)	0.5 (0.6)	0.5 (0.6)	1.3 (0.5)
	Frozen	2.0 (0.0)	0.7 (0.6)	0.3 (0.5)	1.4 (0.8)
18 g/L	Fresh	3.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.5 (0.6)
	Frozen	2.3 (0.5)	0.2 (0.4)	0.3 (0.5)	1.4 (0.4)

3.4. Chemical characterization of muscle

Muscle pH was 7.0 ± 0.1 ($n = 40$) and 6.9 ± 0.1 ($n = 40$) fresh and previously frozen cod, respectively. Phosphate addition did not affect pH in light salted fillets 6.6 ± 0.1 ($n = 20$) from fresh raw materials. After processing of previously frozen fillets, the control group had pH levels of 6.5 ± 0.0 ($n = 4$), while the groups injected with 4.5, 9 and 18 g phosphate/L brine had pH levels of 6.6 ± 0.2 ($n = 4$), 6.7 ± 0.1 ($n = 4$), and 6.6 ± 0.1 ($n = 4$), respectively. Water and NaCl content were 81.2–81.4 g/100 g and 0.17–0.23 g/100 g (Table 3) for both raw material groups. Overall, NaCl concentration declined with increasing phosphate concentrations in light salted samples ($F_{3, 23} = 4.01$, $p = 0.023$), from 4.9 to 4.2% for fresh and from 6.4 to 5.7% for previously frozen cod. In general, NaCl levels were higher in previously frozen, rather than fresh raw material ($F_{1, 23} = 67.10$, $p < 0.001$). Iron contents were higher in fresh cod (10.3 ± 8.3 mg/kg) compared to previously frozen raw materials (3.7 ± 1.0 mg/kg), while the iron levels in light salted samples were similar (<1.0–2.1 mg/kg) (Table 3) for both groups. Despite the calcium content being in the same range for both raw materials, it was significantly higher in light salted fillets from previously frozen (163–204 mg/kg) than for fresh raw materials (108–140 mg/kg) ($F_{1, 15} = 51.26$, $p < 0.001$). All processed samples had higher calcium contents than raw materials (Table 3). Calcium levels decreased with increasing phosphate concentration in samples from fresh raw materials ($F_{1, 11} = 15.45$, $p = 0.001$). For the previously frozen group, no trends in calcium levels were observed with increasing phosphate concentration (Table 3).

3.5. Muscle phosphate content

Overall P_2O_5 muscle levels were higher in fresh (3.8 ± 0.3 mg/g) rather than previously frozen (3.2 ± 0.6 mg/g) raw materials ($F_{1, 23} = 6.14$, $p = 0.021$) as shown in Table 4. For light salted samples produced from fresh raw materials, the total phosphate content increased along with phosphate concentration in the brine, from 2.6 ± 0.1 to 4.4 ± 0.2 mg/g ($F_{1, 11} = 60.61$, $p < 0.001$). Significant differences were found between all phosphate concentrations used

Table 4
Phosphate content in fresh or previously frozen cod muscle ($n = 5$) and light salted fillets ($n = 3$) processed from both raw materials (ave $\pm$ stdev shown). Fillets were injected with brine (180 g/L NaCl) containing, 0, 4.5, 9 or 18 g Carnal 2110/L. (ND = not detected, TL = Trace levels detected below quantification limit ≤ 0.7 mg P_2O_5/g).

Phosphate	Total P_2O_5 (mg/g)		Pyro phosphate (mg/g)		Tri polyphosphate (mg/g)	
	Fresh	Frozen	Fresh	Frozen	Fresh	Frozen
Raw materials	3.8 (0.3)	3.2 (0.6)	ND	ND	ND	ND
0 g/L	2.6 (0.1)	2.9 (0.1)	ND	ND	ND	ND
4.5 g/L	3.3 (0.1)	3.8 (0.5)	ND	ND	ND	ND
9 g/L	3.6 (0.1)	2.0 (0.3)	ND	ND	TL	ND
18 g/L	4.4 (0.2)	4.9 (0.3)	ND	ND	TL	TL

($p < 0.005$) except between the 4.5 and 9 g/L group ($p = 0.298$). For the phosphate content within the previously frozen raw material group however, a corresponding relation to the level of phosphate in the brine injected was observed, from 2.9 ± 0.1 to 4.9 ± 0.3 mg/g (Table 4). Regarding pyro and tri polyphosphate levels in the muscle, only trace amounts of tri polyphosphate were detected (≤ 0.7 mg P_2O_5/g) for the two groups injected with the highest addition of Carnal 2110 (Table 4).

3.6. Oxidation of muscle

Oxidation levels, measured as PV and TBARS, were markedly higher for previously frozen compared to fresh raw materials (Table 5). In the fresh group, the peroxide values were higher in processed products than raw materials, while the opposite was observed for the previously frozen group. When considering TBARS, significantly lower values were found in the fresh compared to the previously frozen group, both for raw materials and light salted fillets. After storage of processed samples (3 months), TBARS levels in the fresh group remained low for all phosphate treatments. For the previously frozen group however, the levels increased considerably from raw materials to light salted samples,

Table 3
Chemical composition of fresh or previously frozen cod muscle ($n = 5$) and light salted fillets ($n = 3$) (ave $\pm$ stdev shown). Fillets were injected with brine (180 g/L NaCl) containing, 0, 4.5, 9 or 18 g CARNAL 2110/L.

	Water content (%)		NaCl (%)		Iron (mg/kg)		Calcium (mg/kg)	
	Fresh	Frozen	Fresh	Frozen	Fresh	Frozen	Fresh	Frozen
Raw materials	81.2 (0.3)	81.4 (0.8)	0.23 (0.3)	0.17 (0.0)	10.3 (8.3)	3.7 (1.0)	93 (9)	92 (8)
Light salted								
0 g/L	83.5 (0.2)	81.6 (0.1)	4.9 (0.3)	6.4 (0.2)	1.5 (0.8)	1.0 (0.0)	140 (4)	204 (60)
4.5 g/L	83.2 (0.5)	81.6 (0.2)	4.8 (0.4)	6.7 (0.2)	1.3 (0.5)	1.7 (1.2)	121 (9)	163 (12)
9 g/L	83.1 (0.2)	81.1 (0.4)	4.3 (0.2)	5.9 (0.5)	1.0 (0.0)	1.3 (0.5)	110 (8)	190 (30)
18 g/L	82.7 (0.3)	81.7 (0.7)	4.2 (0.4)	5.7 (1.2)	2.1 (1.1)	2.1 (1.1)	108 (4)	223 (14)

Table 5
Muscle oxidation levels measured as peroxide value and TBARS in fresh or previously frozen cod muscle ($n = 5$) and light salted fillets ($n = 3$) processed from both raw materials (ave ± stdev shown). Fillets were injected with brine (180 g/L NaCl) containing, 0, 4.5, 9 or 18 g Carnal 2110/L and stored at − 30 °C for 3 months.

	Peroxide Value (meq. 0_2/kg fat)		TBARS (μmol MAD/kg)	
	Fresh	Frozen	Fresh	Frozen
Raw materials	<2.0 (0.0)	306.7 (120)	2.0 (0.5)	9.4 (2.0)
0 g/L	211.1 (7.3)	31.5 (20.9)	2.7 (0.5)	48.7 (15.5)
4.5 g/L	105.0 (14.7)	24.8 (5.2)	3.2 (0.7)	39.7 (17.3)
9 g/L	70.1 (31.0)	35.5 (9.0)	2.3 (1.2)	4.1 (0.9)
18 g/L	113.4 (46.2)	23.7 (6.5)	2.1 (0.7)	2.3 (0.8)

but phosphate addition seemed to reduce TBARS values for the groups treated with 9 and 18 g/L Carnal.

4. Discussion

4.1. Yield calculations

The 10% difference in yield between light salted fillet processed fresh compared to previously frozen raw materials has also been registered for heavy salted cod (Bjørkevoll et al., 2014) and can be explained by increased brine uptake in previously frozen raw materials. Since no differences in pH were registered, alterations of the water holding capacity could not be the reason for the observed yield variations. An explanation for this could however be that water loss from the denaturation of muscle proteins during frozen storage and thawing (Castell, Smith, & Dyer, 1974; Cormier & Léger, 1987; Shenouda, 1980), could have been regained during brine injection. A thaw drip of 15% has earlier been reported as not uncommon (Mahon, Schlamb, & Brotsky, 1970), while others state that properly handled fish normally have minor thaw drip (Aitken, 1975). However, a newer study showed a thaw loss of 4.1−9.4% for optimally handled and thawed cod fillets (Esaiassen, Carlehøg, Eilertsen, Breiland, & Østli, 2011). Another explanation could be that frozen storage causes shrinking of muscle fibers, resulting in increased extracellular space between fibers (Hurling & McArthur, 1996; Jarenback & Liljemark, 1975; Sigurgisladottir, Ingvarsdottir, Torrissen, Cardinal, & Hafsteinsson, 2000). Due to this shrinkage, the available space for the injected brine would be increased compared to fresh raw materials, rendering a higher brine uptake and explaining the increased yields in the frozen and thawed group. Unfortunately, since weights were not registered prior to and after frozen storage and thawing, this hypothesis cannot be verified in our trials.

The use of phosphate additives did not seem to influence yields or drip loss during thawing of light salted fillets. These findings have previously been registered both for unsalted (Cormier & Léger, 1987) and light salted cod (Thorarinsdottir et al., 2004). Still, these results are unexpected since phosphates are often added to foods due to their water binding properties (Aitken, 1975; Hunt, Kim, Park, & Schnee, 2004; Schnee, 2000). The effects of phosphate on drip loss could have been influenced by the high salt concentrations in the products (Desmond, 2007; Esaiassen et al., 2005). The neutral effect of phosphate on yield results could partly be explained by the large increase in yields by brine injection for both the fresh (22%) and frozen (32%) group. The relatively low phosphate concentrations used in our trials, as well as an initial salting out effect caused by the high salt concentration in the brine, could also be an explanation for the lack of registered effect of added polyphosphates on yields.

4.2. Quality effects of raw materials and phosphate addition

The freezing of cod raw materials seemed to result in a whiter flesh than fresh cod muscle as described in Bjørkevoll et al. (2014). In light salted samples the opposite was however registered, as observed in brined samples (Esaiassen et al., 2005). Brine injection with increasing levels of phosphate enhanced sensorial and muscle whiteness (instrumentally determined) for the light salted fillets both from fresh and previously frozen raw materials in most cases. Recorded levels of muscle phosphate in the samples were correlated with this increase in muscle whiteness which could be explained by the reported antioxidative effects of phosphates in fish products (Nguyen et al., 2012; Turan, Kaya, & Erkoyuncu, 2003; Ünal, Erdogdu, Ekiz, & Ozdemir, 2004). Phosphate addition seemed to reduce yellow color only in the light salted fillets from the frozen group, probably due to the antioxidative effect mentioned above. This effect was not observed for the fresh group, most likely explained by low oxidation levels, as determined by PV and TBARS.

Gaping seemed to be reduced with increased phosphate addition, especially for the fresh group added the highest phosphate concentration. This quality defect is caused by degradation of the connective tissue, where enzymatic breakdown of proteoglycans and glycoproteins weakens the collagen fibers (Ofstad, Olsen, Taylor & Hannesson, 2006). The reduction of gaping could be caused by inhibition of proteases due to the sequestering properties of phosphate (Dziezak, 1990). The overall quality of the samples treated with the highest phosphate concentration for both raw materials was increased compared to control samples. This seems to indicate that phosphates may have a positive effect on light salted cod fillet quality. However, the effect of phosphates on quality in heavy salted products is less clear (Bjørkevoll et al., 2014; Nguyen et al., 2012; Thorarinsdottir, Arason, Bogason, & Kristbergsson, 2001). This is likely due to the significantly different levels of NaCl and phosphate uptake between these two products.

4.3. Muscle phosphate content

As reported earlier, the phosphate content was slightly higher in fresh rather than previously frozen raw material (Bjørkevoll et al., 2014). Natural phosphate levels in cod raw materials are reported in the range of 2.0−6.0 g P_2O_5/kg (NIFES, 2013; Schröder, 2010; Thorarinsdottir, Arason & Thorkelsson, 2010; Thorarinsdottir et al., 2001).

Brine injection resulted in a reduction of the muscle phosphate content compared to the raw materials, as reported in previous work on heavy salting of cod (Bjørkevoll et al., 2014; Schröder, 2010; Thorarinsdottir, Arason et al., 2010; Thorarinsdottir et al., 2001). Addition of increasing levels of phosphate in the injection brine contributed to higher phosphate levels, both for fresh and previously frozen raw materials. Under the experimental conditions, phosphate levels remained below present regulation limits in all samples. In addition to the natural phosphate level in a product, standardized at 5 g/kg (FAO/WHO Food Standards, 1991), the regulation states that up to 5 g/kg of P_2O_5 (EC, 2013) can be additionally supplemented. However, present methodologies do not differentiate between natural and added phosphate (Bjørkevoll et al., 2014; Reboredo & Porro, 2012).

4.4. Chemical composition and oxidation of muscle

The light salted fresh group exhibited higher water contents compared to the previously frozen group, despite similar levels in their respective raw materials. Due to increased brine uptake during injection, NaCl levels were higher in the previously frozen

group. This could have led to a reduction in water holding capacity for this group of light salted fillets as optimal water binding is in the range of 4.5–5.8% in meat (Honikel, 1989; Xiong, Lou, Harmon, Wang, & Moody, 2000). However, no differences in drip loss after thawing were observed. The results show that injection of brine containing 180 g/L NaCl results in high salt concentrations in the final light salted products for both fresh and previously frozen raw materials. However, the final salt concentration in the samples is also dependent on the injection parameters and should be adapted to market preferences.

An explanation for large differences in the iron content between the raw material groups could partly be explained by the significant variations of this mineral within each group, probably caused by individual differences in blood levels (variable bleeding efficiency). The iron contents in light salted fillets from both raw material types were identical, while calcium levels were significantly higher in samples processed from previously frozen fillets compared to the fresh group, as previously reported for heavy salted cod (Bjørkevoll et al., 2014). Variation in calcium levels can be explained by the final NaCl levels in the fillets.

Significant oxidation detected after only 10 weeks of frozen storage of the raw material at temperatures as low as -30 °C, shows somewhat unexpectedly, that important quality loss can occur even at these storage conditions. Phosphate treatment seemed to reduce primary oxidation (peroxide value) in light salted samples from fresh raw materials. Even though high primary oxidation was observed during frozen storage of headed and gutted cod, the use of phosphates during light salting significantly inhibited further secondary oxidation (TBARS) during storage. The peroxide value method is adapted to fish with higher fat contents than cod and due to challenges with fat extraction, the peroxide value data could contain some uncertainty.

In heavy salted cod, the antioxidative effects of phosphates have not previously been verified. Nguyen et al. (2012) registered reduced oxidation, while others found opposite results (Bjørkevoll et al., 2014). The relationship between fat oxidation and the development of yellow muscle discoloration has been reported in heavy salted cod (Lauritzsen, Martinsen & Olsen, 1999). This seems also to be the case for light salted cod, since a whiter and less yellow color was positively correlated with lower oxidation.

5. Conclusions

Light salted, net caught cod from fresh raw materials results in higher overall fillet quality, but 10% lower yields after thawing compared to previously frozen raw materials from the same catch. From an industry perspective, the results demonstrate the importance of gaining more knowledge on how product quality and yields can be optimized by choosing the right raw materials.

Brine injection is an efficient method for introducing phosphate into cod fillets. Addition of increased levels of polyphosphates enhances overall light salted fillet quality by reducing yellow discoloration. Increasing concentrations of Carnal 2110 decreased oxidation levels, showing that the polyphosphate has an antioxidant effect in these products. In our trials, drip loss or yields in the final products are not affected by the treatment of this commercial polyphosphate blend.

Despite effective phosphate absorption, only trace levels (below quantification limit) of the added pyro and tri polyphosphate were detected in the final products. The degradation of these compounds into ortho phosphate is a challenge to current official control methodologies, since they do not enable differentiation between natural and added ortho phosphate. To achieve sufficient control of polyphosphate use in fish, new analytical methods are needed where added phosphates can be detected and quantified.

Although only trace levels of the added polyphosphates were detected in the light salted products, our findings suggest that this practice resembles the use of food additives more than technical aids because polyphosphates affect chemical and sensorial quality and stability of light salted cod fillets.

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- The article is the only other paper studying phosphate treatment of light salting of cod fillets that we have found, and therefor very valuable for comparison to our work.

*Lauritzsen, K., Martinsen, G., & Olsen, R. L. (1999). Copper induced lipid oxidation during salting of cod (*Gadus morhua* L.). *Journal of Food Lipids, 6*, 299—315.
- This article describes the lipid oxidation in heavy salted cod and how the natural process in this product is increased by the oxidant copper. This article is important for comparison with our investigations of antioxidative and chaelating effects of phosphate what could reduce lipid oxidation in cod products.

*Esaiassen, M., Østli, J., Joensen, S., Prytz, K., Olsen, J. V., Carlehøg, M., et al. (2005). Brining of cod fillets: Effects of phosphate, salt, glucose, ascorbate and starch on yield, sensory quality and consumers liking. *LWT - Food Science and Technology, 38*, 641—649.
- The article monitors the effects on quality and yields of adding phosphates during brining of cod. This article is relevant for the comparison the methods for addition (brining) and phosphate type with our choice of salting method and phosphate blend.

*Nguyen, M. V., Arason, S., Thorkelsson, G., Gudmundsdottir, A., Thorarinsdottir, K. A., & Vu, B. N. (2012). Effects of added phosphates on lipid stability during salt curing and rehydration of cod (*Gadus morhua*). *Journal of the American Oil Chemists' Society, 90*(3), 317—326.
Like Lauritzsen, et al., 1999 - Like Lauritzsen, et al., 1999, this article studies the lipid stability during heavy salting of cod. Similar to our article, Nguyen et al., 2012 monitors the antioxidative effect of phosphate salted cod products.

*Schröder, U. (2010). Changes in phosphate and water content during processing of salted pacific cod (*Gadus macrocephalus*). *Journal of Aquatic Food Product Technology, 19*, 16—25.
- Schröder (2010) describes the changes in phosphate content during processing of heavy salted Pacific cod which could be comparable to our work on Atlantic cod. The work of Schröder is also relevant because the same phosphate and salting method as in our work are used.