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EFFICIENCY OF HURDLE TECHNOLOGY APPLIED TO RAW CURED MEAT(SI-RAW) PROCESSING

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INTRODUCTION

Si-Raw is one of the raw cured meat products (raw cured meat fermented with steamed rice) that is produced in Taiwan. The aboriginals in Taiwan prefer this type of traditional food. Some people who consumed this type of raw cured meat were poisoned and three of them died of botulism (Shih and Chao, 1986). In order to prevent botulism food poisoning a new method based on HURDLE technology was used to produce this kind of product, as an alternative to the traditional method.

MATERIALS AND METHODS

Using the traditional method, meat was cured in steamed rice and salt and placed in a jar for about one month. Using the new method, citric acid, sodium hypophosphite, Monascus anka mash, plum paste or lactic acid bacteria inoculum were separately added to salt and steamed rice to lower the Aw and pH values of the meat in order to control microbial growth. Sodium chloride, non-protein nitrogen, amino acid nitrogen, amines, ATP and its related compounds and total bacterial counts, anaerobic bacterial counts and lactic acid bacterial counts were determined and a panel test was also performed.

RESULTS AND DISCUSSION

Si-Raw (raw cured meat) is a fermented meat product that is produced by salt cutting followed by sealing in a container. Curing meat with sodium chloride is an ancient preservation method, and together with drying is the only method in many countries that ensures availability of meat throughout the year.

The major effect of this method is to reduce water activity and inhibit microbial growth so as to obtain the preservative effect. During processing the cured meat was placed on the bed of steamed rice in the container to create an anaerobic environment and to encourage a fermentation that favours anaerobic of facultative bacterial growth. The level of saltiness was decided on the basis of the pretest result and 10% salt was used for the test. There was a significant difference ($P<0.01$) in salt contents among the treatments, although salt was used at the same level for the precuring. This indicated that the additives or treatment influenced the salt distribution in the meat. The samples from the Si-Raw produced by the traditional method, and the citric acid, sodium hypophosphite plus citric acid, Monascus anka mash, plum paste, organic acid spray and lactic acid bacteria inoculation treatment methods were found to have salt contents of 6.67%, 6.02%, 6.2%, 6.29%, 6.36%, 5.46%, 6.49%, respectively. It is generally recognized that the factors affecting growth and toxin production of *Cl.botulinum* are pH, composition of food, redox potential and the presence of inhibitors (such as nitrite and nisin), Aw, temperature and competitive flora. A pH below 4.6 and a redox potential above-150mV can inhibit *Cl.botulinum* growth. As Aw is lowered below 0.93-0.92 the organisms are incapable of growth (Baird-Parker and Barbara, 1967;Pierson and Smoot, 1982; Hauschild, 1989). Si-Raw is generally recognized as safe because

at least three factors are exploited to inhibit *Cl.botulinum* growth: low Aw value, reduced pH and the inhibitory effect of food additives. With respect to the pH value, some lactic acid bacteria naturally grow on the meat surface. Schillinger and Lucker (1989) isolated 221 species of lactic acid bacteria from meat and meat products. Si-Raw is cured in a container and sealed, which encourages anaerobic fermentation. The decline in pH of the products was caused by microbial action. Endogenous enzyme action can cause the pH drop, resulting in the creation of a desirable condition for microbial growth. This type of microorganism has been identified as lactic acid bacteria. The steamed rice was added to the cured meat to supply carbohydrate to the Si-Raw as it was manufactured. In Table 1 it is shown the pH of samples from all treatments, which dropped from 6.7 to 4.3. This low pH could inhibit *Cl.Botulinum* growth. The pH value(4.08)for the sample inoculated with *Lactobacillus plantrum* as the lowest amongst all treatments. There was a significant difference ($P<0.01$) in pH level between the traditional sample and all other treatments except for that with added plum paste. The difference in pH values amongst the treatments might be due to naturally occurring lactic acid bacteria, which would be explained by their influence on the rate of acid production.

With respect to water activity (Aw), the results presented in Table 1 revealed that all treatments could reduce the water activity of Si-Raw from 0.97-0.98 in fresh meat to approximately 0.92. However, there also were differences amongst the treatments. It was found that the Aw for the control was not different from that of the treatment with 3% Na-hypophosphite, but it was significantly different from that of all other treatments. As well, Aw between the treatments with plum paste and anka mash were not significantly different, but they were significantly different from the other treatments. It is recognized that the effect of low Aw is to inhibit microbial growth, and *Cl.botulinum* cannot grow if the Aw is below 0.93-0.94. The data presented in Table 1 indicates that the level of Aw for all treatments could inhibit *Cl.botulinum* growth. The Aw of raw meat that is precured with 10% salt could decrease (approximately) to 0.95. In contrast, the Aw for the different treatments under anaerobic fermentation was decreased to below 0.94, which indicates that all treatments apparently were effective in reducing the Aw of Si-Raw.

The observed concentrations of nonprotein nitrogen (NPN), amines and amino acid nitrogen are shown in Table 2 indicating that changes in the muscle proteins took place during fermentation. Proteolytic enzymes break down muscle proteins producing NPN compounds that contribute to flavour and slightly increase pH (Mihalyr and Kormendy, 1967,Demeyer *et al.*, 1979). In this experiment, concentrations of NPN were obtained from the total nitrogen content by subtracting the protein nitrogen content. The result shown in Table 3 indicates that the NPN content of the sample from raw meat sprayed with organic acids was highest, the anka mash treated sample was second highest, and that of the traditional Si-Raw was third. Amino acid nitrogen content was highest in the sample treated with anka mash, that of the traditional sample was second, and the lactic acid bacteria inoculated sample was third in order. The NPN and amino acid nitrogen contents of the anka mash treated sample, were higher than was found in the other treatments. This finding may have been caused by the initial concentrations of NPN and amino acid nitrogen in the anka mash. Monascus anka has proteolytic activity that has been reported by Tsai (1980) and Lin (1986). Traditional Si-Raw is not inoculated with microorganisms which favours the fermenting process and proteolytic activity that is necessary for acid production and pH-drop. Nevertheless, the sample of lactic acid bacteria inoculated sample exhibited the same trend as Si-Raw produced using the traditional process. The conditions applied in the other treatments all had inhibitory effects on microbial growth. As a result of NPN and amino acid nitrogen contents it could be noted that the NPN content of the raw meat sample sprayed with organic acid was higher, its amino acid nitrogen was lower, and the amide nitrogen and ammonium nitrogen contents were higher.

These results indicate that the quality of this sample was undesirable. There was a very significant difference in NPN and amino acid nitrogen contents between the anka mash-added sample and samples from the other treatments. However, the rest of the treatments, except for the anka mash-added treatment, did not produce significantly different NPN and amino acid nitrogen contents. Data for biogenic amines in Si-Raw are presented in Table 3. Putrescine, cadaverine, tryptamine, beta-phenylethamine, spermidine, spermine, histamine, tyramine and agmatine were detected using high performance liquid chromatography (HPLC) analysis. From these results it could be noted that the treatments caused some differences in the levels and kinds of amines that resulted from the fermentation. Food scientists now pay more attention to the levels of biogenic amines present in food since it has been demonstrated that these compounds are harmful to the consumers. This is especially true of the amines, such as putrescine, cadaverine, histamine, bata-phenylethamine, spermine, spermidine, tryptamine and tyramine which have been reported to be higher in fermented

foods.

In general, decarboxylation of amino acids is caused by microbial decarboxylase, which produces biogenic amines. This can also be used as an indicator of the putrefaction of food (Yen, 1986; Yen and Wei, 1990). With the exception of the high tryptamine concentration in the sample of Monascus anka mash treated Si-Raw, amine concentrations resulting from all the treatments were lower when compared to those of other fermented meat products (reported by Yamanaka, 1989; Lakritz *et al.*, 1975; Vandekerckhove, 1977). The amino acid nitrogen content was higher in the product from the raw meat treated with organic acids, but lower in products obtained from other treated meats.

In Table 4, the results on the effect of treatments on ATP and its related compounds are presented. ATP in muscle dropped with the post-mortem time, but the result was not in agreement with the expected changes. In general, IMP appeared at a lower level and ATP and hypoxanthine remained at higher levels. The cause of these changes might be that during cured meat fermentation the ATP in muscle was hydrolyzed and converted into ADP>AMP>IMP>inosine>hypo-xanthine. The later stage of fermentation favours lactic acid bacteria growth under anaerobic conditions, and an increase in microbial ATP production when pH dropped below 5.0 ATPase may be inactivated, causing ATP and hypoxanthine to remain at higher levels. More research should be done on this aspect of the process. From statistical variance analysis ATP, AMP and hypoxanthine levels were found to be significantly different amongst the treatments. The ATP concentration was higher in the sample with added anka mash that was inoculated with lactic acid bacteria. This might help explain the possibility that ATP might originate from the treated microbial cells. The AMP in the anka mash treated sample was significantly higher($P<0.05$) than that in any of the other treatments. Hypoxanthine in the sample of the traditional, organic acid sprayed, Na-hypophosphite and citric acid were not significantly different, while the other treatments were significantly different ($P<0.05$). Hypoxanthine in the citric acid sample was lowest and its inosine content was highest. This may have been caused by the initial pH during the early fermentation period and by inactivated enzymatic activity, resulting in the inosine level of the citric acid sample being higher than that of the other treatments.

No *Trichinella spiralis* was detected in any of the treatment samples. From the pH, Aw,and salt content data values for the final products of Si-Raw, *Trichinella spiralis* should have been killed in this experiment. Crouse and Kemp (1963) indicated that *Trichinella spiralis* was not detected when pork was cured with 6% salt, smoked, then aged at 25°C for two weeks. Lotszch and Rodel (1974) reported that if Aw fell below 0.949 *Trichinella spiralis* could not survive in sausage, but they suggested that it was better to keep the Aw below 0.94. *Cl. botulinum* was not detected growing in Si-Raw samples of all treatment types and the control. Total aerobic bacteria counts for all samples were very low, as shown in Figure 1. Lactic acid bacteria and anaerobic bacteria were the dominant flora in the raw cured fermented meat. This may have been due to the inhibition of mesophilic aerobic bacterial growth by environmental factors such as redox potential, reduced pH, high salt content, reduced Aw, and bacterial competition during fermentation for one month. Surviving mesophilic aerobic microorganisms might be mainly yeasts and other adaptable facultative anaerobic bacteria. The lactic acid bacteria and anaerobic organisms determination may have found that there was a group of facultative or anaerobic organisms (not lactic acid bacteria) growing in/on the Si-Raw-fermented meat product. With the exception of the lactic acid bacteria inoculated sample, *bacilli*, *cocci* and Clostridium (Gram(+)) were detected in/on all Si-Raw samples using light microscopy.

The sensory evaluation was conducted by three different groups: the aboriginals, high school students, and the students working in our laboratory. All products were acceptable to the aboriginals and the agricultural school students, but were not acceptable to our laboratory students. However, the samples with Na-hypophosphite were acceptable to most of the test panellists. Fermented meat products are generally recognized as safe. More precisely, if critical points are properly controlled, this type of the fermented meat product -- Si-Raw should be safe because either the low Aw-value or a combination of reduced Aw-value and reduced pH will inhibit the growth of undesirable microorganisms. As pointed out earlier, these factors play a major role. Nevertheless, other factors also contribute to the safety of raw fermented meat products.

The purpose of this study was to improve the method of processing Si-Raw and its quality,and to prevent a reoccurrence of incidences of botulism. At the same time, it was our purpose to study the characteristics and quality of this kind of

traditional fermented meat product. As mentioned above, there was no significant difference between Si-Raw produced by the traditional method and by the new methods used in this experiment. Both the traditional method and the new methods could inhibit *Cl.botulinum* growth. However, according to Dr.Listner's HURDLE technology (Hecheiumann and Kasprowiak, 1992) the pH, Aw, saltiness, and bacterial competition are inhibitory factors or "HURDLES" for microbial growth, which may be used to ensure Si-Raw product safety.

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Table 1. Water activity, pH and salt content of Si-Raw.

	Aw	pH	Salt (%)
Traditional	0.922 ± 0.0005a*	4.27 ± 0.03a	6.67 ± 0.07a
Citric acid	0.939 ± 0.0038^{b}	4.18 ± 0.07^{dc}	6.02 ± 0.06^{b}
Na-hypophosphite	0.922 ± 0.0020^{a}	4.13 ± 0.03^{def}	6.20 ± 0.04^{c}
Monascus anka mash	0.913 ± 0.0023^{c}	4.20 ± 0.04^{bc}	6.29 ± 0.07^{d}
Plum paste	0.915 ± 0.0018^{c}	4.24 ± 0.02^{ab}	6.36 ± 0.07^{e}
Sprayed with organic acid	0.932 ± 0.0021^{d}	4.12 ± 0.04^{ef}	5.46 ± 0.04^{f}
Lactic acid bacteria	0.926 ± 0.0016^{e}	4.08 ± 0.01^{f}	6.49 ± 0.09^{g}

* Figures in the same column with the same letters are not significantly different ($P<0.05$).

Table 2. Non-protein nitrogen MPN and amino acid nitrogen AAN contents of Si-Raw.

	MPN (%)	AAN (%)
Traditional	0.83	0.18 ± 0.07^{ac*}
Citric acid	0.63	0.16 ± 0.02^{ac}
Na-hypophoshpite	0.61	0.16 ± 0.02^{ac}
Monascus anka mash	0.889	0.54 ± 0.02^{b}
Plum paste	0.67	0.13 ± 0.03^{c}
Sprayed with organic acid	1.262	0.17 ± 0.3^{ac}
Lactic acid bacteria	0.712	0.21 ± 0.09^{a}

* Figures in the same column with the same letters are not significantly different ($P<0.05$).