

Effect of Brine Concentration and Brining Time on Quality of Smoked Rainbow Trout Fillets

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ABSTRACT: Frozen rainbow trout fillets were brined in 8.7 or 17.4% sodium chloride solutions for various periods. Brine uptake, brined and cooked pH, cook yield, shear force, total and water-phase salt content, and brined and cooked proximate composition were determined. Fish mince was used for texture (hardness and cohesiveness) and protein solubility (total soluble and myofibrillar proteins) evaluations. Increasing the brine concentration increased fillet weight loss after brining, cook yield, water-phase salt content, shear force, brined fat, brined and cooked ash, brined pH, and brined and cooked moisture.

Keywords: trout, brining concentration, smoked fish quality, composition, myosin solubility

Introduction

OUTBREAKS OF BOTULISM, LISTERIOSIS, AND SALMONELLOSIS resulting from smoked fish have been reported for over 30 y (CDC 1979; Heinitz and Johnson 1998; Heinitz and others 2000). Outgrowth of the causative organisms may be due to insufficient salt and also to temperature abuse during processing, storage, and distribution. To minimize the risk of botulism, water-phase salt in vacuum-packed, hot-smoked fish must be at least 3.5% when no nitrite is added (FDA 1997). Brine concentrations that range from 15 to 24% (w/v) salt or a long brining time are used to achieve at least 3.5% water-phase salt. However, these conventional brining processes provide smoked products that are too salty and unacceptable to most consumers (Nketsia-Tabiri and Sefa-Dedeh 1995).

In addition, it is difficult to achieve uniform sodium chloride concentrations in large batches of fish or in parts of the same fish during brining operations. Salt uptake depends on many factors including species, fish dimension, weight, muscle thickness, muscle characteristics, composition, physiological state, salting method, brine concentration, brining time, and fish-to-salt ratio. Kosak and Toledo (1981) demonstrated a 2-stage brining procedure to minimize differences in water-phase salt between thin and thick sections of mullet, *Mugel cephalus*, after hot-smoking. Mulletts were soaked in 10% sodium chloride for 15 h using 1 lb fish at a fish-to-brine ratio of approximately 1 lb to 1L, followed by soaking in 2% sodium chloride for 24 to 48 h. The authors indicated that variation in weight and fish species might require different brining times to obtain results comparable to those reported in their paper.

Innovative salting techniques, such as vacuum-sealing fish in plastic bags with salt, vacuum-tumbling, and salt injection, have been reported. Goeller and Lanier (2000) studied the effects of vacuum and solute concentration on sorbitol infusion into intact trout muscle and reported that vacuum did not consistently affect sorbitol uptake. Huang (1983) reported that brine injection with and without EDTA and sodium ascorbate produced uniform sodium chloride distribution in mackerel fillets. However, injection followed by dipping in sodium chloride solution was needed in mullet fillets.

Salt promotes dehydration of muscle proteins, altering the balance of protein-protein and protein-water interactions. The

challenge associated with fish curing and smoking is optimal texture development at a water-phase salt content that limits growth of spoilage and pathogenic organisms and complies with regulatory requirements. Therefore, in the context of the hypothesis that increased brine concentration and brining time will promote protein-protein interactions and decrease protein-water interactions, the objectives for this study were to determine: (1) the effect of brine concentration and brining time on protein-water interactions and relate degree of interaction to texture and (2) the effect of brine concentration and brining time on water-phase salt content in smoked products.

Materials and Methods

Fish samples

Seventy-two rainbow trout, *Oncorhynchus mykiss*, weighing 515.0 ± 70.8 g, were harvested, packed on ice, and transported to the West Virginia Univ. Meats Laboratory for processing within 4.5 h. Trout were filleted, vacuum-packed (Ultravacâ, Model UV500; Koch, Kansas City, Mo., U.S.A.) in polyethylene bags, frozen in a blast freezer, and stored for 78 d at -20 °C.

Brining protocol

After thawing overnight at 3 °C, fillets were soaked in separate containers containing 8.7 or 17.4% (w/v) NaCl for 30, 60, 90, or 120 min at 3 °C. The 8.7% concentration was used as a typical industry brine. Three fillets were used in each treatment combination. Light brown sugar was added at 69% (w/w) of the salt required for the brine to counteract salts harsh flavor. A fillet-to-brine ratio of 1 fillet (340 ± 50 g) to 1.4 L of brine was used. Following brining, fillets were placed skin side down on stainless steel expanded metal racks and were drained for 24 h at 3 °C to allow brine equilibration and facilitate pellicle formation during smoking. Fillets were covered with polyethylene wrap 4 h after draining to prevent excessive drying.

Thermal processing

Brined fillets were smoked in a microprocessor-controlled smoke oven (Model CVU-490; Enviro-Pak, Clackamas, Oreg., U.S.A.) to an internal temperature of 65.5 °C and held for 50 min (Federal Regis-

Table 1—Physical, chemical and protein characteristics, and proximate composition, of fillets at various brine concentrations or brining times

Trait	Brine concentration, % (w/v)		Brining time, min				Orthogonal comparison*
	8.7	17.4	30	60	90	120	
Brined pH	6.42 ^b	6.37 ^a	6.39	6.38	6.40	6.40	-
Cooked pH	6.56 ^a	6.55 ^a	6.56	6.56	6.54	6.56	-
Total protein solubility, mg/mL	10.21 ^a	9.36 ^a	10.12	10.12	9.55	9.35	-
Actin solubility, mg/mL	25.41 ^a	30.63 ^b	26.49	28.09	27.67	29.84	L
Brine uptake, %	1.09 ^b	-1.50 ^a	0.15	-0.04	-0.18	-0.76	L
Fillet cook yield, %	63.78 ^a	66.39 ^b	64.98	65.44	64.93	64.99	-
Water-phase salt content, %	4.65 ^a	7.33 ^b	4.79	5.40	6.23	7.53	L
Fillet shear force, g	671 ^a	784 ^b	657	719	786	747	Q
Brined fillets: Moisture, %	75.99 ^b	73.81 ^a	79.55	75.51	74.94	73.60	L
Fat, %	1.05 ^a	1.90 ^b	1.24	0.97	1.69	2.01	-
Protein, %	17.57 ^a	17.79 ^a	17.75	17.65	17.72	17.61	-
Ash, %	2.38 ^a	3.01 ^b	2.58	2.74	2.77	2.69	-
Smoked fillets: Moisture, %	60.73 ^b	59.16 ^a	60.40	60.81	59.79	58.79	L
Fat, %	6.51 ^a	6.00 ^a	6.35	5.96	6.01	6.71	-
Protein, %	27.42 ^a	27.49 ^a	27.72	27.47	27.72	26.89	-
Ash, %	4.11 ^a	5.46 ^b	4.29	4.46	4.91	5.49	L

^{ab}Means within each response with different superscripts are different ($p < 0.05$)

*L: Linear relationship between brining time and response (-3 -1 +1 +3; $p < 0.05$).

Q: Quadratic relationship between brining time and response (+1 -1 -1 +1; $p < 0.05$).

-: Brining time did not affect response ($p > 0.05$).

ter 1995). After cooking, fillets were cooled at ambient temperature for 30 min, at 3 °C for an additional 30 min, and placed in polyethylene bags. Samples were aerobically stored at 3 °C; and analyses were carried out within 24 h. Cooked fillets were used to determine shear force, total and water-phase salt content, pH, water activity, and proximate composition.

Gel processing

One brined fillet from each treatment was skinned and minced in a Cuisinart® food processor for three 15 s intervals, interrupted by two 15 s intervals. Mince was stuffed into two 2.8 cm (ID), 50 mL polypropylene centrifuge tubes. Following stuffing, tubes were placed in a 45 °C water bath until the internal gel temperature reached 42 °C. At this time, water temperature was increased to 72 °C, and gels were removed from the water bath when the internal temperature reached 65.5 °C. After cooking, gels were cooled to ambient temperature before transfer to 4 °C. Gels were used for texture profile analysis, and the remaining raw mince was used for the determination of total soluble proteins, myofibrillar protein solubility, and proximate composition.

Chemical and physical analysis

Proximate composition was determined using AOAC procedures (AOAC 1990). The average raw fish muscle composition (excluding belly flap) was 76.24% moisture, 18.76% protein, 3.69% fat, and 1.30% ash. The remaining raw mince was frozen with liquid N₂ and powdered in a pre-chilled, commercial Waring blender for 1 min for subsequent analyses. Following shear testing, the remaining cooked whole fillets were skinned and chopped in a Cuisinart® food processor for 45 s. Samples were stored at -20 °C until proximate composition determination.

Sample pH was measured using a pH/ion analyzer 350 (Corning Inc., Corning, N.Y., U.S.A.) equipped with a flat-surface combination probe. The pH was measured on brined fillets at the cranial, middle and caudal areas above the lateral line. Values were averaged for each fillet and used as the observation for that sample. For chopped, cooked samples, 5 g was mixed with 50 mL deionized, distilled water before measuring the pH with a general-purpose combination probe.

Individual fillets were weighed before brining, after brining, and

after cooking. Brine uptake was calculated as the difference between the before and after brining weights. Brined and cooked weights were expressed as a percent of the weight before brining. For the fish mince, individual gels were lightly rolled over filter paper to remove excess moisture and weighed to determine cooked weight. Cook yield was calculated as cooked weight expressed as percent of raw weight.

Salt content of cooked fish was determined by the Indicating Strip Method (nr 971.19; AOAC 1990). This method uses the Quantab® chloride titrator (Environmental Test Systems, Inc., Elkhart, Ind., U.S.A.), nr 711196, which has a range of 0.05 to 1.0% NaCl. After 9:1 dilution, the effective NaCl range of the product is 0.5 to 10%. Chopped, cooked sample (10 g) was mixed with 90 mL of hot deionized distilled water, filtered through Whatman nr 41 filter paper, and sodium chloride content was calculated by multiplying the strip reading for sodium by 10. Moisture content was determined by drying a 3 g sample at 103 ± 1 °C for 24 h. Percent water-phase salt was calcu-

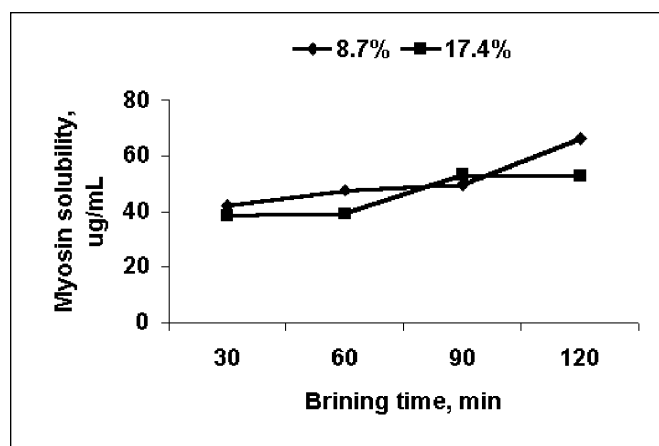


Figure 1—Myosin solubility patterns for 8.7 and 17.4 % (w/v) brine at different brining times. L: linear relationship between brining time and myosin solubility (-3 -1 +1 +3; $p < 0.05$).

lated as (g sodium chloride x 100) / (g sodium chloride + g moisture) (Heinitz and Johnson 1998).

Soluble proteins were extracted by blending 10 g of raw, minced sample with 50 mL of 3.8% (w/v) sodium chloride (ionic strength 0.65) in a Stomacher 400 (Tekmar, Cincinnati, Ohio, U.S.A.) for 30 s. This aqueous-phase salt concentration (3.8%) was calculated based on the estimated salt content in brined fillets (3%) containing 76% moisture content. Amount of myofibrillar proteins was determined by SDS-PAGE as described by Nayak and others (1996).

Gel texture was evaluated according to Texture Profile Analysis (Bourne 1978) using an Instron Universal Testing Machine (Model TM; Instron Corp., Canton, Mass., U.S.A.). Gels were cut into cores (1.91 cm dia, 1.27 cm high). Each core was axially compressed with a 50 kg load cell (Model 1520.50; Daytronic, Miamisburg, Ohio, U.S.A.) at 127 mm/min to 50% of its original height for 2 cycles. A 2.54 x 10 cm section of the dorsal musculature was cut for fillet texture analyses. Shear force was measured with a Warner-Bratzler shear attachment using an Instron Universal Testing Machine as previously described. Each piece was skinned, placed pellicle side down, and sheared at 2.54-cm intervals along the length of the strip.

Experimental design

A 2 x 4 factorial, randomized complete block design with d as block was used. Eight treatment combinations, resulting from 2 brine concentrations (8.7 and 17.4% (w/v) sodium chloride) and 4 brining times (30, 60, 90, and 120 min) were replicated 3 times on 3 separate d. Data were analyzed using the General Linear Model (GLM) procedure of SAS (SAS 1999) to establish main and interaction effects. Brine concentration means were separated using the PDIFF of the lsmeans procedure. Orthogonal polynomials were performed to evaluate the relationship between brining time and response variables at a significance level of $p < 0.05$.

Results and Discussion

pH

High brine concentration (17.4%) decreased ($p < 0.05$) brined pH compared to the low brine concentration (8.7%) (Table 1). The increase in brining time from 30 to 120 min did not affect ($p > 0.05$) brined pH (Table 1). Cooked pH was higher than brined pH; however, brine concentration and brining time did not affect cooked pH ($p > 0.05$).

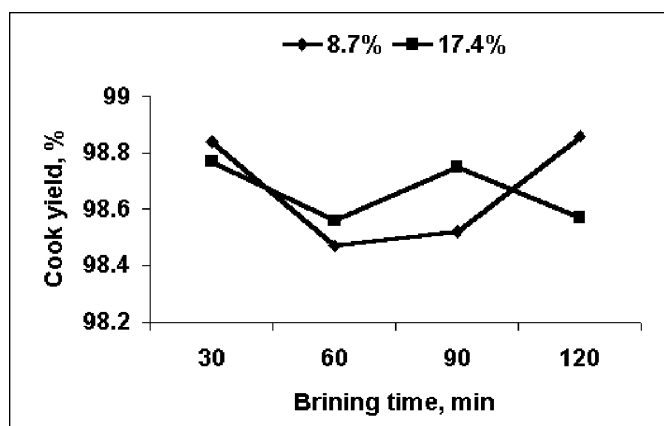


Figure 2—Mince cook yield patterns for 8.7 and 17.4 % (w/v) brine at different brining times. Q: quadratic relationship between brining time and cook yield (+1 -1 -1 +1; $p < 0.05$). -: brining time did not affect cook yield ($p > 0.05$).

Total and myofibrillar protein solubility

Total protein solubility (TPS) did not decrease ($p > 0.05$; Table 1), as brine concentration and brining time increased. Nonetheless, there was a tendency for TPS to decrease while myosin and actin solubility increased. This trend may be caused by the effect of sodium chloride on the salting-out of proteins other than myosin and actin that, in turn, decreased TPS.

In general, and for both brine concentrations, as brining time increased, myosin solubility linearly increased ($p < 0.05$; Figure 1). The lower myosin solubility at a higher brine concentration may be due to the higher ionic strength in raw fish muscle that promotes salting-out of myosin. Sodium chloride increased myosin solubility in the lower brine concentration, particular myosin, perhaps by enhancing electrostatic repulsion (Hamm 1960) and dissociating myosin aggregates (Siegel and Schmidt 1979).

The higher brine concentration increased ($p < 0.05$) actin solubility; and as brining time increased, actin solubility increased linearly ($p < 0.05$; Table 1). An ionic strength up to 0.90 for the 17.4% brine concentration and 120 min brining time did not affect salting-out of actin, even though myofibrillar proteins are generally soluble at 0.3 to 0.6 M sodium chloride (Hultin and others 1995).

Brine uptake

Higher brine concentration caused ($p < 0.05$; Table 1) dehydration of the fillets, due to the difference in solute concentration between the brine solution and inherent muscle water; water migrated from fish muscle to the high brine solution. Soaking in the high brine solution may have caused salting-out of proteins on the fillet surface that led to precipitation and dehydration of proteins and, in turn, exclusion of water molecules. Nketsia-Tabiri and Sefa-Deh (1995) indicated that denaturation of muscle protein facilitated diffusion of water from fish. Decreased brine uptake corresponded with decreased moisture content of brined and cooked fillets. Bligh and Duclos-Rendell (1986) reported that released brine by a vacuum filter-press increased as salt concentration increased from 5 to 28% (w/w) in minced cod. These data are in agreement with our results confirming that water holding capacity of fish flesh decreases with increasing salt content.

Brining time linearly increased fillet dehydration ($p < 0.05$; Table 1). Lautenschlager (1985) reported that diffusion of salt ions through a meat slice was a very slow process and a function of time. Diffusion occurs until sodium chloride concentration of the system

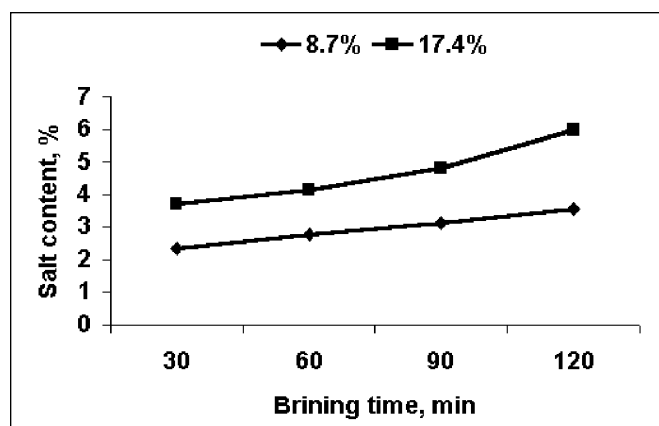


Figure 3—Salt content patterns of smoked fillets for 8.7 and 17.4 % (w/v) brine at different brining times. L: linear relationship between brining time and salt content (-3 -1 +1 +3; $p < 0.05$).

(fish and brine) has equilibrated. A linear relationship in this system indicated that 120 min brining time was not long enough for sodium chloride equilibration.

Cook yield

In the smoked fillets, the 17.4% brine resulted in greater cook yield than the 8.7% brine ($p < 0.05$; Table 1). The high brine concentration decreased moisture content of both brined and cooked fillets. This increased cook yield may be due to less moisture in the high-brined fillets prior to thermal processing; therefore, there was less water to lose during heating. Brining time did not affect ($p > 0.05$) cook yield of smoked fillets (Table 1).

At a high brine concentration, brining time did not affect mince cook yield ($p > 0.05$; Figure 2). In contrast, at the low brine concentration there was a quadratic relationship between brining time and mince cook yield ($p < 0.05$); mince cook yield of the 60 min brining time had the minimum value. Many factors influence mince cook yield. Usually, high protein solubility leads to an increase in cook yield, due to the increased number of soluble proteins present to unfold, leading to protein-protein and protein-water interactions and aggregation in the gelation process (Cheftel and others 1985). However, Cheftel and others (1985) indicated that soluble proteins are not always a prerequisite for gelation. Some aqueous or saline dispersion of insoluble or slightly soluble proteins, such as collagen and some myofibrillar proteins, may also form gels. Hermansson (1978) stated that the slower protein aggregation was relative to partial unfolding of proteins, the better denatured chains orient themselves, and the finer the gel network. Variations in mince cook yield for various treatment combinations were perhaps due to the composition of soluble proteins, a change in protein conformation, and the proportion of soluble and insoluble proteins. In addition, differences in viscosity affect heating rate. In some cases, an increase in aggregation may have occurred so rapidly that the protein did not have adequate time to unfold and form a proper matrix to hold water.

Total and water-phase salt content

Salt preserves fish by dehydrating tissue, increasing water-phase salt, and decreasing water activity. There was a brine concentration by brining time interaction for salt content of smoked products ($p < 0.05$). In general, brining time linearly increased salt content from 30 to 120 min ($p < 0.05$; Figure 3). Salt content of prod-

ucts soaked at the higher brine concentration was greater than of those from the lower brine concentration for the same brining time.

Increasing brine concentration increased ($p < 0.05$) water phase salt content (WPS) of smoked fillets, and brining time linearly increased ($p < 0.05$) WPS (Table 1). Increased WPS corresponded with decreased water activity and increased product ash content.

Texture

Shear force on the fillet was greater for high brine concentration than low brine concentration ($p < 0.05$; Table 1), due to the lower moisture content, which made smoked fillet texture firmer. Texture of fish muscle has been related to water content and lipid content. Dunajski (1979) stated that fish higher in lipid or moisture content were softer in texture. Takahashi (1960) also stated that muscle of species with less water and a higher protein became firmer following cooking. Foegeding and others (1996) indicated that, in fish muscle, relative firmness of cooked fish meats is related primarily to muscle pH and water content.

A quadratic relationship ($p < 0.05$) between shear force and brining time was observed; the 90 min brining time had the maximum shear force (Table 1). Increased shear force from 30 to 90 min corresponded to decreased cooked moisture content. Nketsia-Tabiri and Sefa-Dedeh (1995) reported that salting time influenced sensory hardness of salted-dried tilapia by causing moisture losses and by increasing salt content. Texture development may be initiated during the salting process. Drying provided a 2nd stage of product quality development. According to their suggestion and the findings of Foegeding and others (1996), increased myosin and actin solubility may be involved in shear force increases in the 30 to 90 min brine. A lower shear force at 120 min brine, compared to 90 min, may be due to a higher product fat content (Table 1) and a less than optimum balance of protein-protein and protein-water interactions at the salt concentration achieved by 120 min.

There was an interaction between brine concentration and brining time regarding cooked mince hardness ($p < 0.05$). At the low brine concentration, there was a quadratic relationship between brining time and mince hardness; at 90 min, hardness was maximized ($p < 0.05$; Figure 4). At the high brine concentration, a cubic relationship was evident between mince hardness and brining time ($p < 0.05$).

A brine concentration by brining time interaction was observed

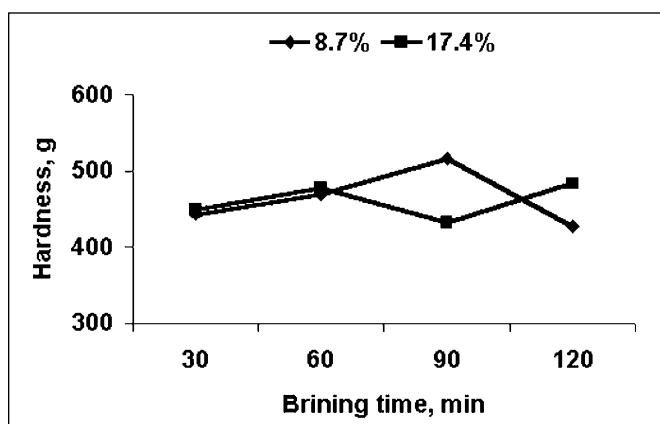


Figure 4—Hardness patterns of cooked mince for 8.7 and 17.4 % (w/v) brine at different brining times. Q: quadratic relationship between brining time and hardness (+1 -1 -1 +1; $p < 0.05$). C: cubic relationship between brining time and hardness (-1 +3 -3 +1; $p < 0.05$).

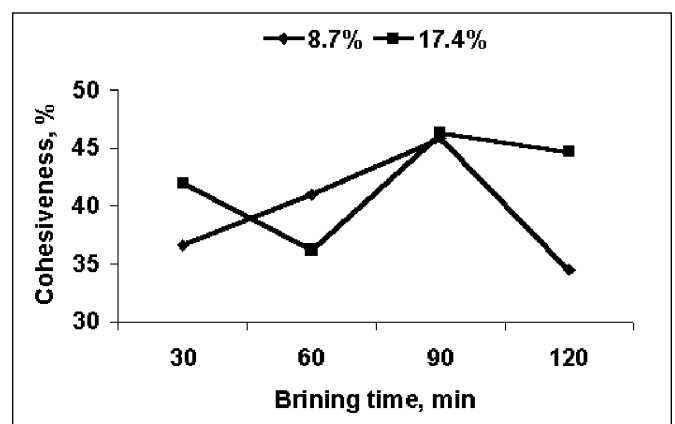


Figure 5—Cohesiveness patterns of cooked mince for 8.7 and 17.4 % (w/v) brine at different brining times. Q: quadratic relationship between brining time and cohesiveness (+1 -1 -1 +1; $p < 0.05$). C: cubic relationship between brining time and cohesiveness (-1 +3 -3 +1; $p < 0.05$).

for cooked mince cohesiveness ($p < 0.05$). At the low brine concentration, there was a quadratic relationship between brining time and cohesiveness, with maximum cohesiveness at 90 min ($p < 0.05$; Figure 5). At the high brine concentration, a cubic relationship was observed between brining time and mince cohesiveness ($p < 0.05$).

At 8.7% brine, increases in hardness and cohesiveness followed the same pattern with regard to brining time, and generally corresponded to increased myosin solubility. However, the highest myosin solubility at this brine concentration did not produce maximum hardness and cohesiveness. At 17.4% brine, hardness and cohesiveness did not relate to increased myosin solubility. Factors other than protein concentration are important to texture development at 17.4% brine. Environmental factors, such as pH, ionic strength and protein conformation, which alters the balance of these interactions, may influence hardness and cohesiveness of cooked mince. Hastings and others (1985) reported decreased myosin transition temperature and endothermic heat when herring was soaked in 14% salt. Increased myosin susceptibility to thermal denaturation may rapidly increase aggregation of protein during the gelation process, thus affecting texture development at 17.4% brine.

Proximate composition

Moisture content of brined fillets (Table 1) was less than that of raw fillets. Increasing brine concentration decreased ($p < 0.05$) moisture content of both brined and cooked fillets (Table 1). Brining time linearly decreased ($p < 0.05$) moisture content of both brined and cooked fillets (Table 1). Nketsia-Tabiri and Sefa-Dedeh (1995) concluded that salting time was an important processing variable influencing product moisture content. Fish salting leads to a reduction in moisture that has been attributed to the denaturing effect of salt on fish proteins.

Fat content of brined fillets increased ($p < 0.05$) with increased brine concentration (Table 1). However, fat content was not different ($p > 0.05$) after cooking (Table 1). Increased salt promoted protein-protein interactions and decreased protein-water interactions that, in turn, decreased the ability of cooked muscle to hold fat. A longer brining time did not affect ($p > 0.05$) brined and cooked fat content (Table 1).

Nketsia-Tabiri and Sefa-Dedeh (1995) reported that salting of tilapia caused losses in some macromolecules, including proteins. Protein content of raw, salted, minced cod decreased inversely with increased salt addition (Bligh and Duclos-Rendell 1986). In the present study, brine concentration and brining time did not affect ($p > 0.05$) brined and cooked protein content (Table 1). This discrepancy may be due to the difference between intact fillet and minced muscle.

Ash content of brined fillets (Table 1) was much higher than that of raw fillet. Increased brine concentration increased ($p < 0.05$) brined and cooked product ash contents (Table 1). Water losses associated with brining resulted in increased ash content. Brining time did not affect ($p > 0.05$) ash content of brined fillets, but it linearly increased ($p < 0.05$) ash content of cooked products (Table 1). This may be due to the differential effect on protein setting associated with time. Increased cooked ash content corresponded to increased salt content in smoked products.

ACCORDING TO TEXTURE AND WATER-PHASE SALT DATA, AN 8.7% brine concentration and a 90-min brining time is the appropriate combination for texture development and for addressing water-phase salt content regulations (= 3.5%) of Food and Drug Administration. However, this brine concentration and brining time combination may vary depending on the quality and size of the raw material, and brining protocol, especially brining time, should be adjusted accordingly.

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