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QUALITY CHANGES IN FROZEN CRAY FISH (*Procambarus Clarkii*) DURING STORAGE

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Abstract

Crayfish sample (*Procambarus clarkii* was divided into whole crayfish (WC) and tail crayfish (TC) after boiling alive in water for 55 min. samples were analyzed chemically and microbiologically during frozen storage for 6 months.

Weight losses of WC and TC were gradually increased by increasing storage period it was higher for TC than WC. The moisture losses were higher for WC (ranged from 1.71 to 2.8%) than TC (ranged from 0.33 to 1.22%) after 6 months of storage at - 18°C. The pH values increased during frozen storage, the increase was higher for WC than TC. While the changes in acidity took the inverse direction of pH values. The amino N; NPN and histamein contents were increased during frozen storage, the increases were higher for WC than TC.

The percentages of myofibrillar; sarcoplasm and stroma proteins were decreased during frozen storage for 6 months for WC and TC from 12.44 to 8.15%, 12.46 to 9.60%, 6.15 to 4.65%, 6.16 to 4.90% and 3.39 to 2.50%, 3.40 to 2.70% (on wet basis), respectively. Therefore, this indicate that denaturation of protein increased by prolonged storage. The TAPC was increased progressively by storage time, which reached $1.4x10^3$ for WC and 3.5×10^2 CFU/g for TC after 6 months of storage. Nevertheless the TAPC was higher for WC than TC. While the fungi count not detected in the TC sample, but it was increased for WC during frozen storage from 0.8×10^2 to 1.6×10^2 CFU/g, moreover the yeast count was increased during frozen storage for all samples.

INTRODUCTION

The muscle proteins consist of myofibrillar, sarcoplasmic and stroma proteins (Moorjani *et al.*, 1962). Myofibrillar proteins from the myofibril or contractible part of muscle are composed of myosin, actin, tropomyosin, troponin moreover ionic strenghs greater than 0.3 are required to solubilize myofibrils actinin (Goll *et al.* (1974). Sarcoplasmic Proteins which are soluble at ionic strenghs of 0.05 or less, consist of water

extractable proteins of albumin type, namely myogen A and B, myoalbumin, glubulin and M-protein. Stroma proteins constitute collagen and elastin, which are considered low-grade because they do not contain the essential amino acid tryptophan, and only a very little amount of methionine. Moreover, they do not readily react with the digestive enzymes and consequently have no food value (Zaitsev *et al.*,1969).

Fish flesh structure is composed of myofibrillar, sarcoplasmic, connective tissue stroma proteins, polypeptides, nucleotides and nonprotein nitrogen compounds. As a preservation technique, freezing of fish muscle results in physical structural and chemical changes for muscle proteins. This is evident from the denaturation and solubility loss (Dyer and Dingle, 1961; Connell, 1964; Sikoreski *et al.*, 1976 and Shenouda, 1980).

Protein denaturation Pacific whiting fillets stored at -8, -20, -34, and -50°C was investigated by Hsu *et al.* (1993) they found that changes in quality of fillet stored at -8°C were significantly greater than those stored at lower temperature. Fillets stored at -34°C and -50°C showed no significant advantage over those stored at -20°C as measured by salt-soluble protein extractability and Ca^{++}ATPase activity.

Lablanc and Leblanc, (1989) isolated the myofibrillar, sarcoplasmic and total extractable proteins from the same lot of frozen cod fillets stored at different low temperatures. It was found that major change was in the frozen stored myofibrillar proteins. Myofibrillar protein consist of actin and myosin, during frozen storage, there was a decrease in the relative amount of myosin heavey chain. And it was observed that the sarcoplasmic protein fraction contains more proteins with covalent disulfide bonds. The changes in the sarcoplasmic enzymatic proteins were, affected by storge and termperature as well as their potential contribution to textural deterioration. Also, it was observed that the determination of total extractable protein, as an indicator, for the deterioration during frozen storage, and it was found considerable changes, that can be seen in total extractable protein for 26 days which decreased with elevated temperatures and storage time.

Loss of water holding properties, or loss of juiciness are recognized as being due to protein denaturation during frozen storage, particularly the myofibrillar proteins (Dyer, 1951).

Connell (1960) reported that a clear relationship between the decrease in protein extractability and the increase in toughness of fish fillets. When comparing myofibrillar proteins to sacroplasmic proteins they seem to be more stable, and their solubili-

ty remains unchanged except after a long storage time. Within the myofibrillar group, myosin is by far the most sensitive protein to denaturation, whereas actin show a very small change.

Tropomyosin is considered the most stable myofibrilliar protein in fish during frozen storage (Matsumoto, 1980).

Baliga *et al*, (1969) studied the changes in soluble protein nitrogen of fresh water fish during iced storage and found that total protein solubiliy decreased 20% after 15 days, while sarcoplasmic protein decreased only slightly.

Low temperatures are used to retard chemical reactions and the action of food enzymes and to slow down or stop growth and activity of microorganisms in food (Frazie, 1958).

During storage, gradual development and accumulation of number of chemical substances, due to the breakdown products of protein and fat. According to the content of these, it is possible to judge the freshness of fish and their fitness for human consumption (Borgstrom, 1961 and Zaitsev *et al.*, 1969), they reported that the destruction of microorganisms at low temperature is due not only to the metabolic disruption, but also as a result of mechanicl rupture of their cells during ice-formation.

Lovell and Barkate (1969) reported the incidence of health related bacteria in crayfish products; however, no studies have been described in the literature on spoilage bacteria in commercially processed crayfish. Most of the commercially processed crayfish is marketed as hand-peeled tails which are stored at low temperature, above freezing, until consumed.

Ibraheim, (1983) studied the effect of supercooling (-2^{o}C) storage on pH; acidity; amino nitrogen; myofibrillar, sarcoplasmic and stroma proteins and microbial count of Bolti fish and Hubbard chicken. He noticed that the supercooling storage decreased acidity; myofibrillar, sarcoplasmic and stroma proteins during storage. However, he observed that the supercooling storage led to decrease in both the total weight and moisture content of Bolti fish and Hubbard chicken.

Chopman *et al.* (1993) stated that extra-cold storage of Hake and Mackerel fillets and mince at -30^{o}C and -40^{o}C. Overtime, the quality of samples decreased according to sensory and chemical indices. They became tougher and generally more cohesive.

The presence of hepatopancreas tissue in other crustaceans has been implicated in similar textural problems (Papadopoulos and Finn, 1985), and collagenolytic enzymes from crustacean hepatopancreas tissue, partially characterized by Nip *et al.*, (1985), were found to degrade tail meat and collagen.

The presence of similar enzymes in crayfish, hepatopancreas could be expected to degrade the texture of crayfish meat as well as gelatin (amorphous collagen).

Presently there are no established crayfish processing procedures, therefore blanch times very considerably. If the enzymes in hepatopancreas are not inactivated by blanching, the presence of this tissue in packages of fresh crayfish meat could cause mushiness (Marshal, *et al.*, 1987).

The crayfish hepatopancreas is rich source of proteolytic enzymes (Kin *et al.*, 1992).

The present investigation was carried out to study chemical, bacteriological and protein fractions changes occurring during frozen storage of crayfish (as whole and tail meat).

MATERIALS AND METHODS

Materials and sampling for analysis

The live red crayfish (*Procambarus clarkii*) between 6-8 cm in length and 40-50 gm in weight harvested from the river Nile in Giza, were purchased from a fisherman in July and transferred immediately to the laboratory. The live crayfish were placed in boiling water for 5 min. and cooled directly in ice water, then divided into two groups. The first group as the whole crayfish (WC); the second group the tail separated from it as tail crayfish (TC). Each group packaged in polyethylene bags and stored in deep freezer (at -18°C) for analysis. The tail separated from first group (WC), the meat obtained by the peeling of the tail and the tail meat of both groups were subjected to chemical and microbiological analysis. The analysis was carried out periodically after 1, 2, 3, 4, 5 and 6 months.

Analytical methods

1- Proximate composition and non-protein nitrogen (NPN): Moisture, crude protein, crude fat, ash and NPN contents were determined according to the methods described in the AOAC (1980).

2- Determination of amino nitrogen (Amino N): The amino nitrogen content of the analyzed samples was determined using the formol volumetric titration method as described by Kolochov (1952).

3- Measurement of pH value and acidity: The pH value was measured in a slurry of sample according to the method recommended by Krilova and Liskovskaia (1961) using a Beckman pH meter with a combined electrode, and acidity was determined according to Keeton and Melton (1978).

4- Determination of histamine content: the histamine content of the analyzed samples was determined by the thin layer chromatorgraphy according to the method described in the E.S. (1990).

5- Determination of protein fractions: Sarcoplasmic protein extract, myofibrillar protein extract, denaturated protein extract and stroma protein were determined by the method of King (1966), using buffer solutions of different values of pH and ionic strength. Extractions were carried out at +2 to +14°C with cold extracting solutions. The total nitrogen of all frations was determined by the standard microKjeldhal method as reported by the AOAC (1980).

6- Microbiological analysis: Sample preparation, (tail meat) was removed from the shell and placed in a sterile blender jar and ground (2 min.) to a slurry. Twenty grams of the ground samples were aseptically transferred into a sterile 0.1% peptone-water solution (180 ml.) and homogenized sterile dilutionsin 0.1% peptone water were prepared for pour plates from the homogenate.

6-1 Total areobic plate counts (TAPC): the TAPC was carried out on plate count agar media (Difco Manual, 1970) incubated at 35 °C for 24-48 hrs. (Refai, 1979) as described in the Food and Drug Administration (1978), were used for the determination of the ATPC content.

6-2 Mould (fungi) count: The total count of fungi was determined according to the method described in Difco Manual (1970), using a malt agar media.

6-3 Yeast count: The total count of yeast was determined according to the method described in Difco Manual (1970), using a potato dextrose agar media.

RESULTS AND DISCUSSION

1- Weight and moisture changes of the WC and TC during frozen storage:

The results illustrated in Table 1 show the effect of frozen storage on the weight and moisture content of the WC and TC. The weight and moisture losses were calculated as percent.

From Table 1, it is clear that the average weight and moisture content of samples were decreased gradually during frozen storage. It was found that the weight losses of WC and TC were increased gradually with prolonged storage and the increase was higher for TC sample than WC sample, probably due to leakage of meat juice and to the evaporation occurred during frozen storage.

Also, from Table 1, it can be observed that the moisture percentage of WC and TC decreased with the same trend that occurred in the weight content, but the % of moisture loss was higher for WC than TC. These results agree with the results obtained by Ibraheim (1983) and Chapman *et al.* (1993).

Table 1. Weight and moisture losses of whole and tail crayfish during frozen storage.

Months of storage	Weight (gm)				Moisture(%)			
	WC	% loss	TC	% loss	WC	% loss	TC	% loss
0*	46.27	0	9.98	0	73.16	0	73.09	0
1	45.67	1.31	9.87	1.80	71.91	1.71	72.85	0.33
2	45.58	1.49	9.80	1.80	71.70	1.98	72.69	0.55
3	45.43	1.82	9.72	2.60	71.55	2.21	72.53	0.77
4	45.20	2.31	9.65	3.31	71.37	2.43	72.43	0.90
5	44.70	3.39	9.45	5.31	71.22	2.64	72.32	1.05
6	44.35	4.15	9.36	6.21	71.10	2.80	72.20	1.22

Data are average of 6 crayfish
* After blanching in water for 5 min.
WC: Whole crayfish TC: Tail crayfish.

2- Quality changes in the WC and TC during frozen storage

Data presented in Tables 2 and 3 show the effect of frozen storage on the quality aspects (pH, acidity, amino N, non-protein nitrogen (NPN) and histamine) of whole and tail crayfish.

It was observed that the pH increased during frozen storage, the increase was higher for whole crayfish (WC) than the tail crayfish (TC), whereas the pH increased from 7.28 to 7.75 for WC and from 7.27 to 7.55 within 6 months of storage. This increase in the pH value during storage could be due to the bacterial breakdown of proteins leading to the accumulation of some basic and alkaline products such as ammonia and amine, or could be attributed to autolysis by tissue proteolytic enzymes (Zaitsev *et al.*, 1969). On the other hand, the results in Tables 2 and 3 revealed that the changes in acidity took the inverse direction of pH values.

Also, from these tables, it is obvious that the amino-N and NPN increased gradually during frozen storage for WC and TC, but the increase was higher for WC sample than TC sample. The amino N increased from 9.68 to 29.93 mg/100g for WC and 9.29 to 18.35 mg/100g for TC within 6 months storage (on dry weight). The NPN took the same trend of amino N for two samples, might be due to the slight activity of some proteolytic enzymes and microorganisms and the breakdown of proteins and amino acids. These results are in agreement with the results obtained by Borgstrom (1961); Zaitsev *et al.* (1969); Baliga *et al.* (1969) and Ibraheim (1983).

From results in tables 2 and 3 it was found that histamine content increased with the storage time for the two samples, the increase was higher for WC sample than TC sample. Nagayama *et al.* (1985) believed that histamine was a good indicator of quality because it started to form before any visible signs of decomposition occured and prior to the changes in pH and volatile basic nitrogen. The toxic level of histamine was reported to be 20 mg/100g (El-Marrakchi *et al.*, 1990)

3- Changes in protein fractions of the WC and TC during frozen storage:

Data in tables 4 and 5 and figures 1 and 2 show the effect of frozen storage on protein fractions of whole and tail meat of crayfish. It was observed that the percentage of myofibrillar, sarcoplasmic and stroma proteins decreased during frozen storage for 6 months of whole and tail meat of crayfish from 12.44 to 8.15%, 12.46 to 9.60%; 6.15 to 4.65%, 6.16 to 4.90% and 3.39 to 2.50%, 3.40 to 2.70%, respectively. While denaturated protein increased in all samples.

Table 2. Quality aspects of whole crayfish (WC) during frozen storage.

Months of storage	PH	Acidity (as Lactic acid)		Aminon (mg/100g)		Non protein N. %		Histamine (mg/100g)	
		A	B	A	B	A	B	A	B
0*	7.28	0.56	2.09	2.60	9.68	0.40	1.49	0.11	0.41
1	7.33	0.48	1.71	2.86	10.18	0.45	1.60	0.59	2.10
2	7.36	0.38	1.34	3.08	10.88	0.50	1.77	1.08	3.82
3	7.45	0.37	1.30	4.65	16.34	0.56	1.97	1.57	5.52
4	7.62	0.36	1.26	5.80	20.26	0.59	2.06	2.08	7.27
5	7.70	0.35	1.22	7.30	25.36	0.64	2.22	2.62	9.10
6	7.75	0.34	1.18	8.65	29.93	0.70	2.42	3.21	11.11

Table 3. Quality aspects of taill crayfish (TC) during frozen storage.

Months of storage	PH	Acidity (as Lactic acid)		Aminon (mg/100g)		Non protein N. %		Histamine (mg/100g)	
		A	B	A	B	A	B	A	B
0*	7.27	0.54	2.01	2.50	9.29	0.37	1.37	0.04	0.15
1	7.29	0.52	1.92	2.70	9.94	0.42	1.55	0.09	0.33
2	7.30	0.47	1.72	2.80	10.25	0.46	1.68	0.14	0.51
3	7.35	0.47	1.71	2.60	13.11	0.48	1.75	0.21	0.76
4	7.42	0.45	1.63	3.85	13.96	0.50	1.81	0.38	1.38
5	7.47	0.44	1.59	4.20	15.17	0.52	1.89	0.49	1.77
6	7.55	0.43	1.55	5.10	18.35	0.55	2.01	0.58	2.09

Data are average of 6 crayfish
* After blanching in water for 5 min.
A: On wet weight basis. B: On dry weight basis.

Table 4. Protein fractions for meat of while crayfish (WC) during frozen storage (ON wet basis).

Months of storage	Myofibrillar P. %		Sarcoplasmic P %		Denaturated P. %		Stroma P. %	
	A	B	A	B	A	B	A	B
0*	12.44	54.99	6.15	27.19	0.64	2.83	3.39	14.99
1	11.65	51.50	5.92	26.17	1.01	4.47	3.30	14.59
2	10.86	48.01	5.69	25.15	1.38	6.10	3.25	14.37
3	10.70	47.30	5.45	24.09	3.65	16.14	3.00	13.26
4	9.35	41.34	5.10	22.55	4.15	18.35	2.85	12.60
5	8.65	38.24	4.90	21.66	5.00	22.10	2.60	11.49
6	8.15	36.03	4.65	20.56	5.50	24.43	2.50	11.05

* After blanching for 5 minutes in water
A: % of fresh meat B: % of total protein of fresh meat.

Table 5. Protein fractions for meat of tail crayfish (TC) during frozen storage (ON wet basis)

Months of storage	Myofibrillar P. %		Sarcoplasmic P %		Denaturated P. %		Stroma P. %	
	A	B	A	B	A	B	A	B
0*	12.46	55.08	6.16	27.23	0.60	2.65	3.40	15.03
1	11.85	52.39	5.98	26.44	0.95	4.20	3.33	14.72
2	11.26	49.78	5.80	25.64	1.26	5.57	3.28	14.50
3	10.66	46.64	5.45	24.09	2.45	10.83	3.20	14.15
4	10.10	44.65	5.25	23.21	3.38	14.94	3.10	13.70
5	9.85	43.66	5.10	22.55	3.55	15.94	2.90	12.82
6	9.60	42.44	4.90	21.66	3.95	17.46	2.70	11.94

* After blanching for 5 minutes in water.
A: % of fresh meat B: % of total protein of fresh meat.

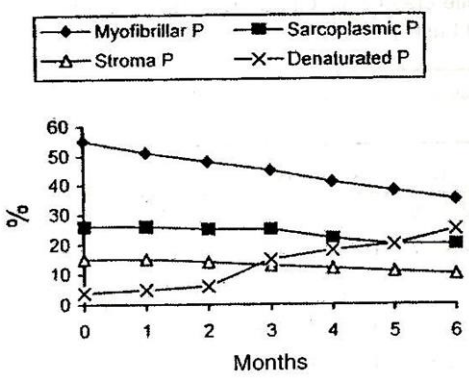

Fig 1: Protein fraction of WC during frozen storage

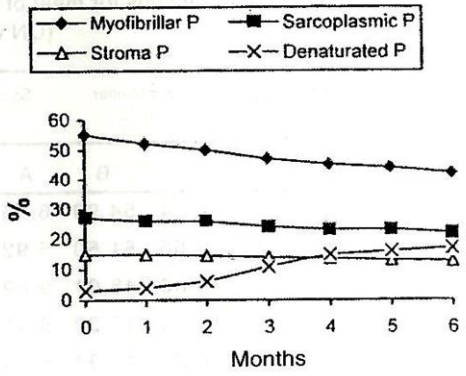

Fig 2: Protein fraction of TC during frozen storage

On the other hand, the decrease in the solubility of myofbrillar and sarcoplasmic proteins was reflected in the increase of the amount of denaturated proteins which ranged from 0.64 to 5.50% for WC and ranged from 0.60 to 3.95% for TC after storage for 6 months. It was demonstrated that the major change was myofibrillar protein, followed by sarcoplasmic protein, and slight change occured in stroma protein. The percentages of changes after 6 months of myofibrillar, sarcoplasmic and stroma proteins of whole and tail meats was 34.49, 22.95%; 24.39, 20.45% and 26.25, respectively.

Although, the changes of tail meat (TC) were less than of whole meat (WC) during frozen storage. The decreasing in myofibrillar and sarcoplasmic proteins during frozen storage might be due to the degradation of the proteins by enzymes and bacteria. (Baliga, *et al.* 1969; Zaitsev, *et al* 1969 and Leblanc and Leblanc, 1989).

These results agree with results obtained by Dyer (1951); Moorjani *et al.* (1962); Zaitsev *et al.* (1969); Goll *et al.* (1974); Ibraheim (1983) and Hsu *et al.* (1993).

4- Microbiological evaluation of WC and TC during frozen storge

The microbiological evaluation of whole and tail crayfish during frozen storage are presented in table 6. It is clear from the results that TAPC for (WC) and (TC) were decreased after one month storage than after blanching probably due to that these or-

Table 6: Microbiological evaluation of whole and tail crayfish during frozen storage. (CFU/g).

Months of	TAPC		Fungi count		Yeast count	
storage	WC	TC	WC	TC	WC	TC
0*	$2.1x10^2$	$1.9x10^2$	-	-	$1.1x10^2$	$0.9x10^2$
1	$1.7x10^2$	$1.5x10^2$	$0.8x10^2$	-	$0.8x10^2$	$0.7x10^2$
2	$1.9x10^2$	$1.6x10^2$	$0.9x10^2$	-	$1.2x10^2$	$0.9x10^2$
3	$2.3x10^2$	$1.8x10^2$	$1.1x10^2$	-	$1.4x10^2$	$1.1x10^2$
4	$2.8x10^2$	$2.1x10^2$	$1.2x10^2$	-	$1.5x10^2$	$1.2x10^2$
5	$1.3x10^2$	$2.8x10^2$	$1.4x10^2$	-	$1.7x10^2$	$1.3x10^2$
6	$1.4x10^2$	$3.5x10^2$	$1.6x10^2$	-	$1.8x10^2$	$1.3x10^2$

* Afer blanching in water for min Total count Incubated at 35°C.
Results are average of three replicated

ganisms were adequated with environment, but TAPC was increased progressively with the increase of storage time, which it reached 1.4×10^3 for WC and 35×10^2 CFU/g for TC. nevertheless, the TAPC was higher for WC than TC.

Also, from the Table 6, showed the total counts of fungi and yeast of whole and tail crayfish, observed that the fungi was not detected in TC during frozen storage, while fungi count was increased during frozen storage for WC which ranged from 0.8×10^2 to 1.6×10^7 CFU/g after 6 months. Moreover, the yeast count was increased during frozen storage but the increasing for WC was higher than TC.

In general, the microbial counts for WC was higher than TC because of the powerful digestive enzymes which perforate the intestine and attack the walls of the body cavity. These results are in agreement with the findings by Zaitsev, *et al.* (1969); Lovell and Barkate (1969); Cox and Lov (1973); Ibraheim, (1983); Papadopoulos and Finne, (1985); Marshal, *et al.* (1987) and Kim, *et al.* (1992).

Hence, it might be concluded that the results of the present work revealed that frozen storage is suitable for crayfish for a long time reached to above 6 months (Frazie, 1958), also it is preferable to store tail meat than whole meat due to the less deterioration in the first, lessening the costs of freezing and utilization of crayfish wastes by processing it to fishmeals.

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تغيرات الجودة لاستاكوزا المياه العذبة (بروكامبارس كلاركي) المجمدة خلال التخزين

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قسمت عينات استاكوزا المياه العذبة (بروكامبارس كلاركي) بعد السلق في الماء المغلي إلي مجموعتين استاكوزا كاملة والذيل فقط، وأجريت التحليلات الكيماوية والميكروبيولوجية لكل مجموعة أثناء التخزين بالتجميد لمدة ٦ شهور.

وجد أن الفقد في الوزن لكل من الاستاكوزا الكاملة والذيل يزداد على مدى التخزين، ووجد الفقد في الوزن أكثر في الذيل عن الاستاكوزا الكاملة، ولكن الفقد في الرطوبة اكثر في الاستاكوزا الكاملة من ١,١٧ إلي ٢,٨٠٪ أما في الذيل ما بين ٠,٢٣ إلي ١,٢٢٪ خلال التخزين لمدة ٦ شهور، ووجد أن قيم الأس الايدروجيني تزداد خلال فترة التخزين بالتجميد. ولكن تلك الزيادة في الاستاكوزا الكاملة تكون أكثر من الزيادة في الذيل، ووجد أن الحموضة القابلة للمعايرة تأخذ اتجاها عكسيا لقيم الأس الأيدروجيني في العينات. أما قيم النتروجين الأميني والنتروجين الغير بروتيني والهستامين فإنها تزداد خلال فترات التخزين، ولكن الزيادة تكون أكثر في الاستاكوزا الكاملة عن الذيل فقط.

تنخفض نسبة بروتينات الميوفيبلار والساركوبلازم والاستروما خلال التخزين بالتجميد لمدة ٦ شهور لكل من الاستاكوزا الكاملة والذيل من ١٢,٤٤ إلى ٨,١٥٪ و ١٢,٤٦ إلى ٩,٦٠٪ و٦,١٥ إلى ٤,٦٥ و ٦,١٦ إلى ٤,٩٠ و ٣,٣٩ إلى ٢,٥٠٪ و ٣,٤٠ إلى ٢,٧٠٪ (على الوزن الرطب) بالترتيب بينما تزداد البروتينات المدنترة خلال التخزين. تزداد تدريجيا الأعداد الكلية للميكروبات الهوائية بزيادة فترات التخزين حيث تكون أعدادها ١,٤ × ١٠^{٣} للاستاكوزا الكاملة و ٣,٥×١٠^{٢} CFU/g للذيل بعد ٦ شهور، والزيادة في أعدادها تكون عالية في الاستاكوزا الكاملة عن الذيل، لم تظهر أي أعداد من الفطر في الذيل، ولكن وجدت زيادة في أعداد الفطر في الاستاكوزا الكاملة خلال التخزين بالتجميد حيث تراوحت أعداده ما بين ٨,٠×١٠^{٢} إلى ١,٦×١٠^{٢} CFU/g علاوة على ذلك وجدت زيادة في أعداد الخميرة للعينات أثناء التخزين بالتجميد.