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Seasonal effects on the physicochemical characteristics of fish sauce made from capelin (*Mallotus villosus*)

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Abstract

Fresh capelin (*Mallotus villosus*) was harvested from the North Atlantic during both summer and winter fishing seasons. Reaction conditions for fish sauce processing were optimized with respect to temperature, salt concentration and reaction time, using a response surface methodology (RSM) experimental design. Whole capelin was minced and samples were ground with increasing salt concentrations. RSM optimizations were conducted, ranging from 5% to 30% salt, and incubating at 5° intervals from 0 to 65 °C. Autolytic activity was estimated by extracting the liquid formed by the mixture with trichloroacetic acid and estimating protein content by the Lowry method. Samples for fish sauce production were then prepared under optimized conditions by mixing ground capelin with 10% salt and incubating at 50 °C for up to 270 days for the summer capelin and up to 360 days for the winter capelin. Samples were collected at regular intervals and analyzed for liquid yield, moisture, protein, soluble solids, specific gravity, pH, colour and amino acid content. Kjeldahl protein content in the fish sauce from summer capelin was 2.03% after 250 days of fermentation and twice as high as that in winter capelin fish sauce. Moisture content and pH were lower in the summer capelin fish sauce, but Brix and density were higher than those in fish sauce from winter capelin. Brown colour formation was very rapid in the summer capelin fish sauce but slow in the winter capelin fish sauce. Summer capelin may be successfully utilized for the production of fish sauce without added enzymes.

Keywords: Fish sauce; Capelin (*Mallotus villosus*); Fish processing; Fermentation; Seasonal changes

1. Introduction

Capelin (*Mallotus villosus*) is a small pelagic fish mainly found in the North Atlantic and Arctic oceans. It is harvested during the winter and summer seasons with an annual catch, in Icelandic waters, of approximately one million tons (ICIMF, 2005). More than 95% has been used for the production of fishmeal and oil, leaving only 5% for human consumption. There is a general and growing interest in increasing the percentage of pelagic fish that is used for human consumption. The justification is both economic and environmental. A small portion of the female capelin catch has been used for human consumption during the spawning season. Female capelin, with a roe content of 15–20% of the body weight, is generally frozen whole with the roes, and marketed for human consumption. The remaining part of the stock, females with less roe content, and all male capelin has mainly been used for fish meal and oil production. Furthermore, after the roe content reaches 50% of the body weight, the roes are separated from the fish, collected and sold separately. The remaining body portion of the female is then, together with the male, used for fish meal and oil production. The life cycle of capelin is highly seasonal and it feeds almost exclusively during the summer season. The feed consist mostly of various planktonic organisms, e.g. some Calanus species such as *Calanus*

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finmarchicus (Vilhjálmsson, 1997). During this time, belly bursting is common during and after harvesting. Although belly bursting has not been directly associated with feeding, it only occurs during the heavy feeding season (Gildberg, 1978). Capelin harvested during the summer has only been utilized for oil and fish meal production. A possible avenue for utilizing capelin for human consumption is by processing it into fish sauce, which is popular in many regions of the world, especially Asia, but has been used by many other cultures, including the ancient Greeks (Kelaiditis, 1949).

Fish sauce is a clear brown ambient liquid with a characteristic flavour and aroma. It is one of the most popular fermented fish products in south east-Asia and constitutes part of the daily diet for a very large population. Fish sauce is mainly used to replace salt in cooking but also to bring characteristic flavour to the food. It is popular as a condiment on rice dishes to give a pleasant flavour (Saisithi, Kasemsarn, Liston, & Dollar, 1966; Adams, Cooke, & Rattagool, 1985; Beddows, 1985). The nutritional value is limited due to the high salt content, but daily consumption of fish sauce renders it one of the main protein sources in some regions where carbohydrates are the fundamental part of the diet (Amano, 1962).

The traditional process for the manufacturing of fish sauce begins with mixing salt together with fish. Small pelagic fish such as anchovies (*Stolephorus* ssp.) have been popular (Saisithi et al., 1966; Adams et al., 1985; Beddows, 1985; Gildberg, 2001; Saisithi, 1994; Lopetcharat, Choi, Park, & Daeschel, 2001). Salt content in the mixture is 20–30%. The fish-salt mixture is stored at ambient tropical temperatures for 6–12 months in large concrete tanks that are usually built into the ground away from direct sunlight. With time, due to the action of digestive enzymes, as well as some bacterial activity at the initial steps of the process, a water-soluble protein-rich fraction is formed, which is referred to as fish sauce. The final processing steps include filtration, followed by bottling or further fermentation (Saisithi et al., 1966; Adams et al., 1985; Beddows, 1985; Saisithi, 1994; Lopetcharat et al., 2001). The quality of fish sauce is rated by total nitrogen content and colour as well as taste and aroma. In Thailand, fish sauce with total nitrogen above 20 g/l (2.0%) is classified as grade 1 but as grade 2 if it contains between 15 and 20 g/l (1.5–2.0%) (Lopetcharat et al., 2001). If the total nitrogen content is below 15 g/l it is forbidden to label the product as pure fish sauce and it must be labelled as diluted or mixed product (Brillantes, 1999). Because of the high salt content ($\geqslant$25% NaCl), salt taste usually overcomes other taste factors (Beddows, Ardeshir, & Daud, 1980).

Fish sauce varies by aroma, taste and colour between different locations and each location uses a specific name, e.g. *nampla* in Thailand, *patis* in the Philippines and *nuoc-mam* in Cambodia and Vietnam (Lopetcharat et al., 2001). Thailand is one of the leading countries in fish sauce production and exports large volumes to the US, Canada, Europe, Australia and Japan. The largest markets are in the US, Japan and Australia. Thailand exported fish sauce worth more than 13 million US$ in 1998 (Brillantes, 1999).

Earlier work on processing fish sauce from capelin was focussed on the utilization of the remaining portion of females after separation from roes or what has been referred to as spent females in the fish processing industry and on utilization of the male capelin during the spawning season in the winter. All known efforts have reported the requirement for added enzymes. Raksakulthai and Haard (1992) were able to produce fish sauce from male capelin with acceptable quality. The initial salt concentration was 25% and 2.5% of squid (*Illex illecebrosus*) pancreas was added and used as an enzyme source. The mixture was fermented for 6 months at 20–25 °C. However, the fish sauce produced without the pancreatic enzymes was unacceptable with respect to protein content. In a study by Gildberg (2001), male capelin was supplemented with 5–10% enzyme-rich cod pyloric caecum. The fish sauce was fermented at 21 °C with an initial salt concentration of 26–27%. Protein recovery was approximately 60% after fermentation for 6 months.

The intensely-feeding summer capelin contains more digestive enzymes than does capelin in other seasons (Gildberg, 1978). Therefore, capelin harvested during the summer should be more suitable for fish sauce processing without supplementation with proteolytic enzymes. The high salt content in fish sauce has been of concern, especially in regions where fish sauce is a significant protein source in the diet. It would therefore be highly beneficial to produce fish sauce with lower salt contents.

The objectives of this work were to investigate physicochemical characteristics of fish sauces made from capelin (*M. villosus*) harvested at two distinctive seasons and to determine optimum conditions for processing.

2. Materials and methods

2.1. Capelin for fish sauce processing

Spawning winter capelin (*M. villosus*) was caught north east of Iceland (fishing zone 363) in February 2000 and was transferred to the Icelandic Fisheries laboratories (IFL) in ice, directly from the landing port. Feeding summer capelin was harvested south east of Iceland (fishing zone 713) in June 2000 and brought to IFL in ice directly from the landing port. All samples were caught by commercial fishing vessels using commercial fishing methods (capelin purse seine). The male winter capelin was separated from the bulk and used as a raw material in fish sauce; both female and male capelin from the summer season were used. Winter capelin was washed to reduce surface contamination prior to mincing; however, it was not possible to wash the summer capelin because of the high autolytic activity. Although kept in ice and brought to shore within 48 h after harvesting, the fish appeared to have very soft flesh.

2.2. Optimization of protease activity

There are three factors that are believed to have the most effect on protease activity in fish sauce production:

temperature, salt concentration and fermentation time (Adams et al., 1985; Beddows et al., 1979). Optimization of these factors for protease activity was conducted by using a central composite design (CCD), where protease activity (Y) was estimated at various levels, for the 3 variables salt concentration (X_1), temperature (X_2) and time (X_3). Optimization was obtained by response surface methodology (RSM) with the aid of Systat 7.0 (SPSS Inc., Chicago, IL). Capelin from both groups was washed and minced through a 6 mm screen, using a mincing machine (type GKM 1.1/1.3 kW, Alexanderwerk AG, Remscheid, Germany) at maximum speed. Minced capelin was mixed with salt at various concentrations from 5% to 30% at 5% intervals and incubated in a heating bath at approximately 5° intervals from 0 to 65 °C. Approximately 40 g samples were deposited on Petri dishes and placed in a heat bath. These were collected after incubation from 30 min up to 150 min at approximately 30 min intervals. At the end of each incubation time period, the reaction was stopped by using 20 ml of 10% TCA. The TCA mixture was homogenized and centrifuged in Sorvall RC-B refrigerated super speed centrifuge (Du Pont Company, Wilmington, DL) at 20,000*g* for 20 min. Soluble nitrogen compounds captured in 10% TCA were estimated by the Lowry method using bovine serum albumin as a standard.

2.3. Preparation of fish sauce

The fish was minced in a mincing machine (type GKM 1.1/1.3 kW) (Alexanderwerk AG, Remscheid, Germany) equipped with a 6 mm screen at maximum speed. Food grade salt was mixed with the mince to reach a salt concentration of 10% (w/w) within 72 h of landing. The fish–salt mixture was kept at 10 °C for 5 h and mixed well, before and after, to ensure a uniform salt distribution. One kilogramme samples of the fish–salt mixture were weighed into plastic containers (1.8 l). The containers were closed with plastic lids and placed in an environmental chamber maintained at 50 ± 1 °C for storage. After each storage period, the fish sauce was pressed from the mince through cheesecloth using 60 kg pressure for 2 h. Fish sauce yield was measured as the ratio of original fish–salt mass in the container to the weight of liquid after pressing. Salt concentration and fermentation temperature were determined with respect to results from the optimization experiments previously conducted.

2.4. Sampling

Unripened fish sauce was obtained by pressing the liquid through two steps of filtration, first through a Whatman # 113 and later through Whatman # 1 (Whatman International Ltd., Maidstone, England). This rinsing process generated a clear brown ambient liquid, which was referred to as unripened fish sauce. All samples were processed in duplicate. Samples were collected as follows: winter-fish sauce, days: 5, 10, 15, 20, 40, 60, 80, 100, 340 and 360; summer-fish sauce, days: 5, 10, 15, 20, 40, 60, 80, 120, 250 and 270.

2.5. Chemical analysis

Total nitrogen content of unripened fish sauce was determined by the Kjeldahl method (ISO, 1979) using Digestion System 40 (1026 Digester, Tecator, Hoganas, Sweden). Crude protein was estimated by multiplying total *N* by 6.25. Salt (NaCl) content was determined according to the Volhard method (AOCS, 1990). Moisture content (g/100 g) was determined by weight difference after heating the sample at 103 ± 2 °C for 4 h (ISO, 1983). Fat content in the raw material was determined by Soxhlet extraction (AOCS, 1998) using absolute ether (Merck KGaA, Darmstadt, Germany). Soluble solids (°Brix) were determined by refractive index with a refractometer (Carl Zeiss IMT Corp. Brighton, Michigan, USA). Specific gravity of unripened fish sauce was measured volumetrically by accurately weighing a specific volume of liquid using a Mettler AE200 (Mettler Toledo, New York, USA) scale. Ten millilitre of unripened fish sauce were pipetted into a beaker and weighed at room temperature; the average of three measurements was used as the weighed value. Specific gravity was calculated as grammes per litre of soluble solids. Browning was determined by mixing 5 ml of fish sauce with 5 ml of 95% ethanol (Merck KGaA, Darmstadt, Germany) prior to centrifugation for 20 min at 2000 rpm in a Sorvall RC-5B Refrigerated super speed centrifuge (Du Pont Company, Wilmington, Delaware) before filtration through Whatman #1. Absorbance was measured at 420 nm (Varian, DMS UV–Visible Spectrophotometer, Mulgarave, Australia). Acidity was measured with a pH meter, Orion combination electrode (model 290A, Orion, Boston). All samples, both raw material and fish sauce, were measured at room temperature.

Free amino acid content was measured using a chromatography system consisting of a Hewlett Packard (HP) 1050 series gradient solvent pumping system, HP auto sampler, Varian 9070 fluorescence detector, Croco-cil column heater and HP Chemstation data handling system. The column (150 × 4.6 mm) (Hichrom, Reading, UK) was packed with 3 μm Spherisorb ODS-2 material and a suitable guard column of the same packing material. Samples (2–3 g) were accurately weighed into centrifuge tubes and 20 ml of hydrochloric acid (0.1 N) and 1 ml of an internal standard (norvaline) were added. After homogenization for 1 min, the samples were centrifuged at 15,000 rev/min for 20 min using a tabletop centrifuge (WIFUG, Bradford, England). A one ml aliquot of the supernatant was then diluted to 25 ml with distilled water before analysis. OPA (*o*-phthalaldehyde) reagent was used for sample dedication, in the auto sampler. Standards were run before samples and all samples were measured in triplicate. The amino acid derivatives were separated by reversed phase chromatography using a binary gradient at 25 °C.

2.6. Statistical analysis

The data obtained from optimization of protease activity were analyzed by response surface methodology, using Systat 7.0 (SPSS Inc., Chicago, IL) and optimized with Design-Expert (Stat-Ease Inc, Minneapolis, MN). Significant differences between groups in the main experiment were determined by the Student's *t*-test using Microsoft Excel 8.00 (Microsoft Inc., Redmond, Wash., USA). The significance level was $p \leqslant 0.05$.

3. Results and discussion

3.1. Optimization of proteolytic activity

In order to increase the protein content and optimize processing conditions with respect to reaction temperature and time for fish sauce production, the effects of salt concentration, temperature and time were optimized, in small scale test tube experiments, with respect to soluble protein formation by running experiments according to a central composite experimental design (Hastings & Currall, 1989). The relationship between salt concentration, processing temperature and time was best described by a quadratic equation ($p > 0.05$) ($r^2 = 0.9437$) and the response surface optimization showed that 10.2% of salt and fermentation at 50.9 °C resulted in the highest proteolytic activity in summer capelin when protein autolysis was analyzed by the Lowry method (Fig. 1). It was evident from the response optimization that the reaction was very dependent on temperature, with reaction rate increasing until a maximum was reached. The dependency on salt concentration was also evident but was not as pronounced as for the temperature, with a slow decrease in the reaction rate with increased salt concentration within the limits tested. One of the objectives of this work was to study the feasibility of reducing salt content in fish sauce driven by nutritional considerations. The results indicate that this is not only feasible but beneficial with respect to reaction rate and processing time. Similar results were obtained for the winter capelin. The results indicated that temperature was the most effective factor in the protein breakdown and that increased salt reduces the reaction rate. The test tube experiments did not indicate time to be a significant factor in the autolysis but the optimization experiments were only conducted for up to 120 min and were mainly designed to optimize temperature and salt content. The effect of time is better demonstrated in the main experiments discussed below.

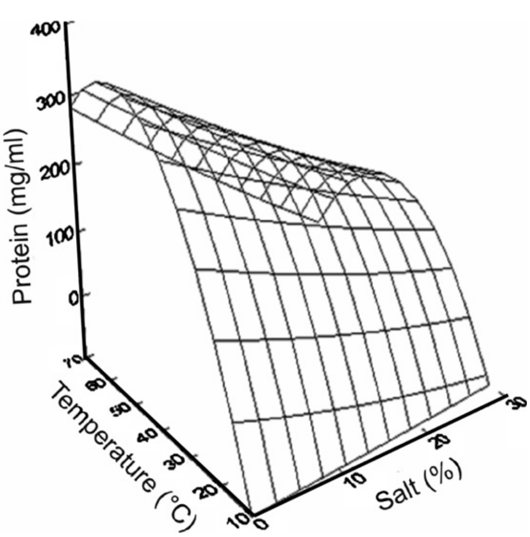

Fig. 1. Response surface plot with a quadratic prediction ($r^2 = 0.9437$) of the autolytic activity in capelin indicated by Lowry protein as a function of temperature and salt concentration.

3.2. Physicochemical properties

3.2.1. Liquid yield

The summer capelin gave higher ($p < 0.05$) liquid yield during fish sauce processing than did the winter capelin (Fig. 2). After 5 days of fermentation, the liquid yield of summer-fish sauce was 50.90 ± 5.80% and this increased to 57.01 ± 0.01% after 20days but then continued to be similar throughout the fermentation time, ending at 59.28 ± 4.86% after 270 d. The liquid yield of the winter fish sauce was lower ($p < 0.05$) or 42.80 ± 1.56% after 5 days of fermentation and remained similar through out the fermentation period, levelling at 42.43 ± 0.52% after 360 d. The results were similar to the observations of Lopetcharat and Park (2002) for Pacific Whiting, where fish samples had also been ground prior to salt addition, increasing the osmotic extraction of liquid from the samples by salt. Beddows (1985) reported a maximum extraction (about 70%) from whole anchovies after 125 days of fermentation of whole fish during Budu extraction. Lopetcharat and Park (2002) concluded that increase in fermentation temperature from 30 to 50 °C and grinding helped to extract more liquid in less time. The reported total liquid yield is naturally highly dependent on the pressure applied to the sample. Lopetcharat and Park (2002) used a laboratory hydraulic press at 1500 psi where our samples were only subjected to a static load of approximately 850 psi.

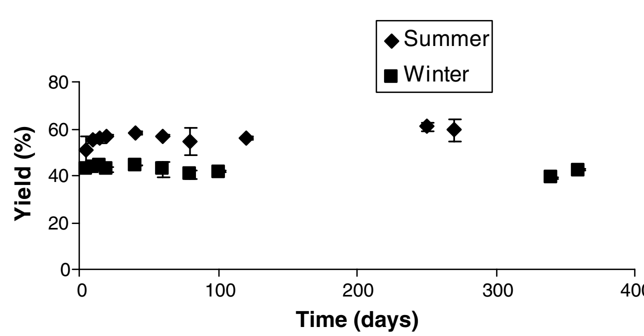

Fig. 2. Yields of fish sauce from summer (◆) and winter (■) capelin as a function of time.

3.2.2. Moisture

The moisture content in fish sauce from summer capelin was lower ($p < 0.05$) than that in fish sauce from winter capelin. The moisture content in the summer fish sauce was $74.82 \pm 0.05\%$ after 5 days of fermentation and this decreased to $72.75 \pm 0.21\%$ after 270 days of fermentation, compared to $83.35 \pm 0.04\%$ after 5 days of fermentation in the winter-fish sauce which decreased to $81.8 \pm 0.28\%$ after 370 days of fermentation (Fig. 3). The difference might be due to more solids in the liquid phase resulting from increased protein hydrolysis in the summer capelin but may also have been partly from the difference in moisture and fat ratio in the raw material. The fat content in the summer capelin was 16.3%, which was significantly higher than the 9.8% measured in the winter capelin. Furthermore, the summer capelin had much lower moisture content at 68.9%, compared to 75.2% in the winter capelin. An inverse relationship between moisture and lipid content has been observed in capelin (Bragadóttir, Palmadóttir, & Kristbergsson, 2002). Our results are in agreement with previously reported values by Bersamin and Napugan (1961) who found 62–74% moisture content in commercially produced patis.

3.2.3. Total nitrogen

One of the most important quality factors for fish sauce is the total nitrogen content in the liquid and regulatory standards for quality have been based on this value. It is the only objective index used to classify the quality of the Thai fish sauce nampla (Wilaipan, 1990). High quality nampla and patis must have 1.5% or higher total nitrogen content, based on the Kjeldahl method (Wilaipan, 1990; Lopetcharat et al., 2001; Lopetcharat & Park, 2002). The total nitrogen content in the summer-fish sauce exceeded the minimum value for second grade fish sauce (1.5–2.0%) set by the Thai Industrial Standards Institute after only 5 days of fermentation (1.64%) and the first grade limit (2%) after 250 d. The total nitrogen content was substantially higher ($p < 0.05$) in the summer fish sauce than in the winter fish sauce (Fig. 4). Nitrogen content in the sum-

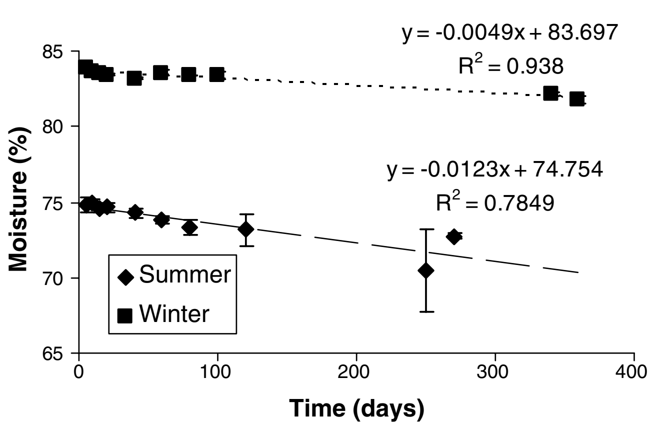

Fig. 3. Moisture contents of summer (◆) and winter (■) capelin fish sauce as a function of time.

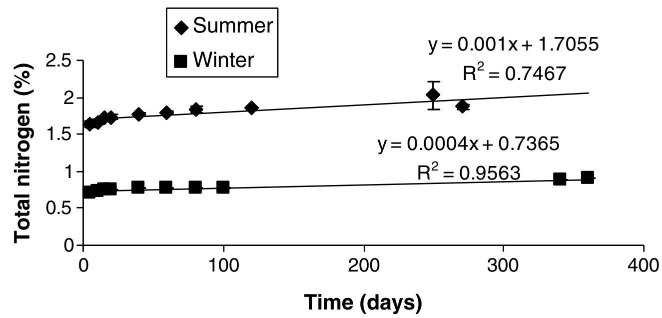

Fig. 4. Total nitrogen contents of summer (◆) and winter (■) fish sauce as a function of time.

mer fish sauce was $2.03 \pm 0.19\%$ after 250 days but the winter fish sauce contained only $0.90 \pm 0.02\%$ of nitrogen after 360 days of fermentation. The protein content of the summer capelin fish sauce was sufficient for the regulatory requirements placed on commercial anchovy fish sauce by the Thai government. The total nitrogen content reported for different types of fish sauce has been variable and may be based on the raw material or the processing conditions. Beddows et al. (1979) found 1.77% total nitrogen in Budu after 154 days of fermentation during a commercial production of the fish sauce. Saisithi et al. (1966) reported 1.8% of total nitrogen in nampla after 9 months of fermentation. The extraction of total nitrogen from summer capelin was slightly faster than that reported for Pacific whiting where comparable processing conditions were used. Lopetcharat and Park (2002) reported 1.57% (15.7 g N/l) total protein for Pacific whiting, which had been mixed with salt after grinding and fermented for 10 d. They concluded that the fast fermentation of fish sauce from Pacific Whiting may have been caused by the combined effects of high enzymatic activity and grinding. The same applies to the summer capelin fish sauce.

During the fermentation process, nitrogen content in the liquid phase increases, due to a breakdown of the fish proteins. The increase in nitrogen compounds during the initial steps of fermentation has been connected to osmosis that leads to the replacement of water and soluble nitrogen compounds from the fish cells (Beddows, 1985). The conversion of insoluble fish proteins into soluble form was estimated by the following equation:

$$\%N\ conversion = \frac{soluble - N\% * volume\ of\ fish\ sauce\ obtained}{N\%\ in\ fish * volume\ of\ fish\ used} * 100$$

After 270 days of fermentation, 54.3% conversion was obtained in the summer-fish sauce, compared to only 18.2% conversion in the winter-fish sauce, after 360 days. These results indicate that the summer capelin may be a suitable raw material for fish sauce processing without the addition of enzymes. Capelin caught during the summer season had been feeding intensely. It has been shown that summer capelin has a high production of digestive enzymes, which accelerate post-mortem autolysis (Gildberg, 1978).

3.2.4. TCA-soluble nitrogen

TCA-soluble nitrogen content in fish sauce from summer capelin was higher ($p < 0.05$) than that in the winter capelin. TCA-soluble nitrogen, measured in summer fish sauce increased from $0.59 \pm 0.01\%$ to $0.64 \pm 0.05\%$ for the first 15 days of fermentation (Fig. 4). Similar patterns but lower values were observed for the initial fermentation of fish sauce from winter capelin, with values ranging from $0.27 \pm 0.02\%$ to $0.37 \pm 0.04\%$. The changes in TCA-soluble nitrogen content were most likely due to diffusion of low molecular weight nitrogen compounds from the fish muscle into the surrounding liquid. Diffusion of non-protein nitrogen compounds into the brine slowly decreased with time to $0.39 \pm 0.01\%$ in the summer fish sauce but a slow increase was observed in the winter-fish sauce to $0.37 \pm 0.01\%$, reaching similar levels to those in the summer capelin fish sauce. This may have been caused by differences in the raw material. Gudmundsdóttir (1995) demonstrated that digestive enzymes were the main cause of the increase in TCA-soluble nitrogen content during a fermentation process for salted herring. Our results indicated that summer capelin might contain higher quantities of digestive enzymes than winter capelin. Tungkawachara, Park, and Choi (2003) demonstrated that protease-like cathepsins were most active during the first month of fermentation. The measurement of TCA-soluble nitrogen indicated that the protease activity in summer capelin was significantly higher than that in the winter capelin, further rendering the summer capelin more suitable as a raw material in fish sauce processing. According to Kiesvaara (1975), TCA-soluble nitrogen content may be a good indicator for monitoring a ripening process when fish is left in contact with salt for some time. Total quantities of soluble nitrogenous compounds, such as peptides and free amino acids, diffuse from the fish muscle into the liquid, mainly due to enzymatic activity. Pérez-Villareal and Pozo (1992) also suggested that non-protein nitrogen compounds could be used as an indicator of a ripening process because of the steady diffusion into the liquid phase.

3.2.5. Soluble solids

The formation of soluble solids (°Brix) in the summer fish sauce was higher ($p < 0.05$) and about twice as fast as in the winter-fish sauce. Soluble solids ranged from 30.77 ± 0.24 to 34.34 ± 0.22 in the fish sauce from summer capelin and from 19.20 ± 0.28 to 22.40 ± 0.05 in fish sauce from winter capelin (Fig. 5). It was suggested by Beddows et al. (1979) that the soluble solids in fish sauce are most likely mainly due to free amino acids and small peptides that are released during protein hydrolysis. A reasonable correlation was observed between the total-nitrogen content and °Brix in the summer-fish sauce ($r^2 = 0.814$), but it was poorer ($r^2 = 0.582$) for winter-fish sauce. This indicates that the total nitrogen might possibly be monitored by measuring °Brix with a refractometer, especially when the process is relatively expedient as in the summer capelin fish sauce. Soluble solids in ground whole fish Pacific whiting fish sauce increased from 32% at day 0 to almost 40% after 60 days of fermentation (Lopetcharat & Park, 2002) which is slightly higher than what was measured in the capelin fish sauce.

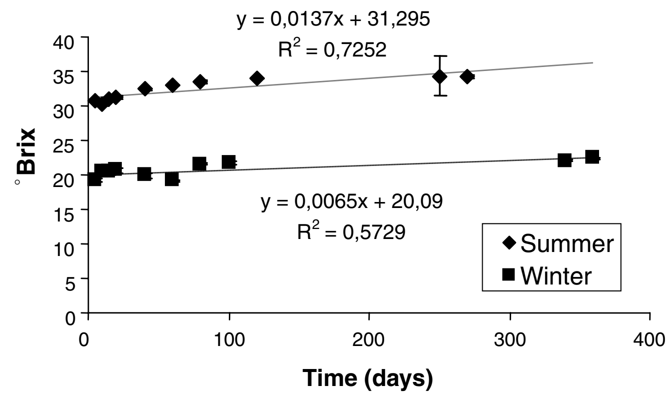

Fig. 5. Soluble solids contents (% Brix) of fish sauce from summer (◆) and winter (■) capelin during fermentation.

3.2.6. Relative gravity

Like many other factors, the relative specific gravity was higher ($p < 0.05$) in the summer-fish sauce at 1.139 ± 0.000 mg/ml and increased to 1.149 ± 0.001 mg/ml after 270 days of fermentation, than in the winter-fish sauce where it was 1.096 ± 0.000 mg/ml with an increase to 1.108 ± 0.001 mg/ml after 360 days of fermentation (Fig. 6). This was slightly lower than the 1.21 mg/ml reported for commercial fish sauce made from anchovies and fish sauce made from Pacific whiting (Lopetcharat & Park, 2002).

3.2.7. pH

No difference ($p < 0.05$) was found between the acidities of fish sauce from winter and summer capelin. The pH of the fish sauce from summer capelin was 5.90 ± 0.00 after 5 days fermentation and it increased to 6.30 ± 0.01 after 15 days but fell gradually after that during fermentation to 5.72 ± 0.03 after 270 days. Similarly, the pH of the win-

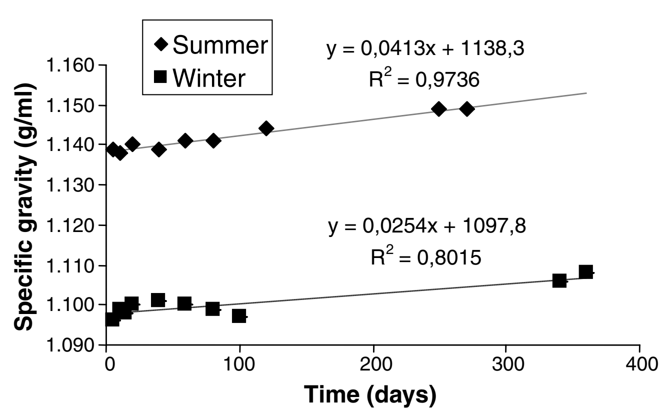

Fig. 6. Specific gravities of fish sauce from summer (◆) and winter (■) capelin during fermentation.

ter fish sauce was 5.98 ± 0.01 after 5 days of fermentation but increased for the first 50 days of fermentation to 6.50 ± 0.0 and fell gradually after that to 5.69 ± 0.01 after 360 days of fermentation. The initial peak during the first days of fermentation was delayed in the winter fish sauce but the pH was not significantly different after approximately 200 days of fermentation in the two types of capelin fish sauce. A greater drop might have been expected in the summer fish sauce than in the winter fish sauce in light of the results of Beddows (1985) who found that an increase with time, in both free amino acids and large polypeptides, reduced pH during fermentation of fish sauce. The fish sauce from summer capelin had a slightly lower pH during fermentation, which may indicate that the rate of hydrolysis of the fish proteins to peptides and free amino acids was faster in the summer fish sauce. The measured pH values in this study do not indicate that pH should be used to distinguish between high and low processing rates during fish sauce processing. However, the observed pH values were slightly higher than the 5.48 ± 0.32 analyzed in commercial fish sauce samples by Lopetcharat and Park (2002); they reported that most commercial nampla is seasoned during ripening with food grade additives, such as citric acid and sorbic acid, to lower pH and to adjust colour. However, the pH of the fermented capelin fish sauce was lower than the 6.0 ± 0.7 average values for pH in commercial fish sauce reported by Mizutani, Kimizuka, Ruddle, and Ishige (1992).

3.2.8. Salt

One of the goals of this research was to produce fish sauce with lower salt content than is generally found in commercially produced fish sauce. Salt content in the summer fish sauce was 13.8 ± 0.21% and this increased gradually to 14.3 ± 0.26% after 270 days of fermentation. This was slightly higher ($p < 0.05$) than the 11.3 ± 0.03% in the winter fish sauce after 5 days of fermentation, which similarly increased gradually to 12.2 ± 0.09% after 370 days of fermentation (Fig. 7). The salt content observed in the summer fish sauce after 270 days of fermentation was about half of what is generally found in commercial fish sauce. Mizutani et al. (1992) found 25.9 ± 3.7 g/dl of salt in commercial fish sauce samples from south east-Asia. The low salt content may have favourable effects on some important factors in the final product as well as on processing. These include an increased rate of protein breakdown, increase in nutritional value due to the lower salt content of the product, and an acceleration of the process without added enzymes. The activation of proteases in fish has been shown to be highly dependent on salt concentration in the environment (Orejana & Liston, 1981). If the initial salt content were too high, it would not only retard enzymatic activity, but also cause the fish tissue to harden too much due to an increase in osmotic pressure, thus further inhibiting attack by proteolytic enzymes (Beddows, 1985). According to (Orejana & Liston, 1981), pepsin is particularly sensitive to high salt concentration and is inhibited

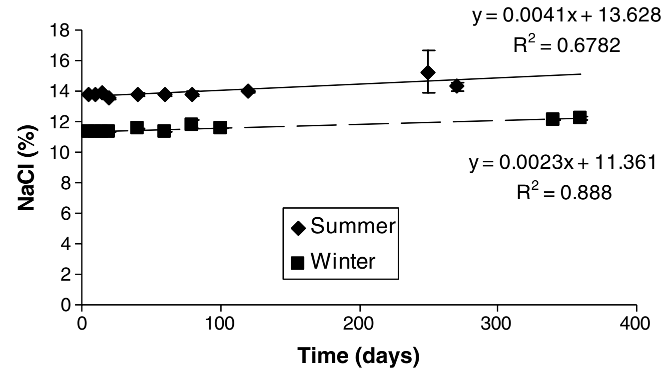

Fig. 7. Salt (NaCl) contents (%) of fish sauce from summer (◆) and winter (■) capelin during fermentation.

at 5% salt. The initial salt content in the fish-salt mass in our study was 10% (w/w). Therefore, it is unlikely that pepsins played a major role in the fermentation process of capelin fish sauce. However, it has been shown that the final quality of fish sauce is not very dependent on the type of enzymes active during the fermentation process (Gildberg, Espejo-Hermes, & Magno-Orejana, 1984).

3.2.9. Colour

The colour formation in the summer fish sauce was very rapid and was similar to that of commercial nampla after 250 days of fermentation without any additives used for increased colour development (Fig. 8) but the amber colour development in the winter fish sauce was very slow. Colour of fish sauce is often determined subjectively (Lopetcharat & Park, 2002) but the most common objective method for determining colour of fish sauce is to measure absorbance at 420 nm. The absorbance after 5 d of fermentation was 0.76 and increased rapidly for the first 40 days to 2.05; after that the increase in absorbance was more gradual and levelled of after 80 days at 2.73, reaching 2.90 after 270 d of fermentation (Fig. 9). An acceptable dark brown colour was only observed in the summer-fish sauce. The absorbance of the winter fish sauce was only 0.93 after 360days of fermentation. The absorbance of the summer capelin fish sauce was the same as the 2.79 ± 0.88 reported as the absorbance at 420 d for commercial Thai fish by Lopetcharat and Park (2002). Browning was most likely due to non-enzymatic browning reactions, mainly the Maillard reaction, but this may be assumed from the formation of pyrazines in the summer fish sauce (Hjalmarsson, 2001) which are believed to be formed by the Maillard browning reaction (Leahy & Roineddius, 1975). Carbohydrate derivatives, such as glucose-6-phosphate among other compounds in the metabolic pathway, may act as reactants in the Maillard browning reaction (Kawashima & Yamanaka, 1996). Wilaipan (1990) concluded that the brown colour of fish sauce was caused by non-enzymatic browning. A good correlation was observed between total nitrogen content and browning ($r^2 = 0.819$), indicating that measurements of browning might be used as a quality indi-

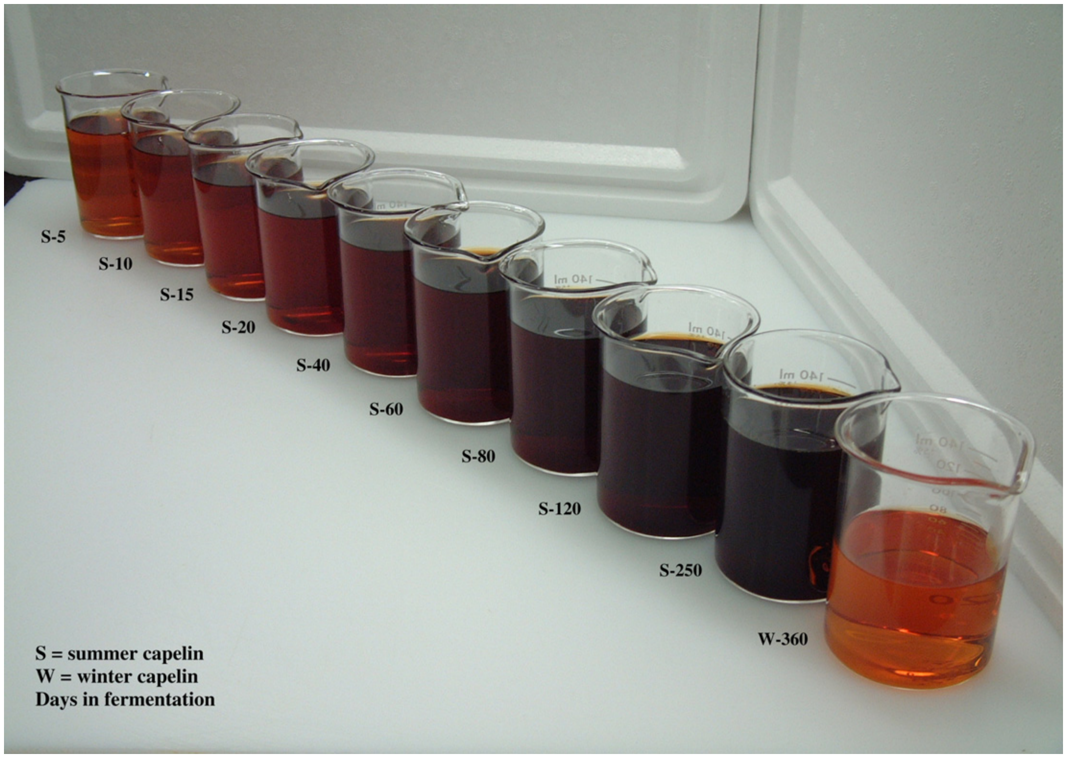

Fig. 8. Samples of summer fish sauce fermented for 5, 10, 15, 20, 40, 60, 80, 120 and 250 days compared to a sample of winter capelin fish sauce fermented for 360 days.

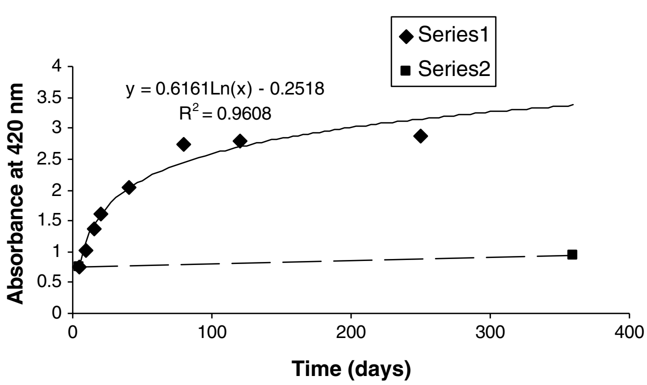

Fig. 9. Development of browning in fish sauce as indicated by absorbance at 420 nm during fermentation of summer (◆) and winter (■) capelin.

cator for monitoring the rate of formation of total nitrogen in the fish sauce during fermentation.

3.3. Amino acid composition

The amino acid composition of fish sauce may be nutritionally important especially in regions where fish sauce serves as a significant source of dietary protein. The concentration of free amino acids in the summer-fish sauce was higher ($p < 0.05$) than that in the winter-fish sauce (Table 1). This difference may be traced to a higher protease activity in the summer capelin where the fish proteins were likely broken down into free amino acids and peptides by proteases during the fermentation process. The fish sauce from summer capelin was found to be high in aspartic acid, glutamic acid, serine, glycine, valine, phenylalanine, isoleucine, leucine and lysine. Threonine and glutamine were not separated under the analytical conditions used and neither were arginine or alanine. We have previously encountered similar problems in unpublished work

Table 1
Free amino acid profiles of summer and winter fish sauce from capelin after 270 days and 360 days of fermentation

Amino acid	Summer fish sauce	Winter fish sauce
	mg/g	mg/g
Aspartic acid	4.55	1.00
Glutamic acid	4.67	1.39
Asparagine	0.09	0.0
Serine	2.59	0.81
Histidine	0.31	0.00
Glycine	2.18	0.61
Threonine + glutamine	2.66	0.76
Arginine + alanine	2.01	0.64
Taurine	1.87	1.62
Tyrosine	0.83	0.37
Methionine	0.98	0.00
Valine	2.70	0.68
Phenylalanine	2.22	0.50
Isoleucine	3.18	0.68
Leucine	5.70	1.73
Lysine	5.69	1.33
Total	72.90	12.12

with separation of amino acids from fish hydrolytes, especially when using new HPLC columns, and when high concentrations of taurine are present in the hydrolysates. Liaset, Julshamn, and Espe (2003) published tables with similar results for amino acid analysis of hydrolysates from salmon with high taurine contents. The amino acid content of the raw capelin was not analysed in this present study but, in previous work by Bragadottir (2001), the concentrations of amino acids in summer capelin were found to be 2–6 times higher than what was found for fish sauce in the results reported here for fish sauce from summer capelin. In that study, threonine was 806 mg/g, glutamine was 649 g/ml, arginine was 617 g/ml and alanine was 1097 mg/ml, in summer capelin from similar fishing grounds. It may therefore be assumed that all of these amino acids should be expected in the capelin fish sauce. It must also be kept in mind that the reverse phase HPLC separation, with fluorescent detection, is not a positive analytical identification method but is based on comparison of samples to standards. The large number of publications in the scientific literature on amino acid analysis are a clear indication of the complexity of their analyses. Abe et al. (1999) analyzed 60 different types of fish sauces from south east Asia for D-amino acids and found D-alanine in all types but its concentration varied largely with products. In fish sauces differing in fermentation time, the concentrations of D-alanine and D-aspartate were highest in the over-aged fish sauce fermented for 22 months. The results indicated that the choice of raw material was an important factor contributing to the final quality of fermented fish sauce.

The amino acid composition of summer capelin fish sauce was comparable to what has been reported for fish sauce by others. It was slightly higher in all amino acids except for alanine–arginine, methionine and histidine, than was fish sauce fermented from Pacific whiting (Tungkawachara et al., 2003). Traditional Indonesian fish sauce was found to be high in alanine, isoleucine, glutamic acid and lysine (Ijong & Ohta, 1996). Lopetcharat et al. (2001) reviewed reports on fish sauce products and manufacturing processes and found that the concentrations of glutamic acid, aspartic acid, lysine, leucine and lysine were generally around 4 g/l and further reported that these amino acids were complimentary to the amino acids derived from cereal. All these amino acids were present in the summer capelin fish sauce in comparable concentrations. Glutamic acid, which has been linked to both umami and meaty taste, was found in both winter and summer fish sauce but was at three times the concentration in the summer-fish sauce. High concentrations of glutamic acid have been linked to high taste quality of fish sauce (Tungkawachara et al., 2003) and it has been implied that high concentrations of glutamic acids in some commercial samples may be from the addition of GSM to boost taste Lopetcharat et al. (2001). However, the high concentration of glutamic acid in the summer fish sauce was natural.

4. Conclusions

Fish sauce with acceptable quality may be produced from whole capelin. Capelin harvested during the summer season was more suitable as a raw material than was capelin harvested during the winter season, due to higher proteolytic activity. Increased fermentation temperature may shorten the fermentation time of fish sauce, as well as reduce the salt concentration needed to produce the protein concentration exceeding 1.5–2.0%. Reduced salt content in processing may help to increase the fermentation rate as well as to improve nutritional properties by reducing the sodium content.

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