Effect of frozen storage on the characteristics of a developed and commercial fish sausages

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Abstract The effect of frozen storage on the physiochemical, chemical and microbial characteristics of two types of fish sausages was studied. Fish sausages developed (DFS) with a spice-sugar formulation and commercial fish sausages (CFS) were stored at -20°C for 3 months. Fresh DFS contained 12.22% lipids and had a 3.53 cfu/g total bacteria count (TBC) whereas, CFS contained 5.5% lipids and had a 4.81 cfu/g TBC. During storage, TBC decreased significantly ($p<0.05$) in DFS whereas it did not change ($p>0.05$) in CFS. A peroxide value (PV) was not detectable until week four and eight of storage in CFS and DFS, respectively. The salt-soluble proteins (SSP) level was stable in DFS but in CFS it declined significantly ($p<0.05$). Colour values did not change significantly ($p>0.05$) in both sausage types. This study showed that the effect of storage at -20°C on fish sausages characteristics varied between formulations and depended on the ingredients of fish sausages.

Keywords Color · Fish · Frozen storage · Rancidity · Sausages

Introduction

Fish sausage is a minced fish or surimi based product manufactured from different fish species with added preservatives and flavors. Due to its relatively short shelf-life (Raju et al. 2003; Santiago 2004), fish sausage currently does not transport well resulting in an inconsistent product available to the consumer. Fish and fish products undergo several chemical and physical changes during frozen storage. These changes adversely affect frozen-fish product quality and storage stability. Although commercially used frozen storage temperatures suppress bacterial growth and spoilage with chemicals such as trimethylamine (Natseba et al. 2005), fish endogenous lipases and proteases remain active causing lipids and proteins deterioration (Siddaiah et al. 2001; Perez-Borla et al. 2002; Aranda et al. 2006).

In addition, frozen storage causes the denaturation of myofibrillar proteins of fish which results in the loss of protein functions such as water holding capacity and gel-forming ability (Benjakul et al. 2003). The use of lower temperatures, such as -80°C , to minimize the frozen storage deterioration is promising (Santiago 2004), but such temperatures have not been applied commercially probably due to their high economic cost. Therefore at a commercial level, fish sausages are frozen at a higher freezing temperature, usually -20°C , in which some quality attributes such as flavour and texture may deteriorate due to changes in lipids and proteins.

Many spices show antioxidant properties that enhance the stability of oils (Gulcin 2005; Tsai et al. 2005). This ability is due to their potential as free radical scavengers

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which may terminate radical chain reactions (Singh et al. 2005). They may also display antibacterial effects against bacterial pathogens such as *Bacillus cereus*, *Staphylococcus aureus* (Banerjee and Sarkar 2003; Kumudavally et al. 2011), *Salmonella* Enteritidis (Benkeblia 2004) and *Shigella* spp. (Bagamboula et al. 2004), as well as against spoilage bacteria such as *Aeromonas hydrophila* (Fabio et al. 2003). The bacteriostatic effects of spices may occur by two mechanisms: the delay and partial inhibition of DNA and proteins synthesis (Feldberg et al. 1988), and the induction of intracellular ATP depletion (Oussalah et al. 2006).

Many carbohydrates also show potential to stabilize proteins during frozen storage as cryoprotectants (Dondero et al. 1996; Jittinandana et al. 2003; Auh et al. 2003). Despite some controversies around the exact mechanisms by which carbohydrates act as cryoprotectants, it is widely accepted that they stabilize proteins by hydrating macromolecules thus reducing the amount of water produced from proteins during freezing (Mackie 1993). In addition carbohydrates, in particular sucrose was reported to inhibit the formation of biogenic amines in dry cured fish at a low storage temperature (4 °C) (Zhang et al. 2011). Although a spice-sugar formulation is used in fish sausages (Rahman et al. 2007), information on the effect of frozen storage on the microbial, chemical and physiochemical characteristics of fish sausages is limited.

It should be noted that commercial fish sausages are produced and consumed in the Middle East, but little is known about their microbial, chemical and physical characteristics and storage stability. This study aimed to study the effect of frozen storage at −20 °C on DFS with a spice-sugar formulation and a CFS type sold in Oman.

Materials and methods

Commercial sausages Commercial fish sausages were produced from fillets of crimson snapper (*Lutjanus erythropterus*), a demersal fish species, with 1.35% sodium sulphite (E 221) as an antimicrobial agent. No spices or other ingredients were included in the formulation. The sausages were produced in a local supermarket, Oman, and packed in sausage casings. A total of 5 kg of fresh sausages were purchased immediately after processing and brought to the laboratory within 30 min.

Developed fish sausage Newly developed fish sausages were produced from fillets of Arabian Sea meagre (*Argyrosomus heinii*), a demersal fish species. Fish fillets were provided by the Oman Fisheries Company, Ghala, Muscat, Oman. Food additives were bought from a local food market. Onion, garlic, and ginger were used in powdered form. The recipe was modified from Rahman et

al. (2007) and consisted of minced fish 61.9%, corn starch 8%, NaCl 2.5%, sucrose 0.7%, vegetable shortening 10%, ice water 16%, white pepper 0.2%, onion 0.17%, ginger 0.133%, garlic 0.083%, cinnamon 0.133% and cumin 0.133%. A total of 5 kg of fish sausage was produced in the laboratory of the Ministry of Agriculture and Fisheries, Muscat, Oman under hygienic conditions at 15–20 °C.

Fish fillets were minced in an industrial client cutter (JICA, Japan) and mixed thoroughly with starch and other ingredients. Once homogenized, the mixture was stuffed manually using a sausage maker (Rost Frei, Japan) into sausage casings (Devro, UK) to yield 10 cm length and 3 cm diameter fish sausage. Immediately, three fresh sausages along with three from CFS were used for analyses at week zero. Fish sausages were packed in polyethylene bags and frozen at −20 °C for 3 months, along with 5 kg of CFS. Both sausage types were analyzed at two-week intervals.

Sample preparation for analyses At two-week intervals, three frozen sausages of each type were defrosted indirectly under running water at 15–25 °C for 30 min. This process of thawing was found to recover the lowest number of microorganisms (Ersoy et al. 2008). Using a sterile knife, chopping board and forceps, defrosted sausage was chopped and mixed. Immediately, sausage (5 g) was removed from each sausage piece for microbial analysis and the rest was used for chemical analysis.

Enumeration of total bacterial count Sausage (5 g) was homogenized with 45 ml 0.1% most recovery diluent (Oxoid, UK) for 1 min in a Colworth stomacher 400 (UK) (ICMSF 1978). 10-fold serial dilutions were prepared and 0.1 ml sausage suspension was spread on plates of standard plate count agar (Oxoid, UK). Total bacteria were enumerated by incubating the plates aerobically at 27 °C for 3 days (Curran et al. 1981).

Proximate composition Water, crude lipids, crude proteins and ash content of fish and sausages were determined according to the Association of Official Analytical Methods, AOAC (Helrich 1990). Water content was determined by drying a sample (5 g) in a 300 plus series atmospheric oven (Gallenkamp, UK) at 105 °C to a constant weight (3 h). Crude lipids were determined by extracting a dried sample (2 g) with 100 ml petroleum ether for 8 h in a soxhlet apparatus. Crude proteins were determined in a micro Kjeldahl (Gallenkamp, UK) by digesting a dried sample (0.5 g), distillation of ammonium sulphate and titrating liberated nitrogen with 0.2 N hydrochloric acid. Ash was determined by burning a dried sample (5 g) to white-gray ash at 550 °C for 3 h in a muffle furnace (Gallenkamp, UK).

Peroxide value Samples for PV determination were dried at 105 °C for 3 h. Peroxide value is an index of rancidity and was determined according to the method of Egan et al. (1981). Dried sample (5 g) was mixed with 25 ml of a mixed solution (acetic acid : chloroform, 3:2). Then, 1 ml of saturated potassium iodide was added and the sample was kept in a dark place for 10 min. A total of 30 ml of distilled water was added and the liberated iodine was titrated with 0.01 N sodium thiosulfate in the presence of 1 ml of freshly prepared 1% starch until the blue color disappeared. Peroxide value was calculated as meq/kg fat according to the following formula:

$$PV = [(A - B) \div S] \times 10$$

Where A is the titration value for the sample, B is the titration value for the blank and S is the weight of the sample.

Protein solubility Salt-soluble proteins index, as a measure of proteins denaturation were determined according to the method of Ironside and Love (1958). Samples (6.67 g) were mixed with 100 ml of chilled 5% NaCl and the pH was adjusted to 7–7.5. The mixture was macerated for 2 min at high speed in a high speed homogenizer (Black and Decker, USA). The homogenate was transferred into a thick-walled 50 ml centrifuge tube by rinsing with chilled 5% NaCl. The mixture was centrifuged at 4,000 rpm for 30 min at 4 °C.

The supernatant was collected and the precipitate was washed with 10 ml of chilled 5% NaCl and centrifuged as above. An aliquot of the combined supernatant (salt-soluble proteins) was mixed with 10 ml of chilled 15% trichloroacetic acid and centrifuged as above to remove non-protein nitrogen compounds. Half gram of precipitated proteins and fish sausages were used to determine the percentages of salt-soluble and total proteins respectively using micro-Kjeldahl analyzer (Gallenkamp, UK). The percentage of SSP was calculated according to the following formula:

$$SSP = (\text{Salt - soluble proteins} / \text{Total proteins}) \times 100.$$

Colour The colour of *Argyrosomus heini* fillets and sausages was determined after defrosting using a color meter (Minolta Chromameter, Model CR-310, Japan) according to the method of Rahman et al. (2002). Three fillets of *Argyrosomus heinii* and fish sausages of both types were used for colour determination. Colour was expressed in Hunter *a*, *b*, and *L* values, where + *a* is intensity of red, −*a* is intensity of green, + *b* is the intensity of yellow, −*b* is intensity of blue, and *L* is lightness or darkness of the sausage, black, *L*=0; white, *L*=100 (Hamre et al. 2003).

Statistical analysis Bacterial numbers are reported as log₁₀ cfu/g. A one way ANOVA was used to evaluate the effect of

frozen storage on the bacterial count, salt-soluble proteins solubility, peroxide value and colour. This test was conducted in Minitab release 15 software (Minitab Inc., USA), and level of *P*<0.05 was considered statistically significant. Data were presented as the mean of two to three determinations.

Results and discussion

Characteristics of fresh fish sausages Tables 1, 2 and 3 show the chemical, microbial and physical characteristics of both types of fish sausage. The bacterial loads on DFS and CFS were lower than the log 5 cfu/g recommended level for good quality products (ICMSF 1986), indicating good microbial quality of fresh fish sausages of both types. This could be attributed to the quality of the raw fish, hygienic processing conditions and effect of the spice-sucrose recipe. In particular the antimicrobial activity of the spices may have played a role. Some spices such as garlic were found to kill 93% of *Staphylococcus epidermidis* and *Salmonella* Typhi within 3 h of incubation (Arora and Kaur 1999). Many spices show antimicrobial activity even at very low concentrations. For instance, the minimum inhibitory concentration of garlic was found to be 6–10 mg/ml for some bacteria such as *S. aureus* (Benkeblia 2004).

Due to its higher lipids content it might be expected that rancidity would commence earlier in DFS than CFS since, for example, horse mackerel with a relatively low lipids content (0.7–1.85%) was found to develop rancidity after 3 months of storage at −20 °C (Aubourg et al. 2004). Variations in characteristics and composition between sausage types will, of course, affect their sensory properties. Amano (1965) reported that routine examination of fish sausages revealed contents of 67–68% water, 5–6% lipids and 14–15% protein. In addition, Chuapoehek et al. (2001) found 74.50% water, 3.16% lipids, and 13.73% proteins in catfish sausages. The results of these studies were similar to those obtained for CFS in the present study.

Table 1 Proximate composition of developed (DFS) and commercial (CFS) fish sausages

Content	DFS	CFS
Water%	53.2±1.62 ^a	68.3±0.03 ^b
Crude lipids%	12.2±0.58 ^a	5.5±0.00 ^b
Crude proteins%	15.7±0.14 ^a	19.7±0.24 ^b
Ash%	2.5±0.09 ^a	1.8±0.00 ^b

Each mean was compared with that of other sausage type. Means with different alphabetical superscript are significantly different (*p*<0.05), *n*=3

Table 2 Microbiological and chemical changes in fish sausages during storage at -20°C

Storage (week)	Total Bacterial counts (log cfu/g)		Peroxide value (meq/kg fat)		% Salt-soluble proteins	
	DFS	CFS	DFS	CFS	DFS	CFS
0	3.5±0.07 ^a	4.8±0.14 ^a	ND	ND	37.5±1.98 ^a	37.5±1.98 ^a
2	3.3±0.07 ^a	5.1±0.32 ^a	ND	ND	39.3±3.29 ^a	28.3±2.50 ^b
4	3.3±0.10 ^a	4.9±0.17 ^a	ND	8.0±2.0 ^a	36.2±0.36 ^a	30.4±2.48 ^c
6	2.9±0.24 ^b	5.0±0.12 ^a	ND	10.6±1.15 ^b	36.0±6.95 ^a	22.9±0.37 ^d
8	2.9±0.19 ^c	4.8±0.24 ^a	14.6±1.15 ^a	17.3±1.15 ^c	40.7±2.53 ^a	23.0±0.43 ^c
10	3.0±0.08 ^d	4.6±0.20 ^a	16.6±0.57 ^b	22±2.0 ^d	36.7±4.06 ^a	22.5±.77 ^f
12	2.8±0.04 ^c	4.7±0.61 ^a	25.3±3.0 ^c	22.6±1.15 ^c	35.3±0.53 ^a	–

Each mean was compared with that of 0 h. Means with different alphabetical superscript are significantly different ($p<0.05$), $n=3$

DFS developed fish sausage

CFS commercial fish sausage

Colour is one of the important quality criteria, which determines the acceptability and marketability of many fish mince products (Sachindra and Mahendrakar 2010). The low a and high L values of DFS indicated this product was processed from a low-myoglobin fish and that food additives did not interfere with the colour of DFS. The high a value for CFS, on the other hand, indicated that the product was processed from red muscled fish. Moreover, the L value of 55.24 in CFS indicated that the product started darkening before storage as the L value for darkening onset has been set at 58 (Ochiai et al. 1988). The b values of 16.64 and 12.80 in DFS and CFS, respectively, were considered of low significance in fresh sausages since no colour materials were added to either type.

Changes in the characteristics of fish sausages during frozen storage The TBC for DFS (Table 2) decreased significantly ($p<0.05$) after week four from 3.53 log cfu/g at the beginning of storage to 2.84 log cfu/g at the end of 3 months frozen storage indicating the effect of the antibacterial spices. Frozen storage at -20°C did not result in any change ($p>0.05$) in the initial bacterial load of CFS (4.81 log cfu/g). The antibacterial capacity of the spices in bacterial reduction may be attributed to the effect of

cinnamon, garlic, onion, and cumin (Wendakoon and Sakaguchi 1992; Tabak et al. 1999; Mau et al. 2001; Fabio et al. 2003; Benkeblia 2004; Jirovetz et al. 2005; Thongson et al. 2005; Arici et al. 2005; Das et al. 2011).

Freezing results in the loss of the ability of bacteria to multiply and in sub-lethal injury, and it seems that this effect depends on the freezing temperature and composition of the bacterial community present. This could explain the absence of changes in the bacterial load of the CFS. The absence of change in bacterial load of CFS during frozen storage agreed with the finding of Rota and Gonzalez (2006) who indicated that frozen storage at -18°C was not a significant factor in the bacterial count. In addition, Moorhead and Dykes (2002) found that aerobic bacterial levels did not decrease on beef trimming during storage at -18°C over 84 days.

By contrast, Abd-El-Rahman (2002) found that total bacterial count increased at -20°C during storage for 6 months, and Al-Harbi and Uddin (2005) found that aerobic plate count were reduced 2 log cycles after 1 month on hybrid tilapia at -20°C . Since frozen storage was found to stop microbial activity (Karacam and Boran 1996), it could be expected that the relative high bacteria load would not be involved in any quality deterioration in commercial sausages during frozen storage.

Table 3 Changes in the colour values of fish sausages during storage at -20°C

Storage, week	DFS			CFS		
	a	b	L	a	b	L
0	2.4±0.02 ^a	16.6±0.61 ^a	60.9±0.42 ^a	11.9±1.15 ^a	12.8±0.29 ^a	55.2±0.08 ^a
2	2.5±0.05 ^a	15.5±0.90 ^a	61.9±0.44 ^a	12.0±0.53 ^a	12.8±0.14 ^a	54.9±0.71 ^a
4	2.5±0.05 ^a	15.2±0.26 ^a	59.8±1.51 ^a	12.4±0.23 ^a	12.2±0.14 ^a	56.4±2.10 ^a
6	2.3±0.09 ^a	15.2±0.03 ^a	61.4±0.32 ^a	12.8±0.02 ^a	12.2±0.08 ^a	55.5±0.15 ^a
8	2.7±0.05 ^a	15.4±0.02 ^a	59.5±0.38 ^a	12.2±0.32 ^a	12.9±0.16 ^a	57.0±0.69 ^a
10	2.3±0.17 ^a	15.4±0.39 ^a	61.1±2.35 ^a	12.1±0.19 ^a	11.9±0.57 ^a	54.9±0.57 ^a
12	2.5±0.19 ^a	15.9±0.03 ^a	61.1±2.35 ^a	12.1±0.25 ^a	12.1±0.21 ^a	55.4±0.65 ^a

Each mean was compared with that of 0 h. Means with different alphabetical superscript are significantly different ($p<0.05$), $n=3$

DFS developed fish sausage

CFS commercial fish sausage

The development of lipid oxidation assessed by peroxide value is shown in Table 2. Initially, PV was detectable in DFS at week eight, whereas in CFS it was detectable at week four. Thereafter PV increased significantly ($p<0.05$) in DFS and CFS from 14.66 to 25.33 meq/kg fat and 8 to 22.66 meq/kg fat, respectively, by the end of storage. Despite these significance changes, PV in both sausages were at the low end of 20–40 meq/kg fat for a noticeable rancid taste in oil (Egan et al. 1981).

Although the antioxidant potential of ginger, garlic and cinnamon have been reported (Salariya and Habib 2003; Shan et al. 2005; Al-Assaf and Abdullah 2005), the antioxidant activities of the spices in food systems were found to be low and dose-dependable (Al-Ismail 2002; Yin and Cheng 2003). In studies where, for example, ginger was used to extend frozen fish shelf-life, peroxide values was found to develop at a much lower rate than in untreated samples (George and Perigreen 1999).

In our study, the spice powders were effective in delaying lipid oxidation, however, they were not able to stop lipid oxidation due to low antioxidant concentration. The increase in the peroxide value in CFS agreed with the study of Siddaiah et al. (2001) who found that peroxide value increased significantly in mined silver carp during storage at -18°C . This increase, however, was much higher than it was found in frozen horse mackerel with an initial lipid content of 0.7–1.85% (Aubourg et al. 2004).

Changes in SSP as an indicator of progressive denaturation in both sausages during frozen storage are shown in Table 2. Salt-soluble proteins decreased significantly ($p<0.05$) and linearly after week zero from 37.58% to 22.52% in CFS during 3 months frozen storage. Salt-soluble myofibrillar proteins did not change significantly ($p>0.05$) in DFS, which maintained its SSP content at 37.42% on average throughout 3 months storage, showing a very low impact of storage time on SSP. Protein stability during frozen storage in DFS could be attributed to the positive cryoprotectant effect of added sucrose, as sucrose-sorbitol either alone or as a mixture was found to act as a cryoprotectant in the manufacture of surimi (Mackie 1993).

In surimi the cryoprotectants have been shown to preserve the structural stability of myofibrillar proteins and reduce the exposure of buried hydrophobic residues on the proteins surface, thus slowing down the kinetics of aggregation of proteins (Herrera and Mackie 2004). Among the factors that were found to decrease myofibrillar protein solubility, the interaction of lipid oxidized products with proteins was found to be important (Shobana and Naidu 2000). This process involves the interaction of oxidized lipids with the cystine—SH group, the NH_3 group of lysine, and N—terminus group of aspartic acid, tyrosine, methionine, and arginine (Kussi et al. 1975, cited by Siddaiah et al. 2001).

It is apparent from Table 2 that both PV detection and SSP reduction occurred at the early stages of storage which could show this interaction thus explaining the significant reduction in the solubility of CFS proteins. In addition, the significant reduction in protein stability in CFS was in agreement with the findings of Siddaiah et al. (2001), Benjakul et al. (2003) and Tokur et al. (2006) who found significant reduction in solubility of fish and fish finger proteins. Protein denaturation in CFS may lead to toughness of the product (data not shown), particularly since toughness in myotomal tissues in fish and fishery products have been attributed to the denaturation of myofibrillar proteins and the decrease in Z lines (Dunajski 1979; Shenouda 1980, cited by Siddaiah et al. 2001).

The colour values of sausage during frozen storage can be seen in Table 3. *a*, *b*, and *L* values in both sausages did not change significantly ($p>0.05$) throughout the storage period, indicating no significant effects of the food additives and storage conditions on the products colour. On average, DFS had *a*, *b*, and *L* values of 2.44, 16.64, and 60.93 respectively, whereas, in CFS these values were 11.90, 12.80, and 55.24 respectively during the 3-month storage period. The *b* and *L* values were significantly higher ($p<0.05$) in DFS than in CFS, whereas the *a* value was significantly higher ($p<0.05$) in CFS than in DFS.

These values agreed with that of Al Bulushi et al. (2005) who found no significant changes in the colour values of fish burgers processed from *Argyrosomus heinii* and stored under the same conditions as in this study. Lipid oxidation has been found to be associated with an increase in *b* value (yellowness) causing discoloration of herring fillets and squid (Hamre et al. 2003; Thanonkaew et al. 2006) via the interaction of oxidized products with amines in proteins. However, our study did not support this finding as the increasing peroxide values did not correlate with an increase in *b* values during frozen storage.

Conclusion

Developed fish sausages with a modified spices-sucrose mix maintained their microbial and chemical characteristics for 3 months at -20°C . In contrast, the characteristics of commercial fish sausages deteriorated during frozen storage. The efficiency of commercial frozen storage temperature of -20°C in maintaining the characteristics of fish sausage during storage was found to be sausage-ingredient dependent.

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