Fishery Technology
2007, Vol. 44 (1) pp : 43 - 48

Effect of Different Organic Acids in Silage Preparation from Shrimp Shell Waste

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The comparative effect of three commonly used organic acids in the production of silage from shrimp shell waste is hereby reported. Formic acid, lactic acid and citric acid were used at 5%, 6%, and 9% concentrations respectively. Samples were kept for 16 days and at an interval of three days, changes in pH, viscosity, protein content and mineral content were analyzed. Formic acid silage had the highest liquefaction and mineral content. Amino acid profile showed highest proportion of essential amino acids in formic acid silage. In silage prepared with lactic acid and citric acid, protein content and mineral content were very low. Chitin prepared from the shell residue of formic acid silage had good quality compared to others as supported by FTIR spectrum and mineral status.

Key words : Shrimp waste, acid silage, lactic acid, formic acid, citric acid

Seafood holds a very unique position in our daily menu. But, the main curse of seafood industry is the large leaps of waste materials delivered by the processing plants. Amongst them, shrimp waste constitutes about more than one lakh tonnes per year in India (Philip & Nair, 2006). They are highly putrescible and quickly become colonized by spoilage microorganisms with the result of becoming a public health hazard (Sini *et. al.*, 2005). The best way to minimize environmental pollution is the conversion of waste materials to beneficial products. Shrimp waste contains about 10-20% calcium, 30-40% protein and 8-10% chitin (Legarreta *et. al.*, 1996). Chitin, a nitrogen containing polysaccharide, is the most valuable component in shrimp shell with numerous potential applications (Meibom *et. al.*, 2004). Chitin can be converted into chitosan, which has profound applications in various fields (Santhosh *et. al.*, 2006). Protein extracted from the shrimp shell waste has been proved to be an excellent animal feed supplement (Meyers & Benjamin, 1987).

Shrimp waste can be effectively converted into silage through an environment-friendly safe technology by using organic acids alone and in combination with *Lactobacillus* (Raa & Gildberg, 1982; Dapkevicius *et. al.*, 1998; Haard *et. al.*, 1985)

For the preparation of acid silage, choice of acid is very important. This work aims to study the effect of three most commonly used organic acids in the preparation of acid preserved shrimp silage and consequent production of chitin from the residual shell waste.

Materials and methods

Shrimp heads and shells of *Parapenaeopsis stylifera* was obtained in fresh condition from a local processing unit in Cochin, Kerala. The waste brought to the laboratory was thoroughly washed and minced in a Sumeet SP-16 electronic food preparation machine.

Formic acid, lactic acid and citric acid used were of analytical grade and procured from Sigma chemicals (USA).Potassium sorbate of

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food quality grade was obtained from SRL, Mumbai.

Silage preparation:

Formic acid silage:

About 500g of the shrimp waste was mixed well with 100ml water in glass container. To this, 30 ml (5 % of the total weight) formic acid was added gradually with constant stirring to keep the pH at 4 -4.2. It was kept for 16 days; samples were taken at intervals for analysis.

Lactic acid silage:

About 36 ml (6 % of the total weight) lactic acid was added to 500g shrimp waste, which was mixed with 100ml water and pH was maintained at 4 -4.2. 5g potassium sorbate was added as preservative. The silage thus prepared was stored for 16 days and samples were taken at intervals for analysis.

Citric acid silage:

A mixture of 500g shrimp waste and 100ml water was taken, 54 ml (9 % of the total weight) of citric acid was added with stirring and a pH in the range of 4-4.2 was maintained. 5g potassium sorbate was added for preservation.

Preparation of chitin

Shell residue obtained by the preparation of silage was converted into chitin. Residue was washed well with water and treated with 0.5 N HCl for 1 h at room temperature. Demineralised residue was washed free of acid and then boiled with 1.5 % (v/v) aqueous NaOH in the ratio of 2:3. Alkali was drained off, washed the residue with water till alkali free and dried.

Moisture, ash and crude protein were determined as per AOAC (2000). For the determination of pH values, 5ml of the sample was taken and pH was measured using Cyberscan 510 pH meter.

Viscosity of the samples was measured using Brookfield DV-E Viscometer with ULA spindle (SMC- 0.64) at 100 rpm.

Amino acid composition was analyzed after treating the hydrolysates with 6N HCl for 24 h at 110^{0}C as described by Moore & Stein (1963) using Shimadzu Amino acid analyzer. Tryptophan was determined colorimetrically (Fischl, 1960).

FTIR spectral analysis of the chitin prepared from shell residue of the three silages were carried out using Nicolet AVATAR 360ESP FTIR Spectrometer.

Calcium, Sodium, Potassium and Magnesium were analyzed for chitin using Varian SpectrAA 220 Atomic Absorption Spectrometer.

Results and Discussion

The proximate composition of shell waste used for silage preparation is given in Table 1.

The changes in the protein content of different silages prepared is indicated in Fig.1. It was observed that protein solubilisation was maximum during the initial 3-4 days in all the three silages and afterwards it remained almost constant. Maximum protein content was obtained in formic acid silage. About 45% protein of the raw material was liquefied in silage prepared using formic acid. But only 27% and 15% of the protein of the raw material were solubilised in lactic acid and citric acid used silages respectively.

The liquefaction of the protein is effected by means of the autolytic enzymes present in the shell. During the formation of silage, autolysis takes place and shrimp shell waste gradually liquefies as the protein matrix partially solubilizes due to its break down by endogenous enzymes (Raa & Gildberg, 1982). Since formic acid has higher pKa value (3.75) than the other two acids, it facilitates greater enzyme action and as a result more protein is leached into the silage.

Table 1. Proximate composition of shrimp shell waste

Composition	Percentage(wet weight basis)
Moisture	74.07± 2.10
Protein	11.48± 1.02
Ash	13.91±1.11
Fat	0.54±0.01

Acids used in the silage show their preservative action by the passage of

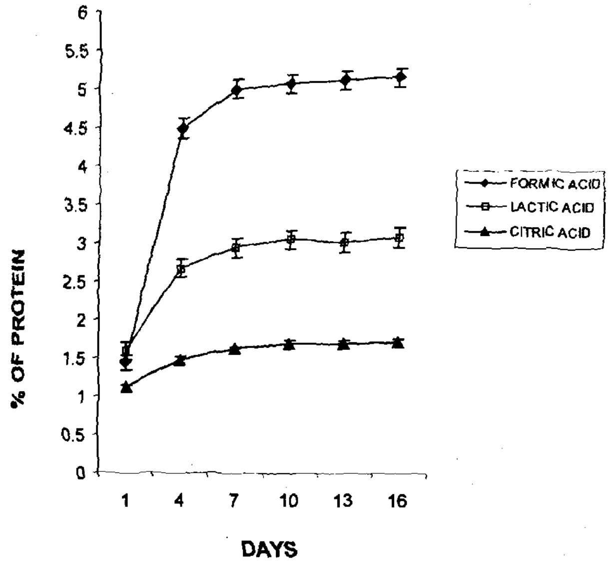

Fig. 1. Changes in protein content of the three different types of silage

undissociated acid molecule into the bacterial cell where it dissociates and lowers the pH to kill the cell (Raghunath & Gopakumar, 2002). Because of higher pKa value of formic acid, a greater proportion of the acid molecules are undissociated at higher pH and thus storage life of formic acid preserved silage is more than the other two types of silage.

To preserve the quality of the lactic acid silage and citric acid silage throughout the duration of the experiment, potassium sorbate was added to them.

Ash content of shrimp silages were very high. This was due to the high mineral content of the shrimp shell waste mainly in the form of calcium carbonate. Demineralisation of the shell waste takes place when organic acids are added to it during silage preparation. The extent of demineralization depends on the type of the organic acids and its concentration. Formic acid is stronger than lactic acid and citric acid. So, formic acid reacts strongly with the minerals, present in the shell waste, than the other two acids and as a result greater extent of demineralization was obtained by the use of formic acid. From the experiment, it was found that 75% demineralization of the shell took place when formic acid was used for silage preparation. About 72% and 67% minerals were removed from the shell with lactic acid and citric acid respectively. Lesser mineral content is more beneficial when silages are used for feed purpose. Here, citric acid silage has comparatively less mineral content than the other two silages. Changes in ash content of the silage is shown in Fig.2

Initial pH was adjusted to 4 – 4.2 in all the samples with gradual addition of respective acids. In the case of silage prepared using lactic acid, there was a reduction in pH from 4.1 to 3.67 in the first 3 days and thereafter pH remained more or less constant. This may be due

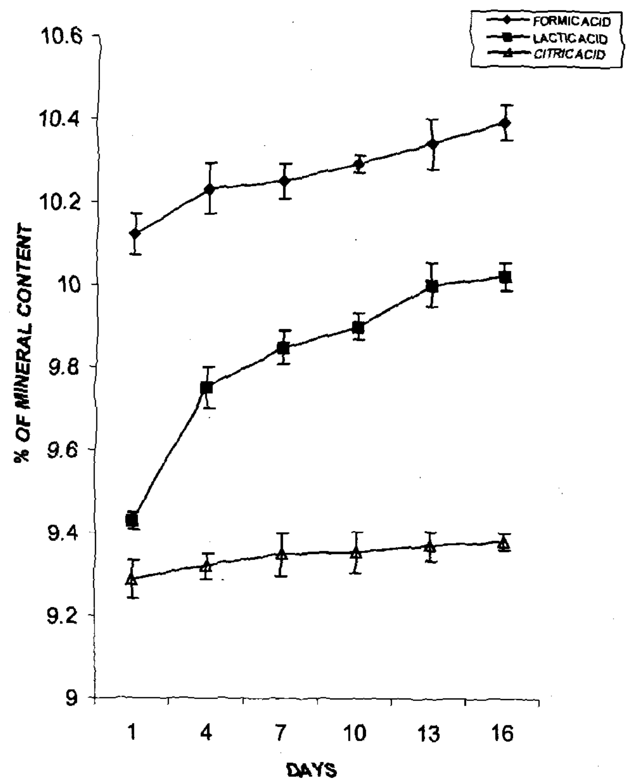

Fig. 2. Changes in mineral content in different types of silage

to the activity of the native lactic acid bacteria present in the raw shell waste. But in formic acid and citric acid treated samples, there was no significant change in pH during storage. pH changes are shown in Fig.3.

Viscosities of the samples were also measured, but it was found below 10 cp.

Amino acid compositions of the three types of silage were given in Table 2. Compared with the other two acid silages, formic acid silage has good composition of essential amino acids. Reduction in the levels of amino acid components in lactic acid silage and citric acid silage may be due to the chemical reactions between a-amino and aldehyde groups present in them (Johnson *et al.*, 1985).

Shell residue obtained after protein removal was also analyzed. The shell residue was generally found to be suitable for chitin preparation. In formic acid silage, shell waste residue had 25% ash and 55% residual protein. In lactic acid treated one, shell waste contained 28% ash and 73% protein. But in citric acid treated silage, 33% ash and 85% protein remained in the shell residue.

FTIR spectrum of chitin produced from the shell residues of the three silages are given in Figs 5a, 5b & 5c.

Chitin prepared from the shell residue of formic acid silage showed a better spectrum than that from other two acid treated residues. In lactic acid and citric acid treated chitins, the peaks (2361 cm^{-1} and 2365 cm^{-1}) responsible for hydroxyl groups, which determine the functional quality of chitin showed a gradual depletion.

Calcium, Sodium, Potassium and Magnesium were analyzed for chitin made from shell residue. Mineral composition of the chitin is given in Fig.4.

Chitin prepared with formic acid treated shell residue has less mineral content than the other two acids treated chitins. This is may be because of the high ionization value of formic acid. Chitin from citric acid has profound minerals because of the weak strength of citric acid. Lesser the mineral content, better the quality of chitin (Tolaimate *et. al.*, 2003).

Formic acid has the highest liquefaction effect and demineralization effect than lactic acid and citric acid. It has more antimicrobial activity so preservative effect is also more in the case of formic acid. More over, formic acid silage has good essential amino acid composition also. Waste residue obtained with silage can be economically utilized by its conversion into good

Table 2. Amino acid composition (g/100g crude protein) of three types of silage.

Amino acids	Formic acid silage	Lactic acid silage	Citric acid silage
Histidine	5.80	4.75	4.72
Lysine	7.18	6.96	6.98
Valine	5.72	5.56	5.49
Methionine	5.98	4.95	4.86
Leucine	7.95	7.80	6.89
Isoleucine	5.00	4.86	4.82
Phenylalanine	4.27	3.98	4.10
Arginine	2.14	1.93	1.56
Threonine	4.92	4.53	4.49
Tryptophan	0.74	0.72	0.69

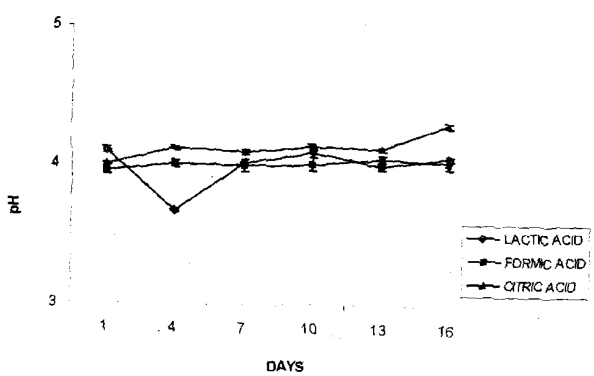

Fig. 3. Changes in pH of the three silage during storage period

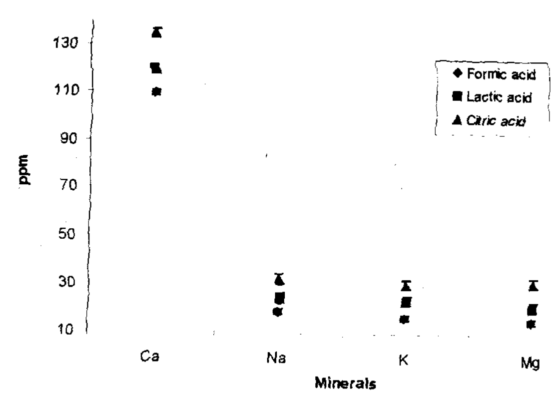

Fig. 4. Mineral composition of chitin prepared from the shell residue of different silages

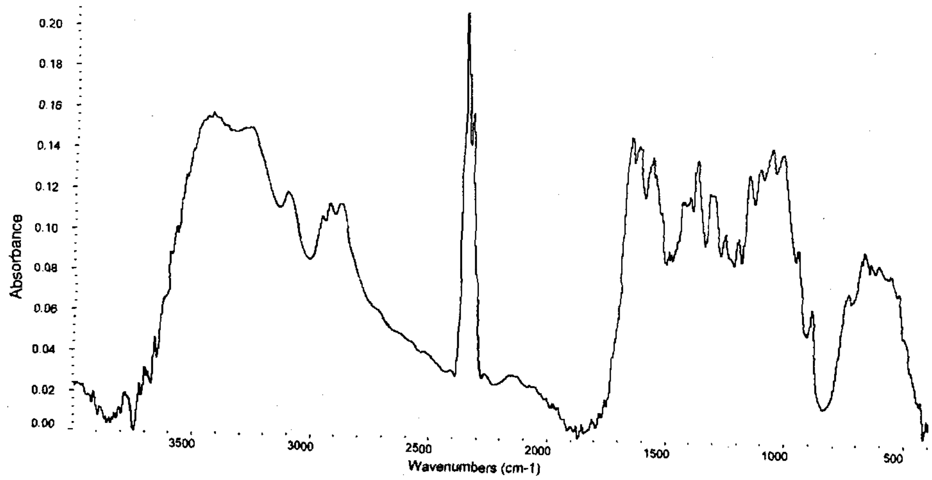

Fig. 5 a. FTIR spectrum of chitin prepared from formic acid treated shell residue

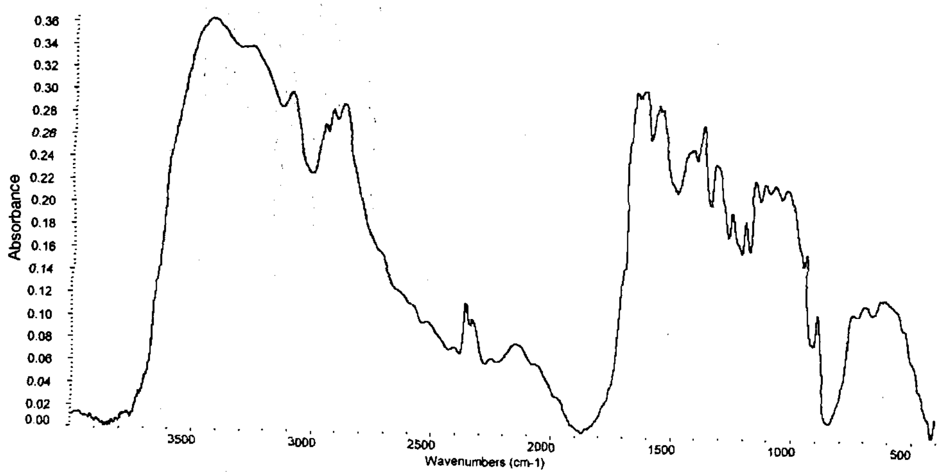

Fig. 5 b. FTIR spectrum of chitin prepared from lactic acid treated shell residue

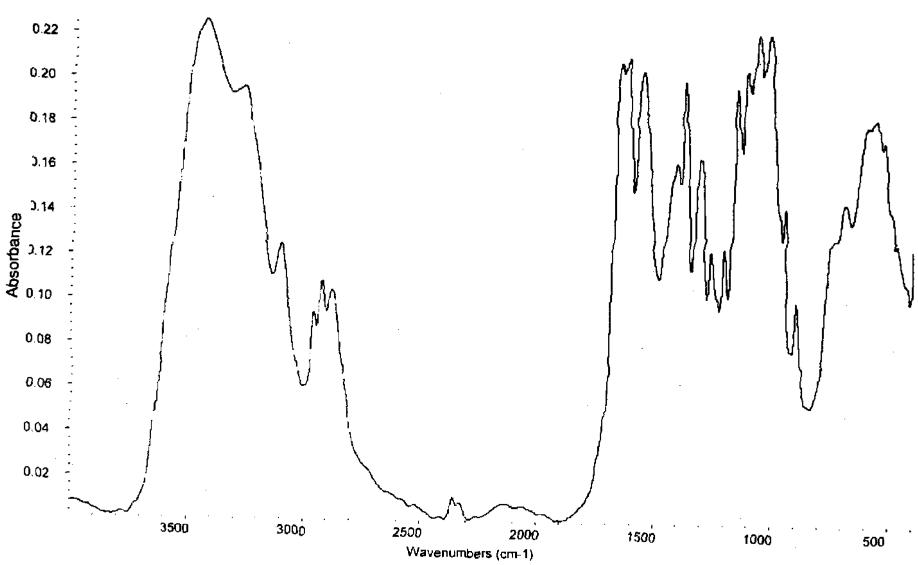

Fig. 5 c. FTIR spectrum of chitin prepared from citric acid treated shell residue

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quality chitin and chitosan. Chitin prepared with the shell residue of formic acid silage has good quality as supported by FTIR spectra and mineral status.

We are grateful to Dr.K.Devadasan, Director, Central Institute of Fisheries Technology, Cochin for providing us facilities to carry out this work.

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