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Quality Assessment of Formic Acid Silage Developed from Squilla *Oratosquilla nepa* (Latreille)

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Abstract

Silages were prepared by mixing formic acid with homogenized whole squilla *Oratosquilla nepa* at 4.5, 5.0, 5.5, 6.0 and 6.5% (v/w) levels. Samples were subjected to storage for 21 days at 28±2°C and changes in moisture, crude protein, ash content, crude lipid, chitin content and crude fibre were analyzed. Results indicated that moisture, chitin and ash contents were increased while, crude protein content decreased in the silage during the storage period. Crude lipid content decreased in 4.5% formic acid treated silage, while it was found to be increased in all other silage treatments with respect to the increase in storage period. Biochemical quality indices such as total volatile base nitrogen (TVBN), non-protein nitrogen (NPN), α amino nitrogen (AAN), peroxide values (PV) and free fatty acids (FFA) increased in all silage treatments with increase in experimental duration. The values were higher in silage prepared with 4.5% formic acid inclusion when compared with the low values in other silage treatments. The silage prepared from 5.5% formic acid inclusion maintained steady pH range of 3.82-3.87 during the experiment than the other silage treatments. Organoleptic analysis with respect to colour and odour showed better consistency in 5.5% formic acid silage than other silage treatments and remained in good condition throughout the storage period.

Keywords: Trawl discards, *Oratosquilla nepa,* formic acid, silage

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Introduction

Silage is fermented, high moisture stored fodder which can be fed to livestock. Squilla *Oratosquilla nepa* is a marine, epibenthic, predatory crustacean belonging to the order Stomatopoda. The species is widely distributed and is reported from Arabian Sea and Bay of Bengal (Sukumaran, 1980; Rajeswary, 1996). Around 12% of total trawl bycatch landings along south-west coast of India was constituted by stomatopods and non-edible biota (Menon et al., 2006). Crustacean bycatch landed at Chennai fisheries harbour during 2005 to 2009 comprised 23% of squilla (Pillai et al., 2014). It has been reported that squilla landed in bulk quantities formed a major bycatch component of shrimp fisheries (Raffi, 2000) and constituted considerable quantities in trawl discards from the south-west and east coast of India (Shanbhogue, 1973; Sukumaran, 1980; Nair & Iyer, 1990; Pillai & Thirumilu, 2012; Dineshbabu et al., 2012; Pillai et al., 2014). *Harpiosquilla indica* and *O. nepa* were recorded from trawl discards collected from south-east cost of Tamil Nadu (Prabhu et al., 2013). Studies conducted on invertebrate bycatch composition indicated that squilla was the second dominant group of trawl discards followed by gastropods, juvenile shrimps, fin fishes and hermit crabs (Kurup et al., 2004; Thomas & Kurup, 2005; Menon et al., 2006). Zacharia (2006) recorded 90% stomatopod bycatch landings from Single Day Fishing (SDF) trawlers along Mangalore coast.

Stomatopods (squilla) are rich in proteins and minerals. Presence of spines in their exoskeleton is a major constraint that hampers easy removal of the meat. Boiling the organism along with certain chemicals is a solution to this drawback (Raffi, 2000). This shellfish is readily available and very cheap owing to its bycatch status and can hence be considered as a source for silage preparation.

Utilizing squilla for pickle preparation has been reported (Rajeswary & Hameed, 1998). Studies on preparation of squilla meal for use as alternate protein sources have extensively been conducted (Naik et al., 2001; Rao & Kumar, 2005; Reddy et al., 1997). It has got high demand in poultry feed industry (Mohamed, 2004). Squilla landed at Karwar coast were utilized for the preparation of fertilizer and poultry feed (Bhat, 2003; Bhat & Shetty, 2005). Frozen stored squilla for a period of 5 months remained in good condition which was utilized for the preparation of value added products (Rajeswary & Hameed, 1998). Acid fish silages were prepared from different sources such as shrimp head waste (Nwanna et al., 2004); lizard fish and blue crab (Mach et al., 2010); marine bycatch (Al-Noor et al., 2014); Atlantic cod fillet offal (Haard et al.,1985); rainbow trout viscera (Goosen et al., 2014). However, attempts on developing silage from stomatopods have not been reported so far. The present study was undertaken to investigate the quality of acid silage prepared from squilla and to ascertain optimal levels of formic acid inclusions required.

Materials and Methods

Trawl discards were collected from Munambam fishing harbour, Kerala, India and transported immediately to laboratory in insulated containers with sufficient ice. They were washed repeatedly with freshwater and drained. Stomatopods were sorted out from discards and were identified as squilla *O. nepa* as described by Rajeswary (1996). Squilla was washed with potable water and was homogenized using a mechanical homogenizer. One kilogram each of the homogenized material was used to prepare batches of formic acid silages (FAS) at 4.5, 5.0, 5.5, 6.0 and 6.5% (v/w) levels of formic acid inclusions. The prepared silages were kept in air tight, food grade plastic containers for storage at room temperature (28±2°C) for 21 days. Aliquots were drawn from the respective containers for further analysis.

Proximate composition of homogenized whole *O. nepa* such as moisture content, crude protein, ash content and crude lipid were estimated using standard procedures (AOAC, 1995). Chitin nitrogen was estimated by the method described by Garg et al. (1977) by alkali digestion and estimating the nitrogen in the digest by Microkjeldahl method. Chitin content was determined by multiplying the chitin nitrogen by 14.5 (Xavier, 2006). Crude fibre was estimated according to the procedure of Maynard (1970).

Biochemical compositions of five types of formic acid silages were estimated on the initial and final days of ensilation. Parameters such as moisture content, crude protein, crude lipid, ash content were estimated using standard AOAC procedures. Crude fibre was estimated according to the procedure of Maynard (1970). Chitin nitrogen was estimated by the method followed by Garg et al. (1977) by alkali digestion and estimating the nitrogen in the digest by Microkjeldahl method.

Quality parameters of five batches of formic acid silages, such as total volatile base nitrogen (TVBN), non-protein nitrogen (NPN), α-amino nitrogen (AAN), free fatty acids (FFA) and peroxide value (PV) were estimated periodically on 0, 3rd, 5th, 10th and 21st days of experimental period by drawing aliquots of samples from each treatment. TVBN was analyzed as per the procedure of Byrene & Conway (1933). NPN and AAN contents of acid silages were estimated by following the standard procedures of AOAC (1995). FFA was detected by following the procedures of AOAC (2000) and PV values were estimated as per Jacob (1958).

The pH values of five formic acid at each silage treatments were measured on 0, 3rd, 5th, 8th, 10th, 13th, 15th, 18th and 21st days of study period by homogenizing 5 g samples in 100 ml distilled water and observing the pH using Oakton digital pH meter (Eutech Instruments, Singapore) according to APHA (1998). Sensory parameters of the five types of formic acid silages such as colour and odour were assessed on 0, 5th, 10th, 15th and 21st days of intervals. Results obtained were expressed as mean ± SD and were subjected to ANOVA and Duncan's test using SPSS 18 software.

Results and Discussion

Proximate composition of *O. nepa* was determined on percentage dry weight basis and the details are given in Table. 1. The moisture content, crude fat, crude protein, ash content, chitin and crude fibre of *O. nepa* were estimated to be 78.54, 4.82, 40.07, 30.29, 11.75 and 2.89% respectively.

The biochemical composition of five types of formic acid silages prepared from *O. nepa* were estimated at the initial and final days of ensilation and the details are given in Table 2. The initial moisture

Table 1. Proximate composition of *Oratosquilla nepa* (whole) in % dry weight basis

Parameters	Mean ± SD
Moisture %	78.54 ± 0.69
Crude Fat %	4.82 ± 1.33
Crude protein % (*TN x 6.25)	40.07 ± 0.68
Ash content %	30.29 ± 1.59
Chitin % (*CN x 14.5)	11.75 ± 0.17
Crude fibre%	2.89 ± 0.54

*TN= Total Nitrogen
*CN= Chitin Nitrogen

content of freshly prepared silages were estimated as 78.74, 79.24, 79.00, 78.54 and 78.98% in 4.5, 5.0, 5.5, 6.0 and 6.5% formic acid inclusions respectively. Slight increase in moisture content was observed in all silage treatments after 21 days of experiment without showing any significant difference ($p>0.05$). Hammoumi et al. (1998), recorded increased moisture content in the fermented silage prepared from chopped waste of *Sardina pilchardus* incubated at 22°C for 20 days. The crude lipid values estimated on initial and final ensilation days showed significant difference ($p<0.05$) between treatments. After 21 days of storage, crude lipid content decreased from 3.79 to 2.19% in silage prepared with 4.5% formic acid inclusion, where as the value increased in all other silage treatments and the highest value was estimated in 6.5% formic acid silage (5.84%). The reduction in lipid content in 4.5% formic acid silage inclusion may be attributed to increased rancidity due to insufficient quantity of formic acid inclusion. The increment in lipid content may be due to increased release of fat from the viscera of the raw material used. Hasan (2003) reported gradual increase in lipid content from 11.2 to 19.23% in 3% formic acid silage prepared from *R. brachysoma* during the ensilation period of 60 days. Continuous increase in lipid content from 2.58 to 3.97% was reported in 2 to 3% formic acid silage developed from marine fishery waste stored for 30 days (Ramasubburayan et al., 2013).

The values for ash content were high in all five silage treatments and were ranging between 28.14 to 30.07%. However, the values did not show any significant difference ($p>0.05$) between treatments. High mineral content in silage may be the result of release of mineral depositions from the exoskeleton of squilla in presence of formic acid. Increased mineral content was observed in shrimp shell waste silage with organic acid which is attributed to increased demineralization of the shell waste in presence of the acid (Santhosh et al., 2007). The crude protein content decreased significantly in all treatments ($p<0.05$) with respect to the increase in storage period and the decrement was more pronounced in silage prepared with 4.5% formic

Table 2. Details of biochemical composition (%) of five types formic acid silages of *Oratosquilla nepa* estimated on initial and final ensilaging days

Parameters	Days	Concentrations of Formic Acid				
		4.5%	5.0%	5.5%	6.0%	6.5%
Moisture %	0	78.74±0.46	79.24±0.80	79.00±0.76	78.54±0.65	78.98±0.76
	21	78.86±0.73	79.33±0.95	79.01±0.74	78.92±0.82	79.00±0.96
Crude lipid %	0	3.79±1.14	3.78±0.82	3.76±0.82	3.93±0.55	3.77±0.66
	21	$2.19±0.61^a$	$4.24±0.73^b$	$4.46±0.93^b$	$5.22±0.77^c$	$5.84±1.16^c$
Ash content %	0	28.14±0.93	28.30±0.98	28.48±1.19	29.09±0.99	29.14±1.55
	21	28.27±1.35	28.56±1.18	29.07±1.29	29.17±1.24	30.07±1.43
Crude protein %	0	39.35±1.15	40.54±1.22	39.52±1.39	40.29±1.99	38.64±1.68
	21	$37.59±1.83^a$	$39.74±1.14^b$	$38.97±0.96^{ab}$	$39.40±0.94^b$	$38.55±1.23^{ab}$
Chitin nitrogen %	0	0.60±0.07	0.63±0.05	0.63±0.04	0.62±0.04	0.61±0.04
	21	$0.71±0.08^a$	$0.70±0.09^a$	$0.67±0.04^{ab}$	$0.67±0.06^{ab}$	$0.62±0.04^b$

Each value is a mean±SD of three replicate samples: values in the same row with different superscripts for each group are statistically significant ($p<0.05$)

acid (37.59%) compared to other silage treatments. Reduction in crude protein content may be attributed to breakdown or hydrolysis of protein during the period of storage. Significant decrease of crude protein content was reported in formic acid silages prepared from processing wastes of grouper *Epinephelus malabaricus* and stored for 30 days (Ramasubburayan et al., 2013). On contrast, chitin nitrogen contents showed increase from initial values in all treatments which differed significantly between treatments (p<0.05) during storage. The values were found the highest in 4.5% silage (0.71%) while, it was at the lowest in 6.5% silage (0.62%) at the end of the study period. The results indicate that higher formic acid inclusions may be negatively affecting chitin nitrogen content as evidenced from the lowest value in 6.5% formic acid silage in contrast to high value in 4.5% silage.

The details of changes in biochemical quality indices of five types (4.5, 5.0, 5.5, 6.0 and 6.5%) of formic acid silages prepared in the present study on storage are given in Table 3. Total volatile base nitrogen (TVBN) content increased in all silage treatments

Table 3. Changes in biochemical quality indices of different formic acid silages (FAS) prepared from *Oratosquilla nepa* estimated at various days of storage

Parameters	Days interval	4.5% FAS*	5.0% FAS	5.5% FAS	6.0% FAS	6.5% FAS
	0	90.76±13.10	81.43±23.30	74.43±27.08	78.40±17.30	78.33±23.92
	3	116.9±33.13	95.90±30.53	99.16±31.64	94.01±20.00	87.26±18.79
TVBN	5	127.4±57.04	92.40±45.46	92.16±47.82	103.6±27.19	97.53±15.74
(mg%)	10	167.3±65.64^{a}	118.06±50.21^{b}	117.13±54.57^{b}	105.23±33.46^{b}	93.80±35.18^{b}
	21	252.93±79.8^{a}	112.7±40.7^{b}	114.80±42.5^{b}	128.33±27.86^{b}	104.76±26.5^{b}
	0	672±42	690±16	588±56	602±96	597±85
	3	868±84	784±84	700±74	606±98	504±84
NPN	5	1092±74^{b}	1026±113^{b}	980±122^{b}	1008±100^{b}	849±70^{b}
(mg%)	10	1418±70^{b}	1138±70^{b}	1036±74^{b}	1026±100^{b}	812±74^{b}
	21	1437±58^{b}	1297±113^{b}	1116±32^{b}	914±113^{b}	737±12^{b}
	0	8.4±1.89	7.64±2.18	7.3±1.92	7.68±8.47	6.67±1.63
	3	11.09±2.24	8.40±2.55	10.01±2.27	9.80±2.80	8.29±2.17
AAN	5	18.64±2.46	15.50±4.21	14.96±4.62	14.32±5.30	14.75±4.30
(mg%)	10	21.75±5.31	18.09±2.45	18.30±2.52	19.38±3.65	17.01±3.01
	21	31.44±3.98^{a}	19.16±3.20^{b}	20.67±2.56^{b}	19.06±3.45^{b}	18.41±2.90^{b}
	0	8.21±3.40	7.69±3.16	9.52±4.22	11.60±3.85	9.04±5.39
	3	10.16±5.49	10.83±3.38	11.92±4.06	11.88±4.33	10.23±4.40
FFA	5	14.67±3.29	13.40±4.90	12.84±5.72	12.69±5.97	11.35±4.99
(mg% oleic	10	16.47±3.57	13.64±4.28	12.51±3.99	14.11±5.08	12.84±6.04
acid)	21	17.59±5.56	13.20±5.05	12.83±5.44	12.09±6.86	13.07±6.97
	0	10.76±3.70	10.52±3.54	10.50±3.18	11.06±4.61	10.10±2.59
	3	13.63±3.56	12.87±4.82	13.04±5.48	11.53±4.03	12.36±2.94
PV	5	16.99±3.80	14.59±3.29	14.23±4.47	15.06±4.21	14.61±3.88
(meq kg^{-1}	10	26.03±6.08^{a}	15.76±4.62^{b}	17.77±4.38^{b}	17.03±4.68^{b}	18.19±7.14^{b}
Fat)	21	34.07±7.57^{a}	17.16±4.95^{b}	16.82±3.41^{b}	16.17±5.40^{b}	18.99±5.59^{b}

Each value is a mean±SD of three replicate samples: values in the same row with different superscripts are statistically significant (p<0.05) with one-way ANOVA test and Duncan test with respect to the increase in storage periods. In 4.5% silage treatment, the initial value of TVBN was recorded at 90.76 mg%, while, the value increased remarkably to 252.93 mg% of the final day of the experiment. The value was comparatively lower in all other silage treatments on the final day of the experiment ($p<0.05$). The increment in TVBN content during storage may be attributed to microbiological and biochemical changes that occurred in the tissue during storage. Babu et al. (2005) reported increased TVBN contents of 2.0, 2.5 and 3.0% formic acid silages prepared from *Leiognathus sp*. as 236, 180 and 116 mg% respectively after a storage period of 21 days at ambient temperature. TVBN values were also reported in the range of 22.20 to 56.46 mg% in smoked *Colisa fasciata* stored at ambient temperature of 30±2°C (Karthikeyan et al., 2007).

Increment ($p<0.05$) in non-protein nitrogen (NPN) and α-amino nitrogen ($p<0.05$) contents were detected during the study period from the respective silages which may be the result of cleavage of peptide bonds and subsequent proteolytic activity. Babu et al. (2005) reported gradual increase in AAN content to 27.30, 21.21 and 24.33% of total nitrogen from 2.0, 2.5 and 3.0% formic acid silages respectively during 21 days of storage. He also reported that the NPN content of respective silages also increased remarkably to 70.72, 56.14 and 50.76 (g 100 g^{-1} of total nitrogen) during the experimental period. Mathew & Nair (2006) also reported similar findings in which the NPN and AAN contents increased from 0.575 to 0.813% and 0.093 to 0.223% respectively in fermented shrimp waste silage stored over a period of 12 days at room temperature.

The free fatty acid (FFA) values increased gradually though mean difference was not significant ($p>0.05$) in all batches of silage treatments with respect to the increase in storage days and it was more pronounced in 4.5% formic acid silage (17.59 mg% oleic acid) on the final day of the experiment. High values in FFA may be the result of lipolysis due to hydrolysis. Similar increment in FFA values were observed in microbial silage produced from *Tilapia niloticus* stored at 30°C for 180 days with and without the addition of antioxidants (Fagbenro & Jauncey, 1998). El-Ajnaf (2009) found similar increment in FFA values up to 5.13% and 7.58% in apple pomace and molasses fermented silages respectively prepared from *Sardina pilchardus* after 30 days of storage. Silage produced from European Sprat *Sprattus sprattus* and stored at 23°C increased FFA values from 6 to 18% over a storage period of 80 days (Tatterson &Windsor, 1974).

The peroxide values (PV) increased in all five silage treatments ($p<0.05$) in accordance with increase in storage period. At the end of the experiment, the value was noted higher (34.07 meq kg^{-1} fat) in 4.5% formic acid silage when compared with the low values in other silage treatments. The higher value in 4.5% silage may be the result of increased degradation of fat associated with low level of formic acid inclusion. Dapkevicius (2002) recorded increment in peroxide values of acid and biological silages prepared from Blue whiting *Micromesistius poutassou* during the storage period of 15 days.

The variations in pH values of five different formic acid silages at different intervals during the experimental period of 21 days is depicted in Table 4. The values were not significantly different ($p>0.05$) between treatments during initial stages of the experiment. When the experimental duration increased, the values showed significant difference

Table 4. Changes in pH values of different formic acid silages (FAS) prepared from *O. nepa* at various days of storage

Conc. of FAS	Days of Storage								
	0	3	5	8	10	13	15	18	21
4.5%	3.86±0.04^{a}	3.98±0.12^{a}	4.06±0.16^{a}	4.17±0.17^{a}	4.22±0.15^{a}	4.37±0.10^{a}	4.55±0.04^{a}	4.76±0.15^{a}	5.07±0.22^{a}
5.0%	3.86±0.04^{a}	3.88±0.06^{ab}	3.91±0.04^{b}	3.91±0.04^{b}	4.01±0.04^{b}	4.00±0.04^{b}	4.01±0.08^{b}	4.00±0.04^{b}	4.02±0.06^{b}
5.5%	3.82±0.05^{a}	3.80±0.04^{bc}	3.80±0.04^{bc}	3.82±0.05^{b}	3.83±0.09^{c}	3.83±0.04^{c}	3.86±0.02^{c}	3.86±0.07^{b}	3.87±0.02^{b}
6.0%	3.83±0.06^{a}	3.70±0.07^{c}	3.66±0.11^{cd}	3.65±0.1^{c}	3.63±0.03^{d}	3.58±0.10^{d}	3.67±0.05^{d}	3.60±0.10^{c}	3.63±0.1^{c}
6.5%	3.82±0.05^{a}	3.56±0.04^{d}	3.58±0.07^{d}	3.52±0.09^{c}	3.55±0.04^{d}	3.57±0.06^{d}	3.58±0.06^{e}	3.53±0.08^{c}	3.51±0.12^{c}

Each value is a mean ± SD of three replicate samples: values in the same column with different superscripts for each group are statistically significant ($p<0.05$) with one-way ANOVA and Duncan test

($p<0.05$) between treatments. It could be noted that the values were more or less steady in silages prepared with 5.0% (3.86 to 4.02) and 5.5% (3.82 to 3.87) formic acid inclusions and the later showed more consistency throughout the study period. Peches (1987) reported that pH should not be below 3.8 if formic acid alone is used for silage preparation. Gildberg (1993) reported that pH should be 4 or lower in organic acid silages made from fish waste or fish by-catch.

The changes in sensory qualities of five types of squilla silages prepared in the present study was evaluated on 0, 5th, 10th, 15th and 21st days of storage at ambient temperatures. Sensory parameters such as colour and odour were observed and it has deep brown colour with characteristic malty odour at the initial day of experiment. With increase in experimental period the silage preapared with 4.5% formic acid inclusion putrified fast with pale brown colour and putrified odours. The silages prepared with 6.0 and 6.5% formic acid inclusions were undesirable due to their high acidic odours. On the contrary, the silage prepared with 5.0 and 5.5% inclusions of formic acid maintained characteristic colour and odour and the latter (5.5%) was more consistent throughout the experimental period. Organoleptic changes in fermented silage prepared from sorghum grain was studied and found good for a storage period of 56 days at 30°C (Ott et al., 2005).

The present findings indicate that *O. nepa* from trawl discard can be used effectively for preparing silage using formic acid. Among the silages prepared with different levels of formic acids, 5.0 and 5.5% showed better storage characteristics in terms of biochemical and organoleptic qualities. Between these, 5.5% formic acid silage showed better consistency throughout the study period. Since squilla is abundantly available, it can be considered as a suitable raw material for preparing formic acid silage, which can be utilized as an alternate source of protein in aquafeeds.

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