Effects Of Modified Atmosphere Packaging On Storage Stability Of Dried Salted Sardines (*Sardinops neopilchardus*)

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Abstract

Australian sardines (*Sardinops neopilchardus*) were salted, dried and packaged with (1) air (LDPE bags) (2) under vacuum, and (3) nitrogen gas-flushed and stored at 30°C, 75% RH for 12 weeks. Storage time was the most important factor affecting the quality of the fish. TBARS values of every sample decreased when storage time increased. Fluorescent products increased dramatically during storage of control-packed (air) samples but remained constant in vacuum and nitrogen-packed samples. Vacuum packaging improved appearance of the products. Nitrogen packaging reduced the leaking of oil from the fish flesh. The appearance of control-packed and nitrogen-packed samples were judged unacceptable after the fourth week of storage while vacuum-packed samples remained acceptable up to 12 weeks of storage. Vacuum and nitrogen packaging did not show any effect on flavour, texture and overall acceptability of the cooked samples. Rancidity scores showed a significant negative correlation ($P \leq 0.05$) to TBARS value and a positive correlation to the level of fluorescent products.

Introduction

Dried salted fish is produced and consumed widely in Southeast Asia due to its simplicity of processing, low investment requirements and agreeable taste.

One problem encountered in dried salted fish is quality loss during storage and distribution, especially in fatty fish which is susceptible to oxidation. An estimated 25% of all dried fish is lost due to spoilage during storage (Bakar, 1983).

Sardines are small, pelagic fatty fish. They can contain up to 22% fat, depending on species, sex, season, age, etc. The shelf life of dried salted sardines is short due to the rapid rate of deterioration, especially from oxidation. Proper packaging must be used to minimize loss. Specifically this must be done to exclude oxygen (a major cause of oxidation and rancidity) to prevent the product from reabsorbing water, and to protect it from dust and undesirable microorganisms.

Typical packaging materials used for wholesale distribution of dried fish in the region are bamboo baskets, cardboard cartons, wooden boxes and hessian sacks. Contamination by dust, moulds and insects cannot be avoided, especially at the retail level where the products are sold unprotected in the markets.

Modified atmosphere packaging (MAP) has been used successfully with fishery products to extend their shelf life. MAP, including vacuum and gas-flushing in a hermetically seal package, can be used satisfactorily with products that are susceptible to oxidation. The products can then be stored at ambient temperature. The exclusion of oxygen can be achieved by vacuum packaging or replacing the air inside the package with oxygen-free nitrogen, and/or by the use of oxygen scavengers. MAP is expected to be an ideal method to extend the shelf life of dried salted fish. It must be kept in mind that high quality raw materials and proper handling must also be used, and that poor quality

products cannot be avoided through the use of packaging materials and methods alone.

The aims of this study are to evaluate the effects of vacuum and nitrogen packaging on the storage stability of dried salted sardines and to determine the correlation between chemical and organoleptic acceptability of the product.

Materials And Methods

Fish Handling

Fresh sardine (*Sardinops neopilchardus*) were purchased and iced for transportation to the laboratory.

Fresh fish were washed and then salted at ambient temperature (17°-22°C) in saturated sodium chloride solution for 12 hr. The ratio of brine to fish was 2:1 (w/w). Additional salt was added to make sure that the solution remained saturated. The salted fish were then dried in a mechanical dryer for 12 hr with air velocity 2 m/sec, temperature 45°C and 30% RH.

Dried salted fish were divided into 3 parts for 3 treatments. Each treatment used 6 fish per bag and 3 bags were prepared. For treatment 1 fish were packed in LDPE bags (305 x 200 mm, 80°-100°C, thickness # F 86/26 purchased from Cello-Pack, Dee-Why, NSW, Australia) which were heat sealed. For treatment 2 fish were vacuum packed in a Vacumatic 282 (Vacumatic Pty Ltd) using Transpak Vacuum Pouch (O_2 transmission @25°C/75% RH-47 cc/m^2/24 hr, moisture transmission @ 38°C/93% RH-8 g/m^2/24 hr purchased from Vacumatic Australia) the package were evacuated to 90% vacuum and sealed for 1.8 sec weld time. For treatment 3 bags, the same type as used in treatment 2, were nitrogen flushed for 8 sec after evacuation to 90% vacuum and sealed for 1.8 sec.

All samples were stored in a relative humidity cabinet which was set to maintain 75% and 30°C for 12 weeks. Samples were removed after 0, 2, 4, 8 and 12 weeks for sensory and chemical analysis.

Analytical Methods

Moisture content

The procedure of the AOAC (1984) was followed.

Water activity

The water activity value of the product was measured by use of a Vaisala humidity meter.

Fat content

A Soxhlet reflux apparatus was used to determine the fat content of dried samples.

Protein

Kjeldalh method was employed where a Kjeltex 1030 Protein Analyser unit was the equipment used.

Ash

The method N[illegible]4009 (AOAC, 1984) was followed.

Salt content

The method used is specified in FAO (1981).

TBARS value

The method of Ke, Cervantes and Martinez (1984) was followed.

Oil extraction

The method of Sheppard, Iverson and Weihrauch (1978), Wills, Balmer and Greenfield (1980) and Lubis (1985) were slightly modified and employed.

Fluorescent product

A slight modification of the method of Fletcher, Dillard and Tappel (1973) was followed.

Free fatty acid

The method used is specified in Paquot (1979).

Sensory evaluation

Uncooked whole dried salted sardines were placed on a white tray, and 15 panelists were

chosen for their familiarity in eating dried salted fish evaluated them for physical damage, sheen, discolouration and overall acceptability. The fish were cut into pieces and deep fried in vegetable oil at 150°C for 3 min. The fried fish were left in air for 15 min to cool then presented to the same group of panelists. The attributes for cooked segments were rancidity, texture, flavour and overall acceptability. The samples were identified by random numbers and rated by the panelists indicating intensity of each attribute on a 100 unstructured line anchored at the end (non to intense).

Statistical analysis

The MANOVA was used to analyse the data obtained through SYSTAT computer software.

Results And Discussion

The characteristics of fresh sardine used for producing dried salted products were 142.1±6.1 mm length, 23.2±3.2 g weight (means from 20 samples), approximately 67.3% moisture, 11.7% fat, 18.7% protein and 2.1% ash (wet basis).

The proximate composition of the dried salted sardines were approximately 45.8% moisture, 12.6% fat, 26.5% protein and 13.8% ash (wet basis).

The characteristics of the dried salted sardine during storage are given in Table 1. In the second week of storage, fish were found floating in oil in the packages due to the separation of oil from the muscle tissue. Much more oil was found in control-packed and vacuum-packed samples than in nitrogen-packed samples. It appears that nitrogen gas-flush packaging was able to reduce the leaking out of oil from dried salted sardine. It is in accordance with a finding by Post *et al.* (1985) that for nitrite-free bacon-like products stored at 8°, 12° and 26°C, nitrogen packaging improved the shelf life by retarding discolouration and minimising exudate. However, odour of these nitrogen-packed products became unacceptable earlier than in vacuum-packed samples.

Table 1 Characteristics of dried salted sardine during storage.

Storage time (weeks)	Packaging type		
	Control	Vacuum	Nitrogen
0	Flesh firm, skin dry and glossy	As control	As control
2	Oil and water (yellow-brown) in the packages, flesh firm	Oil and water (yellow-brown) in the packages, flesh slightly soft	Small amount of oil and water (yellow-brown) in the packages, flesh fairly firm
4	Much oil and water in the packages, flesh very soft, skin and flesh damaged, rancid odour	Oil and water in the packages, flesh soft, skin and flesh damaged	Small amount of oil and water in the packages, flesh slightly soft, pale discolouration
8	Oil and water dried, flesh firm, skin and flesh damaged strong rancid odour	Much oil and water in the packages, flesh very soft, skin and flesh damaged, slightly rancid odour	Oil and water in the packages, flesh slightly soft, pale discolouration, fishy odour
12	Oil and water dried, flesh dry and firm, skin and flesh damaged, very strong rancid odour	Much oil and water in the packages, flesh very soft, skin and flesh damaged, slightly rancid odour	Oil and water in the packages, flesh soft, pale discolouration, fishy odour

Texture change is one of the major problems of cured fish (King, Kamara and Wood 1985). In the present study, the fish turned soft after 2 weeks of storage, and earlier in control-packed samples than in the other two. All the products were damaged mostly by stacking together in the storage chamber.

The process of evacuation of the air inside the packages partly pressed and damaged the vacuum-packed samples. Nitrogen-packed samples seemed to fare better since the gas inside the bags protected the products from compaction. The products in LDPE plastic bags were also damaged from skin sticking to the bag inner walls, while this did not happen to the other two samples. As long-term storage proceeded, the level of oil in control-packed samples decreased, possibly because of the high oil permeability of the plastic. Therefore, LDPE is not recommended as a suitable packaging material for high moisture dried salted fish. Durairaj and Pitchiah (1981) stated that the packaging of dried samples in HDPE required a sample moisture content below 35%, a level impossible for fish with a high fat content.

Rancid odour occurred in control-packed samples from the fourth week of storage, followed by vacuum-packed samples, with a strong fishy odour found in nitrogen-packed samples. Pale skin colour was found in nitrogen-packed samples starting from the fourth week. Presumably, nitrogen gas caused this pale discolouration through a reaction between nitrogen gas and natural pigments in dried salted sardine after a period of time during storage. This is contradictory to the observations of Post *et al.* (1985) who stated that nitrogen packaging used for bacon-like products retarded discolouration. However, more research needs to be done to understand such an effect in dried salted fish.

However, it should be noted that all of the characteristics presented in Table 1 were observed and recorded for the raw fish by the experimenter, and not by the panelists who evaluated the products in only some aspects, ie, the raw samples already taken out of the packages and the cooked samples.

Effects Of Modified Atmosphere Packaging On Chemical Parameters

Significance of F value for several chemical parameters of dried salted sardines are given in Table 2. Two factors, packaging type and storage time, were examined. Packaging type did not significantly affect ($P \leq 0.01$) a_w, salt content and TBARS value while storage time did significantly affect every ($p \leq 0.01$) parameter. The interaction of storage time and packaging type significantly affected moisture content, fluorescent products and FFA. Therefore, it cannot be concluded at this stage that packaging type did not affect the moisture content at all since the significant differences shown in Table 2 may be due to either main effects

Table 2. Result of significance tests (F value) for chemical parameters of dried salted sardines.

Source of variation	Moisture content	Water activity	Salt content	TBARS	Fluorescent products	FFA
Packaging type	NS	NS	NS	NS	S	S
Storage time	S	S	S	S	S	S
Packaging type x Storage time	S	NS	NS	NS	S	S

S = Significant ($P \leq 0.01$)
NS = Not significant

and/or interaction; however, specific conclusions about the main effects are not possible.

Moisture Content, a_w And Salt Content

The average moisture contents of all samples during storage are shown in Table 3. The moisture contents were not constant throughout the storage but were affected significantly ($P \leq 0.01$) by the interaction between storage time and packaging type. These findings were contradictory to other studies, eg, Lubis (1989) found small differences in moisture content of dried salted sardines packed in PE bags and stored at different temperatures for 24 weeks, but they were not significant ($P \geq 0.01$).

All the samples gradually gained about 4-5 % moisture. It was obvious that products packed in LDPE bags gained moisture content faster than did the other two samples starting from the second week and maintained those levels up to the end of storage. Obviously the LDPE bags were more permeable than were the laminated ones.

In this present study, the RH in the storage chamber was not controlled constantly, falling sometimes below 75% RH to between 55-60% RH. If the RH were controlled at 75% constantly throughout the storage time, the products could have gained more moisture than those examined in this study. However, at the twelfth week, the moisture content of control-packed samples was lower than the other two. The water absorbed by product in laminated bags used for vacuum- and nitrogen-packed samples may arise from the condensation of water vapour in the packages since, after drying, fish were left in air for a short period of time then packed. It was concluded that the fish might not have been completely cooled.

The a_w values of all the samples during storage are given in Table 4. The a_w values were significantly affected ($p \leq 0.01$) by storage time. The longer the storage time, the lower the a_w values, in contrast to the moisture contents which increased as storage time proceeded. These results also contrasted with other studies. Lubis (1989) conducted a study on dried salted sardine packed in PE bags and stored at 5°, 20° and 30°C for 24 weeks and found stable a_ws throughout the storage. The cause of this peculiar trend is probably due to the variability of the Vaisala probes used in the experiment. The use of a probe different from that used for a previous analysis may cause a significant difference in the values. Even the same probe can give different readings at different times and consistency also depends on care in handling and calibration of the instrument.

Table 3. Means[A] of moisture content (%) of dried salted sardines during storage.

Storage time (weeks)	Packaging type		
	Control	Vacuum	Nitrogen
0	45.8 ± 0.0a	45.8 ± 0.0a	45.8 ± 0.0a
2	48.5 ± 0.5cd	47.5 ± 0.2bc	46.6 ± 0.1ab
4	48.6 ± 0.3de	47.0 ± 0.1b	46.8 ± 0.0ab
8	49.4 ± 0.4def	49.6 ± 0.5ef	50.7 ± 0.2g
12	49.3 ± 0.2def	49.8 ± 1.2fg	50.7 ± 0.2g

[A]Means of two replicates ± standard deviation.
Means followed by identical letters are not significantly different ($P \geq 0.01$).

Table 4. Means[A] of water activity of dried salted sardines during storage.

Storage time (weeks)	Packaging type		
	Control	Vacuum	Nitrogen
0	0.80 ± 0.01d	0.80 ± 0.01d	0.08 ± 0.01d
2	0.81 ± 0.00e	0.82 ± 0.01f	0.81 ± 0.00e
4	0.82 ± 0.01f	0.81 ± 0.01e	0.81 ± 0.01e
8	0.74 ± 0.01a	0.74 ± 0.00a	0.75 ± 0.00b
12	0.74 ± 0.01a	0.74 ± 0.00a	0.76 ± 0.01c

[A]Means of two replicates ± standard deviation.
Means followed by identical letters are not significantly different (P≥0.01)

Table 5. Means[A] of salt content (%) of dried salted sardines during storage.

Storage time (weeks)	Packaging type		
	Control	Vacuum	Nitrogen
0	16.3 ± 0.5abc	16.3 ± 0.5abc	16.3 ± 0.5abc
2	15.6 ± 0.2abc	15.3 ± 0.1a	15.7 ± 0.4abc
4	15.5 ± 0.1ab	16.5 ± 0.4abcd	15.1 ± 0.5a
8	17.6 ± 0.0de	17.8 ± 0.3e	17.7 ± 0.4de
12	16.6 ± 0.7bcde	16.8 ± 0.6cde	16.7 ± 0.9bcde

[A]Means of two replicates ± standard deviation.
Means followed by identical letters are not significantly different (P≥0.01).

Salt contents of all samples during storage are shown in Table 5. Storage time significantly affected (P≤0.01) the salt content. There was no precise trend (increase or decrease) among the data. The fluctuation in salt content may be caused by a lack of uniformity of the end-point colour during the analysis since even small amounts of $AgNO_3$ can alter the result dramatically. One would expect, as a trend, that the longer the storage time, the lower would be the salt content since the moisture content of the samples increases with storage time. However, the actual trend was small and can be neglected.

TBARS Value

Tables 1 and 6 indicate that only storage time significantly affected (P≤0.01) TBARS values. In the first four weeks of storage, the TBARS values declined dramatically. Similar results have been

Table 6. Means[A] of TBARS value (%mol/kg) sample of dried salted sardines during storage.

Storage time (weeks)	Packaging type		
	Control	Vacuum	Nitrogen
0	6.54 ± 1.20c	6.54 ± 1.20c	6.54 ± 1.20c
2	4.07 ± 0.38b	5.24 ± 0.42bc	4.05 ± 0.70b
4	1.62 ± 0.39a	1.95 ± 0.49a	1.76 ± 0.38a
8	1.34 ± 0.10a	1.35 ± 0.03a	1.28 ± 0.19a
12	1.24 ± 0.07a	1.17 ± 0.08a	1.52 ± 0.07a

[A]Means of two replicates ± standard deviation.
Means followed by identical letters are not significantly different ($P \geq 0.01$).

found by several scientists. Lubis (1989) reported a decrease of TBARS values of dried salted sardines packed in PE bags and stored at 5°, 20° and 30°C for 24 weeks, and the samples stored at the higher storage temperature showed a faster decrease. In the present study, the samples were stored at 30°C and 75% RH which was quite a severe condition, hence the values declined rapidly. The decline in TBARS values can be explained by the slower rate of autoxidation of unsaturated fatty acids and the instability of the malonaldehyde produced or malonaldehyde reacted with amino groups or other carbonyls, or oxidation of this aldehyde during storage yielding the lower TBARS values (Gokalp *et al.*, 1983). In the case of frozen sardine, Numbudiry (1980) reported a constant increase in TBARS values in stored sardine frozen at -5°C through 40 days of storage. In the present study, after the fourth week of storage, TBARS values were constant or not significantly different ($P \geq 0.01$) up to the twelfth week, indicating that TBARS value is not a reliable means of assessing the degree of rancidity in dried salted fish products in long term storage. However, many scientists insist on the use of TBARS value as a good index for measuring the extent of lipid oxidation. Therefore, it can be concluded that TBARS value is probably not a reliable means for measuring lipid oxidation in products stored at high temperature for long term storage, but that it can be used successfully for products stored at low temperatures.

Packaging type did not significantly affect ($P \geq 0.01$) TBARS values in the dried salted sardine in this study. The results are contradictory to those of Hwang, Bowers and Kropf (1990), who found that TBARS values ($P \leq 0.01$) were higher for air-packed cooked beef loin slices than for vacuum and gas mixture-packed (80% N_2 and 20% CO_2) samples stored at -20°C for 11 weeks. The overall mean TBARS values of air-packed beef slices were four to five times higher than that of vacuum- and N_2/CO_2 packed beef. However, the temperature used for storage was much different to the present study. If there was a difference in the rate of oxidation among packaging types in this present experiment, TBARS value was not a good indicator for following lipid oxidation. On the other hand, during high temperature storage vacuum and nitrogen packaging did not significantly affect the rate of oxidation of the products.

Fluorescent Products

Table 7 shows the significant effect ($P \leq 0.01$) of packaging type and storage time on fluorescence values of dried salted sardines. Packaging type,

Table 7. Means[A] of fluorescent products (%g/g dry sample) of dried salted sardines during storage.

Storage time (weeks)	Packaging type		
	Control	Vacuum	Nitrogen
0	5.79 ± 0.31a	5.79 ± 0.31a	5.79 ± 0.31a
2	14.26 ± 2.93cd	8.00 ± 0.52b	14.91 ± 0.16d
4	10.68 ± 3.08bc	10.33 ± 1.11bc	10.44 ± 1.33bc
8	19.51 ± 2.53e	10.30 ± 0.33bc	11.07 ± 1.23bcd
12	25.09 ± 0.16f	9.14 ± 1.63b	7.38 ± 1.25b

[A]Means of two replicates ± standard deviation.
Means followed by identical letters are not significantly different (P≥0.01).

storage time and their interaction significantly affected the fluorescence level. The level of fluorescent products in control-packed samples significantly increased from the fourth week and constantly increased up to the end of the storage period.

For the vacuum-packed samples, the level of fluorescence increased in the first 2 weeks then remained stable through 12 weeks of storage. The level in nitrogen-packed samples fluctuated slightly in the first 4 weeks, however, after that the values remained low and constant. It can be concluded at this stage that, the longer the storage time, the higher the level of fluorescent products produced in dried salted sardine packed in LDPE bags. Vacuum and nitrogen packaging can retard fluorescent products production in these products.

It is contradictory that TBARS values of the products were found constantly low after the fourth week of the storage in every type of packaging but levels of fluorescent products increased rapidly in only control-packed samples after the fourth week. The trend of TBARS value reduction in samples packed under three packaging atmospheres were similar, but for the level of fluorescent products a rapid increase was found only in control-packed products, while a constant level was revealed in vacuum and nitrogen-packed samples. Lubis (1989) recommended the use of the level of fluorescent products for the measurement of rancidity in dried salted sardines since it has been used successfully as an analytical method for quantification of peroxidation damage to biological tissue (Fletcher *et al.*, 1973).

The level of fluorescent products is probably a more sensitive indicator than is the TBARS value in the assessment of lipid oxidation in dried salted sardine in long term storage, because this parameter distinguishes the differences in oxidation rate among different packaging types while TBARS value just showed the same trend of reduction in the values as storage time proceeded. Further research on the sensitivity of these two methods is needed to confirm these findings.

Free Fatty Acid

Table 8 reveals that the interaction of packaging type and storage time significantly affected (P≤0.01) the FFA contents of the products. After week 4 to week 8 of storage, FFA contents significantly increased while during the first 4 week there was no significant difference in FFA content. The same trend was observed in the level of fluorescent products but was contrary to that for TBARS value. After the eighth week, the FFA contents decreased rapidly. This phenomenon was quite peculiar. Numbudiry (1980) conducted a

Table 8. Means[A] of FFA content (%) of dried salted sardines during storage.

Storage time (weeks)	Packaging type		
	Control	Vacuum	Nitrogen
0	4.98 ± 0.23ab	4.98 ± 0.23ab	4.98 ± 0.23ab
2	5.10 ± 0.11ab	5.23 ± 0.34abc	4.91 ± 0.11a
4	5.76 ± 0.33abc	5.95 ± 1.05c	4.93 ± 0.07a
8	10.19 ± 0.25e	11.25 ± 0.22f	10.39 ± 0.08ef
12	6.14 ± 0.34c	7.42 ± 0.61d	4.77 ± 0.28a

[A]Means of two replicates ± standard deviation.
Means followed by identical letters are not significantly different ($P \geq 0.01$)

study on frozen storage of sardine and showed that an increase in FFA content indicates hydrolytic cleavage of the glycerides in lipid and that the longer the storage time the higher the content due to the hydrolysis. The rapid decrease of FFA content after eight weeks was probably caused by an interaction between FFA and other compounds, or by experimental error as the pH meter used to determine the end point of the reaction between acid and base in the last week was not the same as the one used in the previous analysis. Hence, more study needs to be done in order to confirm or reject these results.

Although FFA content showed a similar trend to the level of fluorescent products, no clear trend can be concluded about the effect of packaging type. However, it is quite clear that the critical storage period of the products is the fourth week since all parameters used for oxidation measurement show significant differences around this period of time.

Effects Of Modified Atmosphere Packaging And Storage Time On Sensory Evaluation

Significant F values for several sensory evaluation attributes of dried salted sardine are given in Table 9. Storage time and packaging type significantly affected ($P \leq 0.01$) the appearances of uncooked whole fish but did not affect some attributes in cooked fish segments, ie, texture, flavour and overall acceptability. Only rancidity was significantly affected by storage time.

Uncooked Whole Fish

Four attributes were involved in this evaluation, ie, physical damage, sheen, discolouration and overall acceptability. Table 10 shows that storage time and packaging significantly affected ($P \leq 0.01$) physical damage of the products. There is a clear trend revealing that the longer the storage time, the higher the damage score. Control-packed samples seemed to possess a higher damage level than did the other two samples.

Since the physical damage in control-packed samples was caused by skin sticking to the inner wall of LDPE bags, this type of plastic bags is not recommended for use with these products. The damage found in vacuum packed samples may partly be due to the vacuum packaging process. The major cause of physical damage in these products is stacking-together of the packages in the storage chamber.

All the sensory evaluation scores reveal that the panelists differed in terms of their sensory judgement. Standard deviations show the scatter

Table 9. Significance (F value) for sensory evaluation scores of dried salted sardines during storage.

Source of variation	Uncooked whole fish				Cooked segments			
	Physical damage	Sheen	Discolour-ation	Overall accept-ability	Rancidity	Texture	Flavour	Overall accept-ability
Packaging type	S	S	S	S	NS	NS	NS	NS
Storage time	S	S	S	S	S	NS	NS	NS
Packaging type x Storage time	S	S	S	S	NS	NS	NS	NS

S = Significant (P≤0.01)
NS = Not significant

Table 10. Means[A] of physical damage score[B] of dried salted sardines (uncooked whole fish) during storage.

Storage time (weeks)	Packaging type		
	Control	Vacuum	Nitrogen
0	36.1 ± 24.5abcd	36.1 ± 24.5abcd	36.1 ± 24.5abcd
2	51.4 ± 24.2cde	40.4 ± 21.6abcd	34.2 ± 24.1abc
4	54.1 ± 25.0def	32.2 ± 18.6ab	50.4 ± 22.3bcde
8	71.3 ± 13.9f	25.6 ± 16.9a	49.1 ± 13.4bcd
12	48.5 ± 23.6bcd	53.4 ± 23.7def	67.9 ± 23.0ef

[A]Means of scores from 15 panelists ± standard deviation.
[B]O = absent, 100 = extreme.
Means followed by identical letters are not significantly different (P≥0.01).

of the scores. It should be noted that the judges may not be able to distinguish between samples if the samples do not obviously differ from each other. Panelists must be selected and trained to reduce errors. All the panelists participating in this sensory evaluation were familiar with dried salted fish, nevertheless, their opinions or hedonic responses varied extensively.

Evaluation on sheen scores are presented in Tables 9 and 11. The results fluctuated throughout the storage period even though both storage time and packaging type significantly affected (P≤0.01) the sheen scores of the products. Nitrogen-packed samples possessed significantly lower scores than the other two from week 0 to week 4, but from week 4 to the end of the storage period the scores were not significantly different by LSD test. It may be concluded that nitrogen atmosphere affected the sheen of dried salted sardine.

Table 11. Means[A] of sheen score[B] of dried salted sardines (uncooked whole fish) during storage.

Storage time (weeks)	Packaging type		
	Control	Vacuum	Nitrogen
0	68.6 ± 14.4g	68.6 ± 14.4g	68.6 ± 14.4g
2	43.4 ± 20.8de	46.6 ± 22.3ef	53.6 ± 21.8ef
4	57.8 ± 20.5efg	58.7 ± 16.6fg	27.0 ± 16.1ab
8	31.1 ± 9.9abc	60.8 ± 15.4fg	32.0 ± 18.0abcd
12	53.1 ± 24.0ef	38.1 ± 14.0bcd	20.7 ± 15.1a

[A]Means of scores from 15 panelists ± standard deviation.
[B]O = dull, 100 = bright.
Means followed by identical letters are not significantly different (P≥0.01).

Discolouration scores are given in Table 12. Storage time and packaging type significantly affected (P≤0.01) the scores. The scores given by the panellists were very scattered although the results show an increasing trend as storage time increased, but the scores cannot be judged significantly different by LSD test.

Overall acceptability scores of uncooked whole fish are shown in Table 13. Packaging type and storage time significantly affected the scores. If a score below 50 is regarded as unacceptable, control- and nitrogen-packed samples were unacceptable from the fourth week of storage, while the vacuum-packed samples were unacceptable in the last week. It can be concluded that vacuum-packaging may more greatly enhance the appearances of dried salted sardine than packaging in LDPE bags or nitrogen gas-flushed packaging. A specific recommendation on the shelf life of the products is not possible at this stage because, even though some scores were lower than 50, they did not differ significantly from some scores beyond 50 based on LSD test. However, a rough judgement can be made, viz, the control and nitrogen-packed samples should not be kept longer than 2 months because of undesirable appearance, and vacuum-packed samples should not be kept over 3 months. The major cause of undesirable appearance was the stacking-together of products resulting in damaged fish bodies.

It should be noted that none of the samples in this study were either mouldy or obviously spoiled throughout 12 weeks of storage. The same control-packed samples had been produced and stored under the same conditions by Lubis (1989) spoiled after 8 weeks of storage. Sen *et al.*, (1961) mentioned that sun-dried salted mackerel need to be dried to a moisture content below 37.5% for protection against mould growth. They found that PE bags could not protect this product from fungal attack. For a product with an initial moisture content of 39.7%, fungal growth was observed after 62 days storage at 26°C and 78% RH, and after 85-106 days storage at 37°C and 92% RH. Differences in type, composition, freshness of fish, salting, drying, and storage processes may affect the shelf life of dried salted fish. Many more batch experiments must be conducted to investigate and confirm the estimated shelf life of fishery products.

Cooked Segments

Four attributes used in this study represented sensory characteristics of fried dried salted sardine, ie, rancidity, texture, flavour and overall acceptability.

Lipid becomes rancid as a result of oxidation. Rancidity is considered to be caused by the objectionable tastes and flavours that result from the accumulation of decomposition products of oxidation reactions (Gray, 1978). To minimise bias or human errors, a multi-numbered panel is used.

Tables 9 and 14 show that only storage time significantly affected ($P \leq 0.01$) the rancidity score while packaging type did not show any effect. There were no rancid scores greater than 50 found during storage except for control-packed samples at week 12. However, even though the samples were scored greater than 50 they were not significantly different from the same type of samples at week 8 when LSD test was considered. There was a tendency for an increase of the rancidity score of all the samples when the storage time increased. The scores were not significantly dif-

Table 12. Means[A] of discolouration score[B] of dried salted sardines (uncooked whole fish) during storage.

Storage time (weeks)	Packaging type		
	Control	Vacuum	Nitrogen
0	30.3 ± 19.0ab	30.3 ± 19.0ab	30.3 ± 19.0ab
2	44.8 ± 26.0bcd	30.1 ± 12.5ab	31.1 ± 16.5ab
4	49.0 ± 27.0cd	24.9 ± 14.0a	50.1 ± 21.8cd
8	51.9 ± 23.1d	33.5 ± 20.5abc	40.1 ± 20.0abcd
12	38.8 ± 23.7abcd	46.4 ± 23.0bcd	55.2 ± 24.7d

[A]Means of scores from 15 panelists ± standard deviation.
[B]O = absent, 100 = extreme.
Means followed by identical letters are not significantly different ($P \geq 0.01$).

Table 13. Means[A] of overall acceptability score[B] of dried salted sardines (uncooked whole fish) during storage.

Storage time (weeks)	Packaging type		
	Control	Vacuum	Nitrogen
0	58.6 ± 16.7def	58.6 ± 16.7def	58.6 ± 16.7def
2	45.9 ± 19.2bcde	51.8 ± 15.6cdef	60.2 ± 15.1ef
4	39.7 ± 23.7bc	58.1 ± 15.0def	39.9 ± 13.6bc
8	35.4 ± 14.9ab	64.1 ± 13.7f	49.1 ± 15.7bcde
12	45.1 ± 21.0bcd	37.4 ± 16.0b	21.2 ± 14.7a

[A]Means of scores from 15 panelists ± standard deviation.
[B]O = very unacceptable, 100 = very acceptable.
Means followed by identical letters are not significantly different ($P \geq 0.01$).

Table 14. Means[A] of rancidity score[B] of dried salted sardines (cooked segments) during storage.

Storage time (weeks)	Packaging type		
	Control	Vacuum	Nitrogen
0	10.6 ± 9.5a	10.6 ± 9.5a	10.6 ± 9.5a
2	21.2 ± 14.3abc	13.8 ± 7.5ab	15.5 ± 9.9ab
4	25.7 ± 19.3abcd	20.1 ± 19.4abc	28.2 ± 23.7bcd
8	48.4 ± 21.3ef	32.8 ± 19.1cde	27.8 ± 14.5bcd
12	52.6 ± 31.1f	35.1 ± 27.6cde	40.7 ± 24.1def

[A]Means of scores from 15 panelists ± standard deviation.
[B]O = absent, 100 = extreme.
Means followed by identical letters are not significantly different (P≥0.01).

ferent by LSD test at week 8 up to week 12. Lubis (1989) found that at the end of 24 weeks for dried salted sardine packed in PE bags and stored at 5°C and 20°C, the rancidity score decreased. Possibly at this stage of storage, the non-enzymic browning (NEB) products produced had sufficient inhibitory effect to inhabit lipid oxidation.

Lubis (1989) reported that dried salted sardine stored at 30°C in PE bags became rancid at a faster rate than the samples stored at 5°C and 20°C, and that the rancidity of the 30°C stored samples started with the fourth week of storage. All samples at every storage condition became rancid between 4-6 weeks of storage.

In the present study, it is difficult to interpret the data in order to indicate a specific shelf life of the products by rancidity score because the panelists participating in the sensory testing sessions had not been trained before and had different threshold for rancidity; as can be seen by the standard deviations which varied extremely. However, it could be roughly concluded that the samples revealed a rancid smell after the fourth week of storage. It should be kept in mind that rancid smell is not necessarily an undesirable indicator for all consumers.

Table 15 shows the results of texture scores. Storage time and packaging type did not significantly affect (P≤0.01) the scores in the samples. In this study, the panellists hardly detected the differences, since they examined the cooked samples only after frying. The cooking method resulted in the fish samples losing some water, leading to difficulty in evaluation. A soft texture of the raw samples was observed and recorded by the experimenter (Table 1). Hence, the panelists should be allowed to evaluate the softness of the raw samples as well.

Storage time and packaging type did not show any effect on the flavour of the dried salted samples during storage (Table 16).

Overall acceptability scores are given in Table 17. Storage time and packaging type did not significantly affect (P≥0.01) overall acceptability of the samples. Even though the scores of the products were less than 50 from week 8 for control- and vacuum-packed samples, and at week 12 for nitrogen-packed samples, they were not significantly different (by LSD test) from samples at the previous storage period.

High moisture content is not a desirable characteristic in some kinds of dried salted fish since it leads to unacceptability, even though the quality of dried salted sardine during long term storage were improved or masked by frying. It should be kept in mind that consumers buy the uncooked, not the

Table 15. Means[A] of texture score[B] of dried salted sardines (cooked segments) during storage.

Storage time (weeks)	Packaging type		
	Control	Vacuum	Nitrogen
0	50.6 ± 28.7b	50.6 ± 28.7b	50.6 ± 28.7b
2	46.8 ± 26.1b	51.1 ± 20.7b	37.1 ± 24.7ab
4	46.8 ± 28.4b	35.1 ± 22.8ab	50.1 ± 16.3b
8	45.0 ± 19.7ab	25.4 ± 18.1a	39.3 ± 23.5ab
12	46.5 ± 28.2ab	43.9 ± 28.2ab	46.0 ± 25.8ab

[A]Means of scores from 15 panelists ± standard deviation.
[B]O = soft, 100 = firm.
Means followed by identical letters are not significantly different (P≥0.01).

Table 16. Means[A] of flavour score[B] of dried salted sardines (uncooked segments) during storage.

Storage time (weeks)	Packaging type		
	Control	Vacuum	Nitrogen
0	59.3 ± 21.9a	59.3 ± 21.9a	59.3 ± 21.9a
2	53.4 ± 18.8a	51.9 ± 20.9a	46.0 ± 20.6a
4	56.5 ± 19.2a	56.5 ± 15.9a	57.9 ± 13.2a
8	42.8 ± 21.9a	45.4 ± 18.2a	52.1 ± 17.9a
12	35.7 ± 23.6a	48.7 ± 21.4a	45.2 ± 21.2a

[A]Means of scores from 15 panelists ± standard deviation.
[B]O = very poor, 100 = very good.
Means followed by identical letters are not significantly different (P≥0.01).

fried product, hence it is clear that the appearance of the uncooked products initially must be acceptable, although the acceptability of some products appears to be improved by frying.

Regression Analysis

Guadogni (1968) stated that at our present state of knowledge, it is impossible to list a complete set of rules or requirements which would always ensure success in relating sensory and instrumental results. However, regression analysis does make it possible to interpret relationships between subjective and objective measurements. Table 18 shows simple correlations of rancidity scores with three chemical indices for assessing oxidation of dried salted sardine. Means were calculated from the total scores and values of every

Table 17. Means[A] of overall acceptability score[B] of dried salted sardines (cooked segments) during storage.

Storage time (weeks)	Packaging type		
	Control	Vacuum	Nitrogen
0	56.0 ± 20.9b	56.0 ± 20.9b	56.0 ± 20.9b
2	51.6 ± 15.5b	54.8 ± 21.5b	54.8 ± 19.9b
4	50.7 ± 21.5b	53.2 ± 20.1b	56.1 ± 13.4b
8	44.8 ± 20.9ab	45.6 ± 19.2ab	51.2 ± 16.2b
12	33.8 ± 23.6a	49.5 ± 21.3ab	45.5 ± 19.1ab

[A]Means of scores from 15 panelists ± standard deviation.
[B]O = very unacceptable, 100 = very acceptable.
Means followed by identical letters are not significantly different ($P \geq 0.01$).

Table 18. Correlation coefficients between rancidity scores and TBARS value, level of fluorescent products and FFA content of dried salted sardines during storage.

	Correlation with rancidity score
TBARS	-0.900*
Fluorescent products	0.807*
FFA	0.584

* Significant ($P \leq 0.05$)

packaging type at the same storage time. Means of rancidity scores as well as TBARS values, levels of fluorescent products or FFA contents were plotted against storage time (weeks) as shown in Fig. 1-3.

TBARS showed a significant negative relation ($P \leq 0.05$) with rancidity score. The level of fluorescent products did show a positive significant relationship with the scores. There is considerable evidence for the correlation between sensory evaluation results and TBARS value or the level of fluorescent products; Lubis (1989) found and recommended that the level of fluorescent compounds was a good index for following oxidation in dried salted sardines during storage because of its high positive correlation with rancidity score. He also reported that TBARS value showed only the third highest correlation with rancidity score; fluorescent products was first and polyene index second. The correlation was negative, instead of positive as found by other researchers, possibly because most of the samples used in their experiments were fresh and stored frozen compared to dried slated fish in his study. The trend found is similar to the results in this present study. MacDonald *et al.* (1982) stated that TBARS value

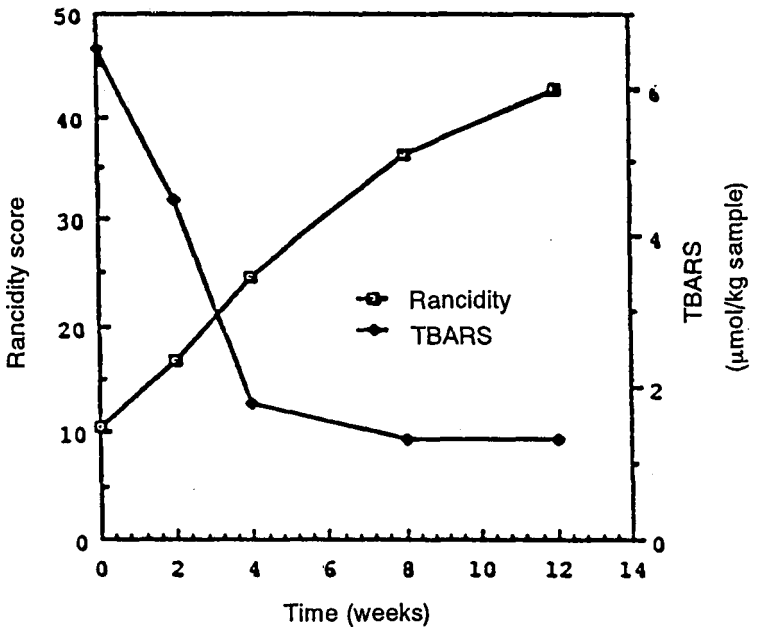

Fig. 1. Rancidity score and TBARS value of dried salted sardine during storage.

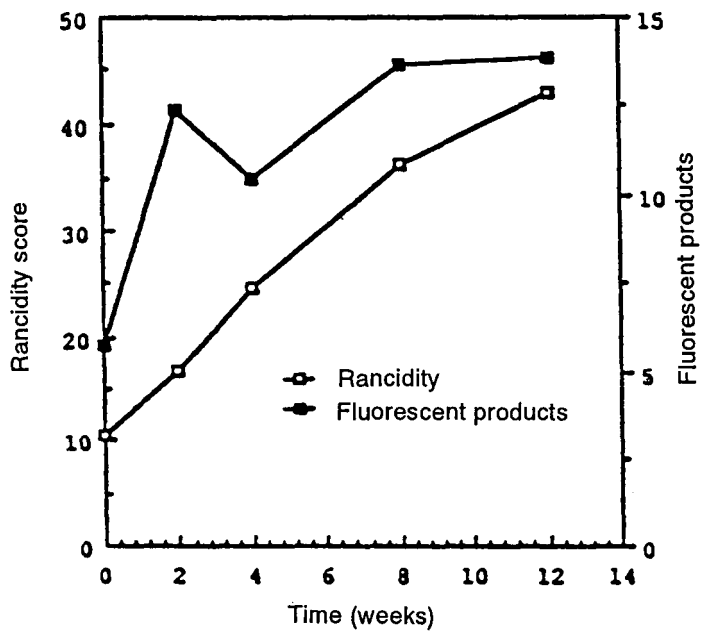

Fig. 2. Rancidity score and fluorescent products of dried salted sardine during storage.

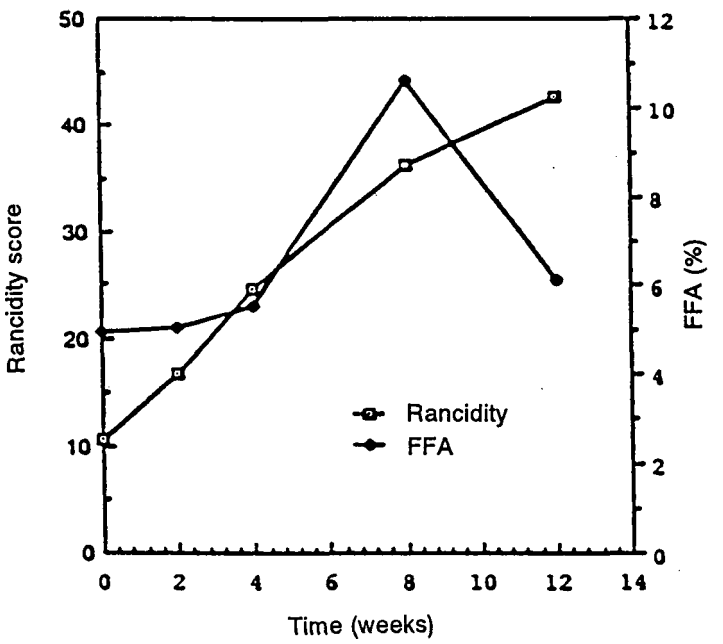

Fig. 3. Rancidity score and FFA content of dried salted fish during storage.

had a significant high positive correlation with both off-odour and off-flavour formation during aerobic storage of pork at 4°C. The controversy can only be resolved by the examination of many batches of samples, to avoid misleading interpretation.

FFA did not reveal any correlation with rancidity score, probably due to the significant decrease in FFA after the eighth week.

Conclusion And Recommendation

Modified atmosphere packaging has shown some effects on the storage stability of dried salted sardine. Storage time mostly affected chemical values and sensory scores of the products.

Storage at 30°C and 75% RH resulted in the fish samples floating in oil inside every package type after the first two weeks of the storage. Further research on storage at the refrigerated temperatures is needed to overcome this problem.

Three different types of packaging had no significant effect ($P \geq 0.01$) on the TBARS values, but storage time showed a significant decrease in this parameter for every sample, especially in the first four weeks of storage. The longer the storage time, the lower the TBARS value. The level of fluorescent products was significantly affected ($P \leq 0.01$) by both packaging type and storage time. TBARS values remained stable after the fourth week of storage in every sample, while the level of fluorescent products increased rapidly after such time in control-packed samples. Small differences in the level of fluorescent products of vacuum- and nitrogen-packed samples were found throughout the storage period. It can be concluded that the level of fluorescent products is a more sensitive indicator than TBARS value of lipid oxidation in

dried salted sardine. Vacuum and nitrogen packaging reduced the rate of lipid oxidation of dried salted sardine due to the exclusion of oxygen in the packages evidenced by the level of fluorescent products. However, the difference was not obviously indicated by TBARS value, FFA content and sensory evaluation. FFA content was significantly affected ($P \leq 0.01$) only by storage time. The longer the storage time, the higher the FFA content in the first eight weeks of storage. However, FFA was not considered a reliable means by which to evaluate lipid oxidation in this study, since a rapid decline was noted after the eighth week in contrast to sensory evaluation results.

The appearance of uncooked whole fish was significantly affected ($P \leq 0.01$) by both storage time and packaging type. The longer the storage time, the more significant the deterioration of the appearance of the products. Nitrogen packaging reduced the leaking-out of oil from the fish flesh and also decreased the sheen of the fish skin. Control-packed samples were mostly damaged by the fish skin sticking to the inner wall of the bags. Vacuum-packed samples were judged to have the best appearance. Since appearance is the most important factor influencing a consumer's decision, it may be concluded that nitrogen-packed samples cannot stay longer in a market than four weeks, control-packed samples not more than 4-6 weeks and vacuum-packed samples about 12 weeks. It is expected that if low temperature storage is involved, it would improve the appearance and extend the shelf life of the products.

Rancidity score was found to be affected significantly ($P \leq 0.01$) by storage time. The longer the storage time, the higher the rancidity score. The panelists were not able to distinguish between the samples of different packaging types since the samples did not differ markedly from each other and the panelists participating in this study had limited sensory judgement because they had not been previously trained. For better results, panelists must be selected and trained before an experiment commences, and the use of *ad hoc* panelists should be avoided.

Other attributes for the cooked samples, ie, texture, flavour and overall acceptability, were found to be improved by frying.

Both TBARS values and the level of fluorescent products showed a significant correlation ($P \leq 0.05$) with rancidity score. In this study, the level of fluorescent products associated with obvious rancid off-flavour was between 16-20 μg/g dry sample, or a TBARS value less than 1.5 μmol/kg sample. Further research is required to determine the possibility of using vacuum packaging with lower-moisture dried salted fish stored at ambient temperature or under refrigeration. The problem of product damage by stacking in the storage chamber must be overcome.

AOAC. 1984. Official Methods of Analysis. 14th ed. Washington, DC: AOAC.

Bakar, J. 1983. Packaging of dried fish in Malaysia in perspective. James, D. ed. The production and storage of dried fish. Proceeding of the workshop on the production and storage of dried fish. University Pertanian Malaysia, Serdang (Malaysia), 2-5 November 1982. Rome: FAO; 1983. FAO Fish. Rep. (279) Suppl. : 265 p.

Durairaj, S. and Pitchiah, P. 1981. Certain studies on the use of high density polyethylene woven sacks as an improved packing for dried fish. Seafood Export Journal 13 (9): 27-33.

FAO. 1981. The prevention of losses in cured fish. Rome: FAO. FAO Fish Tech. Pap. (279). 87 p.

Fletcher, B. L., Dillard, C. J. and Tappel, A. L. 1973. Measurement of fluorescent lipid peroxidation products in biological systems and tissues. Anal. Biochem. 52: 1-9.

Goklap, H. Y., Ockerman, H. W., Plimton, R. F. and Harper, W. J. 1983. Fatty acids of neutral and phospholipids, ranidity scores and TBA values as influenced by packaging and storage. Journal of Food Science. 48: 829-34.

Gray, J. I. 1978. Measurement of lipid oxidation : a review. J. Am. Oil Chem. Soc. 55: 539-546.

Guadagni, D.G. 1968. Requirements for coordination of instrumental and sensory techniques. Correlation of methods in the study of odours and taste. ASTM STP 440. Am. Soc. Test. Mat. 36 p.

Hwang, S. Y. Bowers, J. A. and Kropf, D. H. 1990. Flavour, texture, colour and hexanal and TBA values of frozen cooked beef packaged in modified atmosphere. Journal of Food Science. 55: 26-9.

Ke, P. E., Cervantes, E. and Martinez, R. C. 1984. Determination of thiobarbituric reaction substances (TBARS)) in fish tissues by an improved spectrophotometric method. J. Sci. Food Agri. 35: 1248-54.

King, D., Kamara, V. A. and Wood, C. D. 1985. Salted and pressed sardines. Reily, A., Ed. Spoilage of tropical fish and product developments. Proceedings of a symposium held in conjunction with the Sixth session of the Indo-Pacific Fishery Commission Working Party on Fish Technology and Marketing, Royal Melbourne Institute of Technology, Melbourne, Australia, 23-26 October 1984. Rome: FAO. FAO Fish. Rep. (317) Suppl.: 474 p.

Lubis, Z. 1985. Composition and stability of sardine oil. Kensington: School of Food Science and Technology; UNSW. MAppSc. thesis.

Lubis, Z. 1989. Studies on the stability of lipids in dried salted sardines. Kensington: School of Food Science and Technology; UNSW. PhD. thesis.

MacDonald, B. Gray, J. L., Kakuda, Y. and Lee, M. L. 1982. Role of nitrite in cured meat flavour; Chemical analysis. Journal of Food Science. 45: 889-92.

Numbudiry, D. D. 1980. Lipid oxidation in fatty fish: The effect of salt content in the meat. Journal of Food Science & Technology (Mysore) 7: 176-8.

Paquot, C. 1979. IUPAC: Standard methods for the analysis of oils, fats and derivatives. 6th ed. Part 1. Sect. I and II. Oxford: Pergamon Press.

Post, L.S., Lee, D. A., Solberg, M., *et al.* 1985. Development of botulism toxin and sensory deterioration during storage of vacuum and modified atmosphere packaged fish fillets. Journal of Food Science. 50: 990-996.

Sen, D. P. Anandaswamy, B., Iyegar, N. R. and Lahiry, N. L. 1961. Studies on the storage characteristics and packaging of sun-dried salted mackerel (*Rastrelliger kanagurta* Cuv). Food Sci. (Mysore). 10: 148-53.

Sheppard, A. J., Iverson, J. L. and Weihrauch, J. L. 1978. Composition of selected dietary fats, oils, margarine and butter. Kuksis, A., ed. Handbook of lipid research I. New York: Plenum Press; 341-439.

Wills, R. B., Balmer, N. and Greenfield, H. 1980. Composition of Australian foods. 2. Methods of analysis. Food Technology in Australia. 32: 198-204.

Discussion

A comment was made that in the presentation, the results of the moisture content and water activity appeared to be contradictory; Miss Sophonphong said that she realized that there may possibly have been experimental error.

When asked why the TBARS values decreased during storage and what was responsible for the increase in free fatty acid in storage, Miss Sophonphong said that the increase in free fatty acid could be due to a combination of autoxidation and microbial lipase activity.

Asked why LDPE packing was used as the control rather than unpacked samples, which reflected the actual commercial practice, were not selected, Miss Sophonphong explained that it would be difficult to control the storage conditions of unpacked samples.