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Lipid Oxidation in Charqui (Salted and Dried Beef)*

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ABSTRACT

Charqui, a popular salted and dried beef product consumed in Brazil, was produced using 20% salt (w/w) with the following salt preparations: (1) refined salt, (2) rock salt, (3) refined salt plus BHA/BHT, (4) refined salt plus α-tocopherol, and (5) refined salt plus nitrate. Samples of charqui were analyzed after 0, 15, 30 and 60 days' storage at room temperature. Lipid oxidation was monitored by the TBA test, hexanal development and quantitation of cholesterol oxidation products. Charqui prepared using rock salt had the highest initial TBA numbers, apparently due to contamination by Fe and Cu ions. The antioxidants were generally effective in delaying oxidation for up to 15–30 days, but were ineffective on longer storage. Losses of the unsaturated fatty acids occurred in both the triglyceride and phospholipid fractions, even in samples containing antioxidants. Although the hexanal content and cholesterol oxides increased in all treatments during storage, they tended to be lower in the antioxidant-containing salt treatment. Results indicate that the use of refined salt containing antioxidants retarded lipid oxidation during storage of charqui.

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INTRODUCTION

Charqui is a typical Brazilian dried beef product. It is prepared by exposing salted beef (20% w/w NaCl) to the sun for drying. The product is then ready for consumption and normally is shelf stable for six months when left unpackaged (Pardi, 1961). Charqui is a very popular product in Brazil, since it does not need refrigeration. Therefore, it is of great importance in rural areas where there is often a lack of refrigeration.

Charqui has been prepared and consumed for many years in Brazil, even though the processing and biochemical changes have not been closely examined. Processing conditions used in production of charqui would be expected to promote lipid oxidation. First, the high salt content along with trace metals in the salt could serve as prooxidants in the meat system. Secondly, exposure of the meat to sunlight at temperatures ranging from 25–35°C would also be expected to induce rapid oxidation of the lipid components.

Most products of lipid oxidation, such as malonaldehyde and oxides of cholesterol, have recently been brought to the attention of the scientific community because of their possible relationship to an increased incidence of cancer. This relationship has been reviewed by Pearson *et al.* (1983) and Addis (1986). Thus, the high level of consumption of charqui in Brazil may have a deleterious effect on the health of consumers. The present work addresses the storage stability of charqui and the applicability of using antioxidants as a means of preventing or at least minimizing the degree of rancidity in this popular Brazilian dried beef product.

MATERIALS AND METHODS

Processing of charqui

Freshly excised beef *semitendinosus* muscle was cut in pieces about 5 cm in thickness, and salted (20% w/w salt) using the following treatments: (1) refined salt; (2) rock salt; (3) refined salt coated with a 0·76% BHA/BHT (w/w, 1:1) with the ingoing antioxidant level being 0·02% based on fat content; (4) refined salt coated with 3·8% of α-tocopherol to give an ingoing antioxidant level of 0·1% based on fat content and (5) refined salt plus nitrate (500 ppm based on salt content). The samples were cured for 5 days on trays at room temperature (21 ± 2°C). After curing, the beef was washed in running tap water to remove excess salt and then hung and dried for 2 days at room temperature. The experiment was replicated four times. Samples were analyzed after 0, 15, 30 and 60 days' storage at room temperature. Each sample weighed about 160 g and was sliced and then homogenized.

Measurement of lipid oxidation

The 2-thiobarbituric acid (TBA) assay of Tarladgis *et al.* (1964), which uses an aqueous solution of TBA to replace the acetic acid/H_2O reagent, was modified as described by Crackel *et al.* (1988) using a 10 g sample to follow lipid oxidation. Absorbance of the distillates was measured at 532 nm using a Spectronic 2000 spectrophotometer (Bausch and Lomb, Rochester, NY).

Hexanal was extracted from 50 g of charqui with diethyl ether in a Likens & Nickerson (1964) apparatus. The extracts were stored in screw-top vials at −20°C until analysis. The extracts were thawed and analyzed for hexanal using a Hewlett Packard GC 5890 gas chromatograph fitted with a 0·25 mm (id) × 30 m capillary column coated with Carbowax 20M (AllTech Associates) and an integrator (HP 3392A). The injection volume was 2 μl with a split ratio of 1:20. The GC was operated in the temperature program mode at an initial temperature of 40°C for 10 min, which was then increased at 5°C/min to a temperature of 60°C. The oven temperature was subsequently increased by 15°C/min until 180°C and then held at this temperature for 10 min to remove all other volatiles. The injection port temperature was 200°C, the detector temperature was 275°C and helium was the carrier gas at a flow rate of 20 ml/min. Hexanal was identified by comparing the retention time of the unknown peak to that obtained from a hexanal standard assayed under identical conditions.

Fatty acid analysis

The dry column method of Marmer & Maxwell (1981) was used for simultaneous extraction and separation of the charqui lipids into neutral and phospholipid fractions, although we recognized that there is some carry over of the neutral lipids into the phospholipid fraction. However, Crackel *et al.* (1988) found that the phospholipids of beef obtained by the Marmer & Maxwell (1981) method were quantitatively similar to values obtained by silic acid column chromatography. Furthermore, there was little difference in the fatty acid composition of the phospholipids separated from beef by the Marmer & Maxwell (1988) procedure from those obtained using preparative absorption thin-layer chromatography. Thus, the carry over of neutral lipids under our conditions appears to be minimal and did not adversely affect our results for the phospholipids. Methylation was carried out using the boron–trifluoride–methanol procedure of Morrison & Smith (1964). The methylated samples were analyzed using a HP 5480A gas chromatograph equipped with a flame ionization detector (FID) and a HP 5840A GC integrator. The glass column (2 mm id × 3 m) was packed with 10% SP-2330 on 100/120 mesh Supelcoport. The GC was operated using an initial temperature of 150°C, which was maintained for 1 min and

then increased at 15°C/min to a final temperature of 225°C that was maintained for 10 min. The injection port temperature was 200°C, the FID temperature was 300°C while N_2 was the carrier gas. The component fatty acids were identified by comparing retention times to those obtained from lipid standards assayed under identical conditions.

Cholesterol and its oxidation products

A total of 100 mg of neutral lipids from charqui was extracted using the dry column procedure of Maxwell & Marmer (1981). Then 100 μl 5α-cholestane was added as an internal standard and the sample was cold-saponified with 1 ml 0·1N KOH/EtOH overnight (18 h) at room temperature in the dark (Park & Addis, 1985; Addis, 1986). The non-saponifiables were extracted with diisopropylether. Cholesterol and its oxidation products were converted to their trimethylsilyl derivatives by adding 50 μl bis-trimethylsilyl trifluoroacetamide containing 1% trimethylchlorosilane and 100 μl silylation-grade pyridine (Pierce Chemical Co., Rockford, IL) to a portion of the extract and reacting for 30 min at room temperature. A 1 μl sample was injected in a HP 5890 gas chromatograph fitted with 0·25 mm (id) × 15 m capillary column coated with polydimethylsiloxane (AllTech Associates). The GC was operated with a temperature program at a rate of 10°C/min from 180°C to 230°C, then at a rate of 0·2°C/min from 230°C to 244°C. The injection port temperature was maintained at 300°C and the detector at 275°C. Helium was the carrier gas at a flow rate of 17 ml/min and a split ratio of 1:20. Cholesterol and its oxides were identified by comparison to standards analyzed under identical conditions (Park & Addis, 1985). The GC was connected to an HP A3392 integrator in order to provide quantitative data on the area of the peaks.

Iron and copper analysis

The salt sample was first dissolved in a small amount of distilled deionized water (D–D H_2O) and diluted to 1 g salt/ml. Then 5 ml of concentrated nitric acid were added. The sample solution was diluted with D–D H_2O to give the appropriate concentration of the elements (Cu or Fe) to be measured. The concentrations of copper and iron were determined by flame atomic absorption spectrophotometry (Model IL 951, Instrumentation Laboratory, Inc., Lexington, MA) using aqueous calibration standards and the parameters specified for the instrument according to the manufacturer's recommendations.

Statistical analysis

Wherever possible, the data were analyzed by analysis of variance using MSTAT (1982). Application and interpreation of these procedures was in accordance with the methods described by Steel & Torrie (1980).

RESULTS

TBA numbers

TBA numbers were expressed as milligrams of malonaldehyde per kilogram of sample. The numbers were obtained by multiplying sample absorbance by a factor of 6·32. The factor is smaller than that reported by Tarladgis *et al.* (1960). Recovery in this experiment was 76·50% in comparison to 68·00% in the earlier study.

The TBA numbers of charqui prepared with refined salt were initially low, reached a maximum after 30 days of storage and then generally decreased (Table 1). Similar results have been reported for other meat products where decreases in TBA numbers have been noted to occur during storage. Results can be explained by the observations of Melton (1983) who concluded that the TBA test measures malonaldehyde accurately only at the initiation and propagation stages of oxidation. The added antioxidants (BHA/BHT and α-tocopherol) were effective in decreasing TBA numbers during the first 2 weeks of storage. Thereafter, however, the antioxidant-treated samples showed no significant differences in TBA values in comparison to the other treatments. The initial TBA numbers for the samples containing rock salt

TABLE 1
Effects of Different Salt Treatments upon the TBA Numbers of Charqui Stored for Various Time Periods up to 60 Days

Time (days)	*TBA numbers (mg malonaldehyde/kg sample)*				
	Refined salt	*Rock salt*	*Refined salt + BHA/BHT*	*Refined salt + α-tocopherol*	*Refined salt + nitrate*
0	$1{\cdot}53^{a}$	$7{\cdot}04^{c}$	$1{\cdot}51^{a}$	$1{\cdot}47^{a}$	$5{\cdot}21^{c}$
15	$2{\cdot}02^{a}$	$3{\cdot}76^{a,d}$	$1{\cdot}58^{a}$	$2{\cdot}36^{a}$	$2{\cdot}20^{a,b}$
30	$4{\cdot}31^{b}$	$2{\cdot}00^{a}$	$3{\cdot}06^{b,c}$	$3{\cdot}43^{b}$	$4{\cdot}42^{b,c}$
60	$2{\cdot}47^{a,b}$	$4{\cdot}52^{c,d}$	$3{\cdot}45^{b,c}$	$3{\cdot}09^{b}$	$2{\cdot}74^{a,b}$

Each value is the mean of four replicate samples analyzed in duplicate. Values in the same column and row bearing different superscripts were significantly different at $P < 0{\cdot}05$.

TABLE 2
Effects of Refined Salt and Refined Salt plus BHA/BHT on TBA Numbers During Processing and Storage

Time (days)	*TBA numbers (mg malonaldehyde/kg sample)*	
	Refined salt	*Refined salt plus BHA/BHT*
	Processing	
0	0·98	0·98
1	0·45	0·42
2	0·93	0·29
3	0·77	0·73
4	1·26	0·75
	Storage	
0	1·89	0·98
7	2·51	1·58
15	2·27	1·84
30	5·65	2·30

Each value represents the mean of two replicates that were analyzed in duplicate.

were much higher than other treatments ($P < 0·05$). Trace metal analysis demonstrated that the rock salt contained 115 and 1 ppm of Fe and Cu, respectively. Charqui produced in Brazil is processed with rock salt, but the trace metal content of the salt is unknown. Although the charqui used in this study was produced without exposure to sunlight, it was similar to the Brazilian product in color, texture, appearance, and flavor.

Because of the high initial TBA numbers obtained, another experiment using BHA/BHT was conducted to verify the effects of salting and drying upon TBA numbers with the data being presented in Table 2. Results indicate that the antioxidant effect was partially depleted during processing. Thus, the BHA/BHT treatment was effective in preventing oxidation as measured by TBA numbers during the first 15 days of storage, although there appeared to be some carry over for up to 30 days. The lack of packaging in charqui is another factor that may be a problem in oxidation. Good packaging may be helpful in retarding oxidation of charqui and should be investigated.

Nitrate

The application of nitrate to the charqui did not have any apparent antioxidant effect. Thus, results suggest that the initial high salt level

TABLE 3
Hexanal Content of Charqui Prepared from Different Salt Preparations During 60 Days' Storage

Storage (days)	*Hexanal concentration (ppb)*				
	Refined salt	*Rock salt*	*Refined salt + BHA/BHT*	*Refined salt + α-tocopherol*	*Refined salt + nitrate*
0	$0{\cdot}55^{a}$	$0{\cdot}12^{a}$	$0{\cdot}55^{a}$	$0{\cdot}95^{b}$	$1{\cdot}05^{b}$
15	$4{\cdot}58^{b,c}$	$3{\cdot}40^{b}$	$3{\cdot}60^{b}$	$5{\cdot}40^{c}$	$6{\cdot}00^{c}$
30	$4{\cdot}80^{b}$	$4{\cdot}80^{b}$	$9{\cdot}60^{d}$	$8{\cdot}80^{d}$	$8{\cdot}60^{d}$
60	$8{\cdot}60^{d}$	$11{\cdot}00^{a}$	$9{\cdot}00^{d}$	$9{\cdot}40^{d}$	$7{\cdot}40^{e}$

Values are means of four replicates analyzed in duplicate. Values in same row and line followed by different superscripts are statistically significant at $P < 0{\cdot}05$.

prevented growth of microorganisms that are capable of reducing nitrate to nitrite. It may be possible to prevent oxidation by using nitrite instead of nitrate since it has antioxidative properties in cured meat.

Hexanal levels

Hexanal levels (Table 3) also showed that a high degree of lipid oxidation occurred after 30 days' storage for all treatments. Results of this study indicate that hexanal is a good measure of oxidation. Shahidi *et al.* (1987) investigated the potential of hexanal content as a measure of oxidative stability in cooked ground pork and reported that the hexanal content and sensory scores were linearly interrelated. Results of hexanal analysis also support the work of Fritsch & Gale (1977), who found good agreement between hexanal content and sensory scores in oat products.

Changes in fatty acids

Changes in the fatty acid composition of the triglycerides and phospholipids of the best (refined salt and BHA/BHT) and worst (rock salt) treatments are shown in Table 4 and reflect a loss in the proportion of polyunsaturated fatty acids during storage of charqui, which was accompanied by a corresponding increase in the saturated fatty acids. This was true in all salt treatments in both the triglycerides and phospholipids. The decrease in the percentage of unsaturated fatty acids of the triglyceride fraction during 60 days' storage amounted to 13·9% for the refined salt treatment, which suffered the greatest loss, in comparison to 8·6% and 3·4% for the rock salt and the refined salt + BHA/BHT treatments, respectively. These results

TABLE 4

Changes in the Fatty Acid Composition of the Triglycerides (TG) and Phospholipids (PHOS) in Charqui Prepared with Refined Salt, Rock Salt, and Refined Salt plus BHA/BHT During 60 Days' Storage[a]

	Treatments											
	Refined salt				*Rock salt*				*Refined salt + BHA/BHT*			
	TG		*PHOS*		*TG*		*PHOS*		*TG*		*PHOS*	
	0[b]	*60*[b]	*0*	*60*	*0*	*60*	*0*	*60*	*0*	*60*	*0*	*60*
Fatty acids												
14:0	2·68	4·19	1·10	1·65	3·92	4·28	1·60	3·50	3·77	3·79	1·94	2·36
14:1	5·64	—	—	—	0·42	—	—	—	0·06	—	—	—
16:0	22·48	31·39	21·80	24·22	28·02	29·55	24·13	24·77	27·19	31·10	22·79	24·71
16:1	3·80	—	0·18	—	1·91	—	0·29	—	1·74	—	0·68	—
18:0	10·87	13·53	8·70	10·55	11·83	20·02	10·20	12·43	11·95	11·81	9·86	13·19
18:1	37·50	38·08	30·57	32·85	44·98	38·94	32·85	33·30	44·68	47·82	34·35	36·21
18:2	3·08	4·07	12·25	11·62	2·88	6·69	12·28	10·57	3·25	1·55	13·52	10·58
18:3	—	—	—	—	—	—	—	—	0·14	—	—	—
20:0	2·01	1·91	3·15	3·31	1·65	0·51	2·65	3·44	1·75	0·45	2·09	4·04
20:2	0·07	4·81	2·34	1·93	0·54	—	0·47	2·19	0·25	0·45	0·32	1·13
20:4	1·30	0·86	6·13	4·82	0·14	—	6·79	3·32	0·26	1·67	3·32	2·19
22:0	0·25	1·15	3·05	3·52	0·34	—	1·44	2·04	0·45	1·35	1·65	2·73
22:2	2·99	—	2·70	2·00	0·38	—	2·14	3·29	0·58	—	1·91	1·66
22:4	3·92	—	4·38	3·53	1·23	—	1·74	1·15	1·18	—	2·84	1·21
22:6	3·41	—	3·65	—	1·77	—	3·43	—	2·76	—	4·83	—
Tot. Sat.	38·29	52·17	37·80	43·25	45·76	54·36	40·02	46·18	45·11	48·50	38·33	47·03
Tot. Mono-unsat.	46·94	38·08	30·75	32·85	47·31	38·94	33·14	33·30	46·48	47·82	35·03	36·21
Tot. Di- & Poly-unsat.	14·77	9·74	31·45	23·90	6·93	6·69	26·84	20·52	8·42	3·67	26·74	16·77
Tot. Unsat.	61·71	47·82	62·20	56·75	54·24	45·63	59·98	53·82	54·90	51·49	61·77	52·98

[a] Each value is given in area per cent and is the mean of four replicate samples analyzed in duplicate. Blank values indicate they were not detectable.
[b] Storage time in days.

suggest that the refined salt + BHA/BHT treatment may protect the unsaturated fatty acids in the triglyceride fraction, even though refined salt alone had a more deleterious effect on the stability of the unsaturated fatty acids than the rock salt. Although the charqui cured with rock salt had the lowest initial percentage of unsaturated fatty acids in the triglyceride fraction (54·24%), it was similar to that for the refined salt alone (54·90%).

In the phospholipid fraction, on the other hand, the decrease in percentage of the unsaturated fatty acids during 60 days' storage was greatest for the refined salt + BHA/BHT, amounting to 8·8% in comparison to 6·1% for the rock salt and 5·4% for the refined salt alone. Results suggest that the antioxidant treatment (BHA/BHT) protected the unsaturated fatty acids in the triglycerides but did not inhibit breakdown of the unsaturated fatty acids in the phospholipids. According to Keller & Kinsella (1973) changes in the fatty acid composition may be useful as an indirect measure of lipid oxidation in meat products.

Cholesterol oxides

Cholesterol oxidation products (Table 5) were detected in all samples after 30 days of storage, and included 7α-hydroxy-, 7β-hydroxy-, 4β-hydroxy-, α-epoxide-, triol- and 7-keto-cholesterol. The levels of cholesterol oxides in the charqui samples were lower than those reported by Higley *et al.* (1986) in processed meats but may still be a matter of health concern. The presence of oxides of cholesterol may be viewed with some alarm because of the large quantities of charqui consumed in Brazil.

TABLE 5
Oxides and Cholesterol in Charqui Prepared Using Refined Salt, Rock Salt, and Refined Salt plus BHA/BHT After 30 Days' Storage

Cholesterol oxides (ppb)	*Refined salt*	*Rock salt*	*Refined salt + BHA/BHT*
7α-Hydroxy-cholesterol	—	—	—
7β-Hydroxy-cholesterol	—	3·16	0·38
7-Ketocholesterol	5·54	5·12	0·88
4β-Hydroxy-cholesterol	—	—	0·78
Triol-cholesterol	1·82	3·92	—
α-Epoxide-cholesterol	—	—	0·96
Total cholesterol oxides	7·36	12·20	3·00

Each value represents the mean of duplicate determinations. All blanks indicate non-detectable amounts of the corresponding cholesterol oxide.

DISCUSSION

Overall results demonstrate that charqui suffers from oxidation, which was verified by TBA numbers, hexanal levels and the presence of cholesterol oxidation products. Although the use of BHA/BHT-containing salt retarded oxidation of charqui for 15 days or longer, it was not effective in decreasing oxidation during long term storage, i.e. over 30 days. Generally speaking, refined salt produced charqui that suffered less oxidative degradation than rock salt, which was found to contain trace amounts of iron (115 ppm) and copper (1 ppm). These trace metal contaminants appeared to accelerate oxidation of the lipids in charqui and should, thus, not be used in its production.

The identification of cholesterol oxides in charqui not only raises questions about their possible involvement in human health, i.e. especially in the etiology of cancer, but also in other oxidative reactions. Results suggest that every effort should be taken to eliminate and minimize oxidation in charqui. Other means of reducing oxidative reactions in producing charqui need to be investigated.

The changes in fatty acid composition were small in comparison to the increases in hexanal and malonaldehyde (TBA numbers). Lazarus *et al.* (1977) did not find any major changes in the composition of fatty acids from lamb during 9 days of storage at 4°C. Kunsman *et al.* (1978), in explaining the absence of changes in fatty acids during storage of meat, suggested that the heme proteins may act as antioxidants. Yamauchi *et al.* (1980) have shown that endogenous levels of α-tocopherol can influence the rate of oxidation by exerting an antioxidative effect in meat. These and other factors should be studied in not only charqui but in producing other cured meat products.

SUMMARY

Results indicated that trace amounts of the transition metals, including Fe and Cu, in rock salt may play in important role as catalysts of lipid oxidation in charqui. The use of refined and/or antioxidant containing salt may help in reducing the extent of oxidation. Cholesterol oxides were detected in charqui for the first time, being found in all samples after 30 days of storage irrespective of treatment. These results may be of possible health significance in view of the large amounts of charqui consumed in parts of Brazil.

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