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SELECTED QUALITY ATTRIBUTES OF FROZEN FARMED GIANT GROUPER (*EPINEPHELUS LANCEOLATUS*) AS A FUNCTION OF STORAGE TEMPERATURE

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Key words: giant grouper, frozen storage, freshness, oxidation.

ABSTRACT

The effects of storage temperature (-20°C, -30°C and -55°C) on selected quality-related characteristics of vacuum packed frozen giant grouper fillets (*Epinephelus lanceolatus*) stored for 6 months were evaluated. The following quality attributes were measured: proximate composition, pH, thiobarbituric acid (TBA) value, total volatile basic nitrogen (VBN), ATP related compounds, K value, microbiological analysis, color, texture and sensory evaluation. Formation of TBA was low in the giant grouper fillet, with a value of less than 3.91 mg malondialdehyde (MDA) kg^{-1}, after 6 months storage at -30 and -55°C. No significant differences were found in pH, total plate count (TPC), white index (WI) or color value when fish were stored at different temperatures. All fillets stored under -55°C had K values lower than 20%, indicating the fillets were still in very fresh condition even after four months storage. The texture profile and hardness increased significantly with storage duration. Particularly, the raw giant grouper fillet stored at -55°C showed the greatest increase in hardness after 6 months storage compared to the other two storage temperatures. Significant differences in sensory attributes were barely detected by 56 panelists. For giant grouper fillets, little improvement in quality was found using ultra low storage temperature (-55°C) compared to the storage at -30°C. Accordingly, storage temperature at -30°C is considered the most economical and appropriate practice in preserving giant grouper fillets for commercial purposes.

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I. INTRODUCTION

Fish is one of the most important nutritional sources of animal proteins and ω-3 fatty acids for human consumption. The relatively high nutrient and moisture contents of fish make it an easily perishable commodity. Especially the high degree of unsaturated lipids and strong pro-oxidative systems in fish predispose them to lipid oxidation, leading to quality issues within a short time period storage (Nawar, 1996). Worldwide roughly 100 million tons of fish were caught every year but only around 20% of total catch were consumed fresh as the rest were being frozen (Huss et al., 2000). Freezing is considered the best preservation technique for consumers compared to salting, smoking, drying and canning. Nonetheless, freezing fish can induce physicochemical and structural alterations that influence fillet quality to various degrees (Burgaard and Jørgensen, 2010). Physical changes in meat texture are caused by large ice crystals that potentially induce protein denaturation, lipid oxidation, discoloration, freezer burn and protein oxidation. These undesirable changes are the most common defects found in frozen fish during storage (Burgaard & Jørgensen, 2010). Changes in quality vary largely depending on fish species, the frequency and rate of freezing and thawing, storage temperature and duration, and the presence of oxygen and light (Cappeln et al., 1999; Leelapongwattana et al., 2005). As a result, it is crucial to investigate economic freezing and storage methods that can preserve fillet quality for the fish processing industry. In general, the storage of frozen fish below -40°C is referred as ultra-low temperature storage (Indergård, Tolstorebrov, Larsen, & Eikevik, 2014). Under this condition, only unfreezable bound water remains in flesh, all freezable free water is frozen (Bogh-Sorensen, 2006). Magnussen and Johansen (1995) reported that ultra-low temperature increases the shelf life of frozen salmon fillets compared to products stored between -18°C and -25°C. In order to preserve the best quality of tuna, it has been recommended tuna should be stored at temperatures below -60°C for the raw fish market (Chow, Ochiai, Watabe, & Hashimoto, 1988; Chow, Ochiai, & Watabe, 2004). However, Mørkøre and Lilleholt (2007) found no beneficial effects in farmed Atlantic cod when the freez-

ing temperature was controlled below -40°C. The ideal preservation temperature is different for various fish species. It was suggested that salmon fillets, for instance, should be stored between -45°C and -60°C (Magnussen and Johansen, 1995; Mørkøre and Lilleholt, 2007).

Giant grouper (*Epinephelus lanceolatus*) is currently a high-value species in the fish markets of Taiwan, China, Japan and Vietnam. The giant grouper has become a very important species for the aquaculture industry in Taiwan and is mainly shipped alive to Mainland China for consumption. Aquatic farmers in Taiwan breed about 17,042 tons of giant groupers, accounting for 42% of the global yield (FAO, 2011). Until recently, sumptuary laws have restricted and limited the consumption of giant grouper in China. Consequently, the market price of live giant grouper fish has declined dramatically. In the food industry, freezing is generally used for better preservation. Ultra-low temperature storage has the advantage of maintaining quality attributes, including safety, nutrition, color and appearance, flavor, texture, and the suitability of the fillet for further processing and preservation. However, economic considerations generally preclude temperatures below -30°C for transport and storage, except for high-value fishery products (Potter and Hotchkiss, 1997). Although the giant grouper is a very important and high-value species in Taiwan, studies on the post-mortem changes that occur at different temperatures during storage still remain limited. (Mørkøre & Lilleholt, 2007; Magnussen & Johansen, 1995).

The objective of this study was to investigate the selected quality attributes on the physical, chemical and organoleptic characteristics, and microbial quality of vacuum packed giant grouper fillets using freezer temperatures at -20°C, -30°C and -55°C. Establishing such fundamental knowledge on the chemical composition and storage characteristics of giant grouper will definitely benefit its future applications as a high-value fishery product for domestic consumption and export potential. It also offers alternative marketing strategies for aquatic farmers and the fish processing industry.

II. MATERIALS AND METHODS

1. Materials and Sample Preparation

Giant grouper (*Epinephelus lanceolatus*) was fed commercial feed and purchased from Chung Yuang Aquatic Farm (Touchen Town, Yilan County) in April 2013. Fresh giant grouper of similar weight (4.0-4.5 kg) was selected for this experiment. Upon arrival at the Department of Food Science, National Taiwan Ocean University, Keelung, fish was stored in ice with a fish/ice ratio of 1:2 (w/w) for 30 min. The fish was then killed by a blow to the head, eviscerated, filleted and skinned by hand. The fillets (300 g each) were vacuum-packed in oxygen-impermeable bags and frozen at -35°C in an ultra-low temperature freezer overnight (Law-Chain Computer Tech. Co. Ltd., Kaohsiung County, Taiwan). Fish fillets were then kept at -20°C, -30°C or -55°C for 6 months. Samples were taken at month 0, 2, 4 and 6 of storage and thawed at 7°C overnight. The fillets were divided into three lots, and six fillets from each lot were taken per sampling month to perform the different analyses. All chemicals for thiobarbituric acid (TBA) value and proximate composition analysis were purchased from Merck KGaA (Darmstadt, Germany). ATP related standard compounds were obtained from Sigma-Aldrich (St. Louis, USA).

2. Physicochemical Analyses

The proximate chemical composition of each giant grouper fillet was determined according to the Association of Official Analytical Chemists (AOAC) method (AOAC, 1998) on day 0 of storage. The moisture content of each fillet was determined as per AOAC procedure 984.25 by oven drying at 105°C for 24 h. Total ash was measured by burning the sample at 530°C for 24 h. Crude lipid content of the sample was determined using the ether extraction method. Crude protein (N × 6.25) was measured using Kjeldahl method (AOAC: 955.04). Crude carbohydrate was estimated using the equation: 100 – (moisture + crude protein + lipid content + total ash). Measurements of each analysis were performed in three replications (n = 3) and expressed as mean ± standard deviation (SD) based on wet weight (%).

3. Determination of pH, Thiobarbituric Acid (TBA) and Total Volatile Basic Nitrogen (VBN)

Five g of each sample was added to 45 g of distilled water, blended and filtered with Whatman No. 1 filter paper before pH analysis (pH Mettler Toledo MP-220, Zurich, Switzerland). A combination electrode, standardized between pH 4.0 and 7.0 and attached to a pH/ion analyzer, was inserted into the strained liquid using the method reported by Li et al. (2015) with slight modifications. TBA value was determined according to the method described by Vyncke (1975). A fish fillet sample (5.0 g) was homogenized with 20 ml of trichloroacetic acid extraction solution (7% TCA) at 4500 *g* at 4°C for 20 min (Model Polytron PT 3000, Ultra-Turrax T-25 basic, IKA, Germany). The homogenized samples were then centrifuged 3 times at 4500 *g* for 20 min at 4°C (Hitachi CF TD 2) (TJ-25 Centrifuge, Beckmann Coulter, USA), as in the above procedure. The solution was then filtered with Toyo No. 2 filter paper (125 mm) and topped up to 50 ml with double distilled water. 2 ml of each solution was mixed with an equal volume of thiobarbituric acid (0.02 M) and heated in a boiling water bath for 30 min before being cooled down on ice. The absorbance of the solutions was measured at 532 nm (C-1800 MK-II, Tecan Sunrise, Austria). A standard curve was constructed using 0.01 to 2 μg/ml 1,1,3,3-tetraethoxypropane. The results were expressed as mg of malonaldehyde diethylacetal/g of wet sample. Volatile basic nitrogen (VBN, mg/100 ml) was evaluated using the micro-diffusion method (Siddaiah, Sagar Reddy, Raju, & Chandrasekhar, 2001). Five g of homogenized sample was mixed with 40 ml 0.7% TCA solution and blended for 3 min at low speed (Model Polytron PT 3000, Ultra-Turrax T-25 basic, IKA, Germany). The mixture was filtered with a Toyo No. 2 filter and topped up to 100 ml. One ml of filtrate was transferred to a Conway dish containing 1 ml saturated potassium carbonate solution and allowed to react at 25°C for 120 min. A

boric acid solution (10%) contained methyl red and bromocresol green indicator was used to absorb nitrogen. The solution was titrated with 0.01 N HCl.

4. ATP Related Compounds and K Value

Nucleotides and related compounds were measured using a reverse phase high-performance liquid chromatography (Shimazu LC-10A, Nacalai Tesque Inc., Japan) in accordance with the method described by Guizani et al. (2005). The identification of adenosine 5'-triphosphate (ATP), adenosine 5'-diphosphate (ADP), adenosine 5'-monophosphate (AMP), inosine monophosphate (IMP), inosine (HxR) and hypoxanthine (Hx) was obtained by comparing their retention times with a standard mixture purchased from Sigma-Aldrich Chemicals Private Limited (Bangalore, India) and by the spiking or addition of standards.

The K value was defined as the ratio of the sum of inosine and hypoxanthine to the total concentration of all ATP related compounds (Saito, Arai, & Matsuyoshi, 1959). ATP related compounds were extracted from giant grouper flesh with 6% perchloric acid (PCA). Five g of each fish flesh sample was homogenized with 10 ml of 6% PCA extraction solution for 2 min using a homogenizer (Brinkmann Polytron PT 3000, American Laboratory Trading Inc. San Francisco, USA). The homogenized samples were then centrifuged at 3000 *g* for 20 min at 4°C (Hitachi SCR 20 B, Japan). The supernatant was filtered with Toyo No. 2 filter paper and the bottom substance was extracted with 6% PCA using the homogenizer, as described above. All filtered solutions were topped up to 100 ml with extracted solution. A COSMOSIL packed column 5C 18-MS reverse phase stainless steel column (4.6 mm I.D. × 250 mm) was used for separation of ATP related compounds. Potassium dihydrogen phosphate (50 mM) and dipotassium hydrogen phosphate (50 mM) were dissolved in HPLC grade water (Millipore) and pH was adjusted to 6.5 for the mobile phase. The solution was filtered using a 0.2 μm filter and sonicated for 30 min prior to injection into the column. Flow rate was maintained at 0.8 ml/min. Absorbance of the eluate was measured at 254 nm.

5. Microbiological Analysis

The measurement of the total plate count (TPC) followed the FDA Bacteriological Analytical Manual (FDA, 1998). Ten g of giant grouper flesh sample was transferred aseptically into a stomacher bag containing 90 ml of sterile potassium phosphate buffer and homogenized for 60 s. Further decimal dilutions were prepared and then 0.1 ml of each dilution was transferred onto the surface of a plate count agar plate. The plates were incubated at 37°C for 48 h. Three duplicates of at least three appropriate dilutions per sampling month were enumerated.

6. Determination of Giant Grouper Fillet Color

The color of the giant grouper fillet was measured by a spectrophotometer (TC-1800 MK-II, Tokyo, Japan) using the L (lightness), a (redness/greenness), b (yellowness/blueness) color scale. A white tile and black cup were used to standardize the spectrophotometer. Each sample was loaded onto a quartz sample cup, with three measurements taken for each sample, and triplicate measurements were recorded for each storage temperature. White index (W.I.) was calculated using the following equation:

$$W.I. = 100 - ((100 - L)^2 + a^2 + b^2)^{1/2}$$

7. Instrumental Texture Measurements

Texture profile analyses (TPA) of giant grouper fillets were measured using a TA-TX 2 texture analyzer (Stable microsystem, U.K.) with a load cell of 5 kg. Samples measuring 15 mm × 15 mm × 10 mm, were compressed perpendicularly using a 5 mm cylindrical probe (P/5) as described by Li et al. (2011). The hardness was expressed as the maximum probe force (g) required to compress the fillet to a depth of 2.5 mm at a constant speed of 1.0 mm/s. Springiness measures elasticity by determining the extent of recovery between the first and second compression. The test was repeated in triplicate.

8. Sensory Evaluation

Cooked giant grouper fillets were served to 56 untrained panelists to evaluate color, odor, texture, taste and overall score. 18 male, and 38 female graduate students and faculty members of the Department of Food Science, between the ages of 20 and 44, were participants on the panel. Panelists were instructed to evaluate each attribute using a seven-point hedonic scale ranging from "1 = extremely dislike" to "7 = extremely like". Cooked fillets coded with three random digits were supplied to them. Each data point from sensory analysis represents an average of 56 panelists.

9. Statistical Analysis

A completely randomized block design was used with three or six replications per treatment. Data were analyzed by analysis of variance (ANOVA) using the SPSS statistic program (SPSS, 12.0, 1998). Differences between the means were evaluated using Duncan's Multiple Range Test (Steel & Torrie, 1980).

III. RESULTS AND DISCUSSION

1. Chemical Composition of Giant Grouper Fillets

The moisture content of giant grouper fillets was 70.71 ± 1.63% and the protein content was 18.66 ± 0.80%. The fat and ash content of giant grouper fillets were 7.51 ± 0.37% and 1.62 ± 0.17%, respectively. Giant grouper is a fish species with medium fat (5-15%) and high protein (15-20%) according to Stansby (1963) and Haard (1992). Fillet composition varies greatly from species to species and is influenced by feeding and environmental conditions.

2. Changes in pH, TBA and VBN

pH of giant grouper fillets increased from 6.39 to 6.46 after 6 months storage at -20°C, -30°C and -55°C, as shown in Table 1. The pH values increased ($p < 0.05$) after 6 months storage while there were no statistical differences ($p > 0.05$) between the samp-

Table 1. Changes in pH, volatile basic nitrogen (VBN), thiobarbituric acid (TBA) value, and total plate count (TPC) of giant grouper flesh stored at -20, -30 and -55°C.

Measurements	Storage period (months)			
	0	2	4	6
Giant grouper[A]		At -20°C		
pH	6.39 ± 0.08[a]	6.37 ± 0.09[a]	6.45 ± 0.04[b]	6.46 ± 0.05[b]
VBN (mg/100g)	6.97 ± 1.27[a]	6.81 ± 1.11[a]	10.19 ± 1.50[b]	11.18 ± 0.41[c]
TBA (mg/kg)	1.51 ± 0.42[a]	1.95 ± 0.42[a]	2.35 ± 1.17[a]	3.91 ± 1.09[b]
TPC (log CFU/g)	4.35 ± 0.05[b]	4.20 ± 0.10[b]	3.76 ± 0.30[a]	3.75 ± 0.25[a]
Giant grouper[A]		At -30°C		
pH	6.39 ± 0.08[a]	6.39 ± 0.05[a]	6.43 ± 0.08[ab]	6.45 ± 0.03[b]
VBN (mg/100g)	6.97 ± 1.27[a]	6.72 ± 0.70[a]	9.82 ± 1.71[b]	10.19 ± 1.39[b]
TBA (mg/kg)	1.51 ± 0.42[a]	1.80 ± 0.69[a]	1.97 ± 0.63[a]	3.00 ± 0.77[b]
TPC (log CFU/g)	4.35 ± 0.05[b]	4.14 ± 0.17[b]	3.58 ± 0.46[a]	3.55 ± 0.43[a]
Giant grouper[A]		At -55°C		
pH	6.39 ± 0.08[a]	6.41 ± 0.04[a]	6.43 ± 0.05[ab]	6.45 ± 0.03[b]
VBN (mg/100g)	6.97 ± 1.27[a]	6.83 ± 1.64[a]	7.82 ± 1.02[b]	9.05 ± 0.70[c]
TBA (mg/kg)	1.51 ± 0.42[a]	1.72 ± 0.50[a]	1.94 ± 0.72[ab]	2.67 ± 0.96[b]
TPC (log CFU/g)	4.35 ± 0.05[b]	4.13 ± 0.07[b]	3.55 ± 0.30[a]	3.42 ± 0.36[a]

[A] Data are mean ± standard deviation (n = 6).

Means followed by different letters within each row are significantly different ($p < 0.05$).

ling period and storage temperature. Mørkøre and Lilleholt (2007) observed that flesh pH showed no significant variation in cod fillets stored between -10°C to -70°C. However a tendency ($p = 0.06$) for higher flesh pH in fillets stored at lower temperatures was reported. As a result, pH could not be used for monitoring quality changes. TBA value is commonly used as a marker for the decomposition of secondary lipid oxidation products. The TBA values for all samples were below reported lipid spoilage indices, established at 3 or less mg malonaldehyde equivalents/kg flesh, except for the sample stored at -20°C for 6 months. Results from giant grouper fillets stored up to 6 months showed no significant differences at -20°C, -30°C and -55°C (Table 1). Higher TBA content in horse mackerel fillets stored at -20°C versus those stored at -80°C was reported by Aubourg et al. (2004). On the other hand, the TBA content in farmed Coho salmon (0.54 mg/kg) did not increase until after 15 months of frozen storage. The reported TBA values attributed the low degree of lipid oxidation to stable endogenous antioxidants due to the vacuum packaging. Mackerel showed increased TBA value but Coho salmon and giant grouper did not might be due to the fatty acid composition of fish species. Only poly unsaturated fatty acids of 3 or more unsaturated bonds will be able to form TBA reactive substance upon oxidation. Cultured fish from subtropical area like giant grouper does not contain that much C18:3, EPA or DHA to form TBA reactive substance. Dulavik et al. (1998) measured higher TBA concentrations in saithe light and dark flesh at a higher freezer storage temperature.

VBN is widely used as an indicator of fish deterioration; it consists of ammonia and primary, secondary and tertiary amines. In this study the amount of VBN in giant grouper fillets significantly increased ($p < 0.05$) at all storage temperatures after 4 months (Table 1). Typically a level of 15 mg/100 g of flesh is considered very fresh for raw fish. A level of 15-25 mg VBN is still accepted as moderately fresh. A level above 30-40 mg/100 g of fish is considered spoiled and a level greater than 50 mg/100 g is unsuitable for human consumption (TFDA, 1998, 2011). In this research, the final VBN contents of giant grouper fillet stored at -20°C, -30°C and -55°C for six months were 11.18 ± 0.41, 10.19 ± 1.39 and 9.05 ± 0.70, respectively. This indicates that the fillets preserved their high quality during freezer storage. Lower temperatures can certainly inhibit bacterial growth and reduce its capacity for oxidative deterioration of non-protein nitrogen compounds.

3. ATP Related Compounds and K Value

Changes in ATP related compounds in the fillets of vacuum packed giant grouper during 6 months storage at different freezer temperatures are shown in Table 2. ATP, ADP and AMP concentrations were 0.03, 0.06 and 0.04 μmol/g meat, respectively, after the giant grouper were filleted (Table 2). ATP rapidly disappeared after death and the content (0.18 μmol/g) is higher than that of yellow grouper 12 h post-catch. However, the IMP concentration decreased at -20°C, -30°C and -55°C in accordance with the regression equations $y = -0.0337x + 1.2389$ with an $r^2 = 0.8607$, $y = 0.008x + 102418$ with an $r^2 = 0.631$ and $y = -0.0004x + 1.1887$ with an $r^2 = 0.0011$ calculated from Table 2 by IMP concentration versus storage months, respectively. The resulting evidence indicates the IMP concentration decreased faster at -20°C compared to -30°C and -55°C. A high concentration of IMP shows a quick degradation of ATP to IMP. Varia-

Table 2. Changes in ATP-related components (μmol/g) and K value of giant grouper flesh stored at -20, -30 and -55°C.

Measurements	Storage period (months)			
	0	2	4	6
Giant grouper[A]		At -20°C		
ATP	0.03 ± 0.01^{a}	0.03 ± 0.01^{ab}	0.05 ± 0.01^{bc}	0.06 ± 0.02^{c}
IMP	1.27 ± 0.22^{a}	1.28 ± 0.35^{a}	1.22 ± 0.13^{a}	1.21 ± 0.14^{a}
ADP	0.06 ± 0.02^{ab}	0.07 ± 0.02^{b}	0.05 ± 0.01^{a}	0.05 ± 0.01^{a}
Hypoxanthine (Hx)	0.10 ± 0.03^{a}	0.14 ± 0.01^{a}	0.40 ± 0.08^{b}	0.56 ± 0.06^{c}
AMP	0.04 ± 0.01^{a}	0.04 ± 0.01^{a}	0.05 ± 0.01^{a}	0.05 ± 0.01^{a}
Inosine (HxR)	0.03 ± 0.02^{a}	0.09 ± 0.01^{b}	0.13 ± 0.01^{c}	0.17 ± 0.03^{d}
Total	1.52 ± 0.31	1.65 ± 0.40	1.89 ± 0.25	2.09 ± 0.27
K value (%)	8.72 ± 2.71^{a}	14.22 ± 3.68^{b}	27.74 ± 3.17^{c}	35.10 ± 2.69^{d}
Giant grouper[A]		At -30°C		
ATP	0.03 ± 0.01^{a}	0.05 ± 0.02^{b}	0.06 ± 0.01^{b}	0.06 ± 0.01^{b}
IMP	1.27 ± 0.22^{a}	1.28 ± 0.11^{a}	1.25 ± 0.17^{a}	1.22 ± 0.09^{a}
ADP	0.06 ± 0.02^{a}	0.05 ± 0.02^{a}	0.07 ± 0.01^{a}	0.06 ± 0.00^{a}
Hypoxanthine (Hx)	0.10 ± 0.03^{a}	0.15 ± 0.01^{b}	0.25 ± 0.01^{c}	0.40 ± 0.05^{d}
AMP	0.04 ± 0.01^{a}	0.05 ± 0.01^{ab}	0.07 ± 0.01^{c}	0.06 ± 0.01^{bc}
Inosine (HxR)	0.03 ± 0.02^{a}	0.10 ± 0.02^{b}	0.19 ± 0.01^{c}	0.18 ± 0.02^{c}
Total	1.52 ± 0.31	1.67 ± 0.18	1.89 ± 0.21	1.97 ± 0.17
K value (%)	8.72 ± 2.71^{a}	14.91 ± 1.35^{b}	23.64 ± 1.80^{c}	29.60 ± 1.14^{d}
Giant grouper[A]		At -55°C		
ATP	0.03 ± 0.01^{a}	0.04 ± 0.00^{b}	0.05 ± 0.01^{bc}	0.06 ± 0.01^{c}
IMP	1.27 ± 0.22^{a}	1.27 ± 0.22^{a}	1.24 ± 0.12^{a}	1.26 ± 0.07^{a}
ADP	0.06 ± 0.02^{b}	0.04 ± 0.01^{a}	0.06 ± 0.01^{b}	0.06 ± 0.01^{b}
Hypoxanthine (Hx)	0.10 ± 0.03^{a}	0.11 ± 0.01^{a}	0.22 ± 0.03^{b}	0.35 ± 0.03^{c}
AMP	0.04 ± 0.01^{a}	0.06 ± 0.01^{c}	0.05 ± 0.00^{bc}	0.04 ± 0.00^{ab}
Inosine (HxR)	0.03 ± 0.02^{a}	0.09 ± 0.02^{b}	0.13 ± 0.02^{c}	0.15 ± 0.02^{d}
Total	1.52 ± 0.31	1.62 ± 0.27	1.76 ± 0.19	1.92 ± 0.19
K value (%)	8.72 ± 2.71^{a}	12.82 ± 2.02^{b}	20.08 ± 1.94^{c}	26.32 ± 1.48^{d}

[A] Data are mean ± standard deviation (n = 6).

Means followed by different letters within each row are significantly different ($p < 0.05$).

tions in ATP, ADP, AMP and IMP concentrations did not greatly change during storage time ($p > 0.05$). Hypoxanthine (Hx) and inosine (HxR) concentrations increased dramatically during 6 months storage at all freezer temperatures, compared to the concentrations of ATP, ADP and IMP. HxR concentrations ($y = 0.0817x - 0.0046$ with an $r^2 = 0.7649$ at -20°C, $y = 0.0629x + 0.0443$ with an $r^2 = 0.9553$ at -30°C and $y = 0.0347x + 0.0621$ with an $r^2 = 0.9565$ at -55°C) increased during 6 months storage. Hx accumulation at -30°C and -55°C could be used as a freshness indicator due to the high correlation with its formation at -20°C after 6 months storage. Ocaño-Higuera et al. (2009) reported similar ATP degradation patterns for cazon fish flesh. Howgate (2005) proposed the loss of flavor and freshness in several fish species correlated with decreasing IMP concentrations. Li et al. (2011) suggested Hx accumulation in fish meat reflected the initial phase of autolytic deterioration and bacterial spoilage. However, the total plate count of the fish meat in this study also decreased during 6 months of storage ($p < 0.05$). These results implied the initial phase of autolytic deterioration was more significant than any bacterial spoilage during storage.

Quantification of K value was derived from the ATP content and its degraded products (Mazorra-Manzano, Pacheco-Aguilar, Díaz-Rojas, & Lugo-Sánchez, 2000). A significant linear K value increase ($y = 5.5383x + 4.2609$ with an $r^2 = 0.9843$ at -20°C, $y = 4.0053x + 6.7528$ with an $r^2 = 0.9593$ at -30°C and $y = 3.084x + 6.091$ with an $r^2 = 0.9811$ at -55°C) was observed in giant grouper fillets during 6 months storage. Freshness of fish with K values lower than 20 qualifies as very fresh (Saito, Arai, & Matsuyoshi, 1959). K values for the giant grouper fillets during the first two months were lower than 20% at all three temperatures, indicating they were very fresh (Table 2). Saito et al. (1959) proposed marine products with K values less than 50% were moderately fresh. Using the predictive equation and based on these K value categories, the giant grouper fillets at -20°C and -30°C of this study can be kept at very fresh conditions for 2 months, while the fillets at -55°C can be considered very fresh for at least 4 months. All fillets at -20°C, -30°C and -55°C can be considered moderately fresh after 4 and 6 months storage (Table 2). These

Table 3. Changes in hardness and springness of giant grouper flesh stored at -20, -30 and -55°C.

Measurements	Storage period (months)			
	0	2	4	6
Giant grouper[A]		At -20°C		
Raw flesh				
Hardness (g)	38.37 ± 8.64[a]	47.85 ± 14.59[a]	111.33 ± 64.40[b]	130.48 ± 25.42[b]
Springness	0.90 ± 0.02[a]	0.91 ± 0.02[ab]	0.92 ± 0.01[ab]	0.93 ± 0.02[b]
Cooked flesh				
Hardness (g)	121.15 ± 52.92[a]	158.18 ± 25.27[ab]	221.30 ± 54.85[b]	310.68 ± 78.18[c]
Springness	0.90 ± 0.05[a]	0.94 ± 0.03[b]	0.93 ± 0.01[ab]	0.92 ± 0.02[ab]
Giant grouper[A]		At -30°C		
Raw flesh				
Hardness (g)	38.37 ± 8.64[a]	77.73 ± 40.00[ab]	117.88 ± 13.90[bc]	128.03 ± 54.62[c]
Springness	0.90 ± 0.02[a]	0.91 ± 0.01[a]	0.91 ± 0.02[a]	0.92 ± 0.02[a]
Cooked flesh				
Hardness (g)	121.15 ± 52.92[a]	169.75 ± 86.22[ab]	223.80 ± 32.14[b]	312.59 ± 60.93[c]
Springness	0.90 ± 0.05[a]	0.93 ± 0.02[a]	0.90 ± 0.02[a]	0.92 ± 0.02[a]
Giant grouper[A]		At -55°C		
Raw flesh				
Hardness (g)	38.37 ± 8.64[a]	97.47 ± 12.15[b]	147.47 ± 15.91[c]	174.05 ± 17.05[c]
Springness	0.90 ± 0.02[a]	0.91 ± 0.04[a]	0.93 ± 0.02[a]	0.92 ± 0.02[a]
Cooked flesh				
Hardness (g)	121.15 ± 52.92[a]	192.18 ± 39.64[b]	222.40 ± 82.83[b]	325.59 ± 22.04[c]
Springness	0.90 ± 0.05[a]	0.91 ± 0.03[a]	0.92 ± 0.02[a]	0.92 ± 0.01[a]

[A]Data are mean ± standard deviation (n = 6).

Means followed by different letters within each row are significantly different ($p < 0.05$).

K values have a negative correlation with TBA and the sensory score of fish fillets. A storage temperature of -30°C seemed to be sufficient for the preservation of frozen giant grouper fillet freshness for up to 6 months with comparable attributes to storage temperature at -55°C. These results were in agreement with Li et al. (2011) The K value can be used to evaluate the freshness of frozen fish and correlates well with storage time.

4. Instrumental Texture Measurements

Texture and color are two important qualities in fish fillets (Haard, 1992). Table 3 shows the mean values for the textural characteristics of the frozen giant grouper fillets stored for 6 months. Hardness of the raw fillets and cooked fillets increased with storage time. After the giant groupers have been slaughtered, fish muscle tissue is subjected to spontaneous reactions as mammalian tissue. The duration and extent of rigor mortis depend on the physiological condition of the giant groupers. Several days can pass before the rigor subsides as a result of the activity of endogenous fish proteinases in some species. Ultra-low temperature (-55°C) retarded the rigor mortis in this study, the proteolysis started again on thawing. This may cause increased hardness in texture and water loss in the product of raw and cooked fillets (Table 3). Our results indicated that the aggregation occurred in the myofibrillar protein of giant grouper flesh toughened the fillet during storage. Therefore, the values of hardness in all of three different storage temperatures increased from 2nd month to 4th month (Table 3). The fillets stored at -55°C showed the highest hardness values in both raw and cooked meat at the end of 6 months storage. Similar results were found in white flaky fillets of hake, cod and haddock which became hard, dry and fibrous during freezer storage (Careche, Herrero, Rodriguez-Casado, Del Mazo, & Carmona, 1999; Gill, Keith, & Lall, 1979; Kim & Heldman, 1985). Aggregation of flesh proteins might lead to texture deterioration. Love (1988) observed dehydration and a decrease in water-holding capacity upon freezing and thawing, and proposed high liquid losses; it may result in a tougher flesh after cooking. Refsgaard et al. (1998) reported frozen storage to be associated with the toughening, protein denaturation and water loss of both rainbow trout and farmed Atlantic salmon. The increase in toughness with storage time could be due to the subsequent formaldehyde-protein reaction, which is believed to contribute to the toughening of fish flesh during freezer storage. Dimethylamine and formaldehyde are formed during the breakdown of trimethylamine oxide in marine species by an endogenous enzyme. Formaldehyde induces cross-linking of flesh proteins and renders them insoluble, causing the giant grouper meat to toughen up (Castell & Smith, 1973). The formation of new disulfide bonds in meat proteins may contribute to denaturation. However, this is not always the case, as Martinez et al. (2010) found that storage led to signifi-

Table 4. Changes in Hunter L, a, b value and white index (W.I) of giant grouper flesh stored at -20, -30 and -55°C.

Measurements	Storage period (months)			
	0	2	4	6
Giant grouper[A]		At -20°C		
L	65.39 ± 0.92[a]	65.71 ± 2.28[a]	71.25 ± 3.76[b]	73.44 ± 2.52[c]
A	-8.36 ± 1.06[bc]	-9.98 ± 0.57[a]	-8.02 ± 1.56[c]	-8.93 ± 1.69[b]
B	28.16 ± 0.80[a]	28.27 ± 0.96[a]	31.48 ± 1.18[b]	34.72 ± 3.27[c]
W.I*	54.58 ± 0.65[a]	54.41 ± 1.31[a]	56.47 ± 1.68[b]	55.26 ± 2.92[ab]
Giant grouper[A]		At -30°C		
L	65.39 ± 0.92[a]	67.17 ± 2.63[b]	72.19 ± 1.96[c]	66.90 ± 2.17[b]
a	-8.36 ± 1.06[b]	-10.11 ± 0.24[a]	-10.23 ± 1.10[a]	-9.89 ± 1.53[a]
b	28.16 ± 0.80[a]	28.60 ± 0.96[a]	31.42 ± 2.03[c]	29.82 ± 1.26[b]
W.I*	54.58 ± 0.65[a]	55.24 ± 1.43[a]	56.72 ± 1.87[b]	54.29 ± 1.02[a]
Giant grouper[A]		At -55°C		
L	65.39 ± 0.92[ab]	64.28 ± 3.12[a]	67.67 ± 2.03[c]	66.28 ± 1.84[b]
a	-8.36 ± 1.06[b]	-8.88 ± 0.65[ab]	-9.50 ± 1.63[a]	-8.98 ± 1.57[ab]
b	28.16 ± 0.80[ab]	27.62 ± 1.57[a]	28.72 ± 0.51[b]	27.86 ± 1.76[a]
W.I*	54.58 ± 0.65[ab]	53.94 ± 2.74[a]	55.67 ± 1.30[b]	55.31 ± 2.38[b]

[A] Data are mean ± standard deviation (n = 6).

Means followed by different letters within each row are significantly different ($p < 0.05$).

[1] L value: lightness; a value: redness; b value: yellowness; W.I: white index.

* $\text{W.I.} = 100\sqrt{\left(100\left|\text{L}\right.\right)^2 + \text{a}^2 + \text{b}^2}$

cant reductions in the hardness of salmon smoked with a liquid smoke flavouring and vacuum packed yellow grouper fillets stored at 0°C. Fish flesh can undergo a number of changes during the freezing process and storage (Barroso, Careche, & Borderıas, 1998).

5. Microbiological Analysis

The growth of microorganisms in frozen fish fillets was significantly decreased after 6 months storage for all three freezer temperatures. The initial total plate count for fresh giant grouper fillets was 4.35 log CFU/g, but the population decreased to 3.75 log CFU/g after 6 months at -20°C (Table 1). The greater inhibitory effect might be related to the lower freezing temperature used in this study. In addition, the extra- and intra-cellular ice crystals that formed during storage induced irreversible damage to the cytoplasmic membranes of the bacteria (Uljas & Ingham, 1999).

6. Color Measurement of Giant Grouper Fillets

Considerable variations in color were observed among individual giant grouper fillets. All fillets were white to yellowish in color, which was illustrated by a L value > 60, an a value ranging from -8 to -11 and a b value ranging from 28 to 34 (Table 4). Temperature and duration of storage affected the L and b values of the fillets. The L and b values of the frozen fillets increased after 6 months storage, indicating an increased lightness of the fillet color with extended storage, especially at -20°C. The fillets stored at -20°C for 6 months had significantly higher L values compared to those stored at -55°C (Table 4). Stien et al. (2005) proposed that increasing L* values in chilled, stored pre rigor fillets of farmed cod was related to flesh protein denaturation and a greater loss of liquid. This color change may also be caused by the oxidation of lipids during storage. Tironi et al. (2007) stated this color change may be in line for the ice crystals that formed during the freezing process and storage, leading to the deterioration of cellular components (e.g., protein denaturation). Chaijan et al. (2005) reported the color loss in sardine and mackerel flesh during iced storage to the oxidation of myoglobin and hemoglobin.

7. Sensory Evaluation

Color, odor, texture and overall scores for vacuum packed filleted giant grouper samples stored for 6 months showed a slight decrease in acceptability at the different storage temperatures (Table 5). All panelists still favored the cooked giant grouper fillet although it gave a decreasing tendency in acceptability after 6 months storage. There were no differences at all between the sensory characteristics of cooked meat stored at -30°C and that stored at -55°C for 6 months. Sensory scores for cooked giant grouper fillets stored at different temperatures for 6 months are summarized in Table 5. For all three temperatures, none of the fillet attributes changed after 2 months of storage. Only color, odor and overall score had any discernible effect on the quality of the cooked fillet after 4 months of storage; no significant differences were found between the fillets for any of the attributes for the sensory score ($p > 0.05$).

Table 5. Sensory scores for giant grouper flesh stored at different temperatures for 6 months.

	Color	Odor	Texture	Taste	Overall
2 months storage					
Fresh	6.06 ± 0.63^{a}	5.72 ± 0.60^{a}	5.72 ± 0.56^{a}	5.76 ± 0.55^{a}	5.94 ± 0.36^{a}
-20°C	5.93 ± 0.61^{a}	5.63 ± 0.52^{a}	5.70 ± 0.57^{a}	5.69 ± 0.67^{a}	5.89 ± 0.37^{a}
-30°C	5.96 ± 0.47^{a}	5.67 ± 0.64^{a}	5.69 ± 0.64^{a}	5.70 ± 0.66^{a}	5.91 ± 0.40^{a}
-55°C	5.94 ± 0.53^{a}	5.65 ± 0.62^{a}	5.70 ± 0.46^{a}	5.76 ± 0.51^{a}	5.91 ± 0.35^{a}
4 months storage					
Fresh	5.98 ± 0.49^{b}	5.76 ± 0.47^{a}	5.73 ± 0.53^{b}	5.78 ± 0.50^{b}	5.89 ± 0.31^{b}
-20°C	5.78 ± 0.42^{a}	5.58 ± 0.57^{a}	5.47 ± 0.60^{a}	5.51 ± 0.74^{a}	5.55 ± 0.50^{a}
-30°C	5.78 ± 0.42^{a}	5.62 ± 0.53^{a}	5.44 ± 0.50^{a}	5.55 ± 0.54^{a}	5.60 ± 0.49^{a}
-55°C	5.80 ± 0.45^{a}	5.62 ± 0.49^{a}	5.53 ± 0.50^{ab}	5.56 ± 0.50^{a}	5.78 ± 0.42^{b}
6 months storage					
Fresh	5.88 ± 0.43^{b}	5.80 ± 0.48^{b}	5.80 ± 0.52^{c}	5.79 ± 0.46^{c}	5.88 ± 0.33^{c}
-20°C	5.57 ± 0.53^{a}	5.45 ± 0.50^{a}	5.45 ± 0.50^{a}	5.41 ± 0.56^{a}	5.45 ± 0.50^{a}
-30°C	5.64 ± 0.52^{a}	5.52 ± 0.50^{a}	5.55 ± 0.60^{ab}	5.57 ± 0.53^{ab}	5.50 ± 0.50^{ab}
-55°C	5.66 ± 0.48^{a}	5.57 ± 0.50^{a}	5.66 ± 0.48^{bc}	5.63 ± 0.49^{b}	5.66 ± 0.48^{b}

Data are mean ± standard deviation (n = 56).

Means from the same storage time following different letters are significantly different ($p < 0.05$).

Score 7 = like extremely; 4 = neither like nor dislike; 1 = dislike extremely.

IV. CONCLUSION

Thiobarbituric acid (TBA) value, volatile basic nitrogen (VBN) and K value increased in vacuum packed giant grouper fillets for all tested storage temperatures. An ultra-low storage temperature of -55°C can maintain the physical and chemical properties of the fresh fillet better than the other two storage temperatures (-20°C and -30°C). Having that said, storage at -30°C mitigated the formation of TBA, VBN, inosine, hypoxanthin and texture changes, similar to the results at -55°C. However, the freshness of the frozen fillets changed from very fresh to moderately fresh after 4 months storage for all temperatures by the level of VBN and K values. A slight decrease in sensory score was recorded by the panelists. The VBN of the frozen fish remained at a fresh level for all freezer temperatures after 6 months storage. Nevertheless, the K values of the frozen fish remained at a fresh level (< 20%) for all freezer temperatures after 2 months storage except at -55°C for 4 months storage. Storage temperature at -30°C did not influence the color characteristic or texture profile, compared to the ones at -55°C. Given all the findings, we suggest that ultra-low storage temperature (-55°C) is not necessary to maintain the quality of frozen giant grouper fillets for commercial operations for 6 months storage except for special use such as sashimi product.

REFERENCES

AOAC. (1998). Methods of Analysis of the Association of Official Analytical Chemists (16th ed.). Association of Official Analytical Chemists, Washington DC, USA.

Aubourg, S. P., C. Piñeiro and M. J. González (2004). Quality loss related to rancidity development during frozen storage of horse mackerel (Trachurus trachurus). Journal of the American Oil Chemists Society 81, 671-678.

Barroso, M., M. Careche and A. Borderıas (1998). Quality control of frozen fish using rheological techniques. Trends in Food Science & Technology 9, 223-229.

Bogh-Sorensen, L. (2006). Recommendations for the process and handling of frozen foods. (4th ed.). International Institute of Refrigeration, Paris, France.

Burgaard, M. G. and B. M. Jørgensen (2010). Effect of Temperature on Quality-Related Changes in Cod (*Gadus morhua*) During Short- and Long-Term Frozen Storage. Journal of Aquatic Food Product Technology 19, 249-263.

Cappeln, G., J. Nielsen and F. Jessen (1999). Synthesis and degradation of adenosine triphosphate in cod (Gadus morhua) at subzero temperatures. Journal of the Science of Food and Agriculture 79, 1099-1104.

Careche, M., A. M. Herrero, A. Rodriguez-Casado, M. L. Del Mazo and P. Carmona (1999). Structural changes of hake (Merluccius merluccius L.) fillets: effects of freezing and frozen storage. Journal of Agricultural and Food Chemistry 47, 952-959.

Castell, C. H. and B. Smith (1973). Measurement of formaldehyde in fish muscle using TCA extraction and the NASH reagent. Journal of the Fisheries Research Board of Canada 30, 91-98.

Chaijan, M., S. Benjakul, W. Visessanguan and C. Faustman (2005). Changes of pigments and color in sardine (*Sardinella gibbosa*) and mackerel (*Rastrelliger kanagurta*) muscle during iced storage. Food Chemistry 93, 607-617.

Chow, C. J., Y. Ochiai, S. Watabe and K. Hashimoto (1988). Effect of Freezing and Thawing on the Discoloration of Tuna Meat. Nippon Suisan Gakkaishi, 54, 639-648.

Chow, C. J., Y. Ochiai and S. Watabe (2004). Effect of frozen temperature on autoxidation and aggregation of bluefin tuna myoglobin in solution. Journal of Food Biochemistry 28, 123-134.

Dulavik, B., N. K. Sørensen, H. Barstad, O. Horvli and R. L. Olsen (1998). Oxidative stability of frozen light and dark muscles of saithe (*Pollachius virens L.*). Journal of Food Lipids 5, 233-245.

FAO. (2011). Fish STAT fishery statistical collections global aquaculture production. http://www.fao.org/fishery/statistics/global-aquaculture-production/query/en Accessed 02.03.11.

FDA. (1998). Bacteriological analytical manual (8th ed.). Food and Drug Administration, Gaithersburg, MD, USA.

Gill, T. A., R. A. Keith and B. S. Lall (1979). Textural deterioration of red hake and haddock muscle in frozen storage as related to chemical parameters and changes in the myofibrillar proteins. Journal of Food Science 44, 661-667.

Guizani, N., M. A. Al-Busaidy, I. M. Al-Belushi, A. Mothershaw and M. S. Rahman

(2005). The effect of storage temperature on histamine production and the freshness of yellowfin tuna (*Thunnus albacares*). Food Research International 38, 215-222.

Haard, N. F. (1992). Control of chemical composition and food quality attributes of cultured fish. Food Research International 25, 289-307.

Howgate, P. (2005). Kinetics of degradation of adenosine triphosphate in chill-stored rainbow trout (*Oncorhynchus mykiss*). International Journal of Food Science & Technology 40, 579-588.

Huss, H. H., A. Reilly and P. K. B. Embarek (2000). Prevention and control of hazards in seafood. Food Control 11, 149-156.

Indergård, E., I. Tolstorebrov, H. Larsen and T. M. Eikevik (2014). The influence of long-term storage, temperature and type of packaging materials on the quality characteristics of frozen farmed Atlantic Salmon (*Salmo Salar*). International Journal of Refrigeration 41, 27-36.

Kim, Y. J., and D. R. Heldman (1985). Quantitative analysis of texture change in cod muscle during frozen storage. Journal of Food Process Engineering 7, 265-272.

Leelapongwattana, K., S. Benjakul, W. Visessanguan and N. K. Howell (2005). Physicochemical and biochemical changes during frozen storage of minced flesh of lizardfish (*Saurida micropectoralis*). Food Chemistry 90, 141-150.

Li, K., Y. Luo and H. Shen (2015). Postmortem Changes of Crucian Carp (*Carassius auratus*) During Storage in Ice. International Journal of Food Properties 18, 205-212.

Li, X. P., J. R. Li, J. L. Zhu, Y. B. Wang, L. L. Fu and W. Xuan (2011). Postmortem changes in yellow grouper (*Epinephelus awoara*) fillets stored under vacuum packaging at 0°C. Food Chemistry 126, 896-901.

Love, R. M. (1988). Their Intrinsic Variation and Practical Implications. In: The Food Fishes. Farrand Press, London, UK.

Mørkøre , T. and R. Lilleholt (2007). Impact of freezing temperature on quality of farmed Atlantic cod (*Gadus morhua* L.). Journal of Texture Studies 38, 457-472.

Magnussen, O. M. and S. Johansen (1995). Effect of freezing and storage temperatures at -25°C to -60°C on fat fish quality. In: 19th International Congress of Refrigeration, 249-257.

Martinez, O., J. Salmerón, M. D. Guillén and C. Casas (2010). Effect of freezing on the physicochemical, textural and sensorial characteristics of salmon (Salmo salar) smoked with a liquid smoke flavouring. LWT - Food Science and Technology 43, 910-918.

Mazorra-Manzano, M. A., R. Pacheco-Aguilar, E. I. Díaz-Rojas and M. E. Lugo-Sánchez (2000). Postmortem changes in black skipjack muscle during storage in ice. Journal of Food Science 65, 774-779.

Nawar, W. W. (1996). Lipids. In: Food Chemistry (3rd ed), edited by Fennema, O. R., Marcel Dekker Inc., New York, USA, 225-320.

Ocaño-Higuera, V. M., E. Marquez-Ríos, M. Canizales-Dávila, F. J. Castillo-Yáñez, R. Pacheco-Aguilar, M. E. Lugo-Sánchez, K. D. García-Orozco and A. Z. Graciano-Verdugo (2009). Postmortem changes in cazon fish muscle stored on ice. Food Chemistry 116, 933-938.

Potter, N. N. and J. H. Hotchkiss (1997). Cold preservation and processing. In Food Science (16th ed.), Chapman & Hall, New York, USA, 163-199.

Refsgaard, H. H. F., P. B. Brockhoff and B. Jensen (1998). Sensory and Chemical Changes in Farmed Atlantic Salmon (*Salmo salar*) during Frozen Storage. Journal of Agricultural and Food Chemistry 46, 3473-3479.

Saito, T., K. I. Arai and M. Matsuyoshi (1959). A new method for estimating the freshness of fish. Nippon Suisan Gakkaishi 24, 749-750.

Siddaiah, D., G. V. Sagar Reddy, C. V. Raju and T. C. Chandrasekhar (2001). Changes in lipids, proteins and kamaboko forming ability of silver carp (*Hypophthalmichthys molitrix*) mince during frozen storage. Food Research International 34, 47-53.

Stansby, M. E. (1963). Industrial fishery technology. Reinhold Publishing Co., New York, USA.

Steel, R. G. D. and J. H. Torrie (1980). Principle and Procedure of Statistics (2nd ed.), McGraw-Hill, NeW York, USA.

Stien, L. H., E. Hirmas, M. Bjørnevik, M., Ø. Karlsen, R. Nortvedt, A. M. B. Rørå, J. Sunde and A. Kiessling (2005). The effects of stress and storage temperature on the colour and texture of pre-rigor filleted farmed cod (*Gadus morhua* L.). Aquaculture Research 36, 1197-1206.

TFDA. (1998). Food sanitary standards for frozen foods (Taiwan FDA Food 8143635 Announcement) (3rd ed.), Food and Drug Administration, Ministry of Health and Welfare, Taipei, Taiwan.

TFDA. (2011). Food sanitary standards for raw fish, fruit and vegetable (Taiwan FDA Food 1001302260 Announcement) (3rd ed.), Food and Drug Administration, Ministry of Health and Welfare, Taipei, Taiwan.

Tironi, V., A. LeBail and M. de Lamballerie (2007). Effects of pressure-shift freezing and pressure-assisted thawing on sea bass (*Dicentrarchus labrax*) quality. Journal of Food Science 72, C381-387.

Uljas, H. E., and S. C. Ingham (1999). Combinations of intervention treatments resulting in 5-log10-unit reductions in numbers of Escherichia coli O157: H7 and Salmonella typhimurium DT104 organisms in apple cider. Applied and Environmental Microbiology 65, 1924-1929.

Vyncke, W. (1975). Evaluation of the Direct Thiobarbituric Acid Extraction Method for Determining Oxidative Rancidity in Mackerel (*Scomber scombrus* L.). Fette, Seifen, Anstrichmittel 77, 239-240.