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RESEARCH PAPER

Standardising Preprocess Treatments for Improved Sensory Quality and Storage Stability of Carp Pickle

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Abstract

A study was undertaken for developing a pickled product using meat of the carp, rohu (*Labeo rohita*). The meat pieces were subjected to different preprocesses treatments, viz., salting, drying, frying, baking, smoking and marinating in various combinations. The most acceptable combination was marinating fish meat pieces for 15 min followed by smoking for 3 h at 60°C and frying for 30 sec at 180°C in refined vegetable oil. Vinegar- tamarind juice mixture in 1:1 proportion was found to be the most preferred acid source for preparing the pickle. Three types of pickles were prepared, selected pretreatments and acid mixture, of pH values 4.0, 4.5 and 5.0, and storage studies were conducted for a period of 72 days. Sensory scores for odour, texture, taste and overall quality dropped significantly after 24 days in the case of pickle of initial pH value 5.0, and became unacceptable on the 36th day. Pickles adjusted to pH values 4.0 and 4.5, showed an increasing trend in sensory quality during first few weeks of storage, after which the sensory scores remained more or less steady for rest of the period. The total plate count showed no increase in pickles of pH 4.0 and 4.5 during storage, but it significantly increased in pickle of pH 5.0. This was accompanied by significant increases in total volatile base nitrogen content and pH in the latter. The peroxide value also increased at a greater rate indicating oxidation of tat. Thus, it was concluded that pH of 5.0 is unsuitable. Among the other two lots pH 4.5 was found more suitable as the product could be well preserved and the sourness was found to be less organoleptically compared to the pickle of pH 4.0.

Keywords: Pre-process treatments, rohu pickle, pH, meat, carp, storage, peroxide value

The increasing demand for fish is on account of awareness of the public about nutritional advantages of consumption of fishery products and increasing world population. In developing countries, most people rely on fish for animal protein requirements. In India about two-third of the total fish caught or cultured are consumed fresh, and the remaining is preserved by various methods. All over the world, food technologists are focusing much attention on the development of products based on consumer acceptance. In recent years fish and fishery products have become very popular worldwide, as they are rich in protein, vitamins, minerals and polyunsaturated fatty acids.

Freshwater fishes, although low priced, are not commonly used for pickle making. This may be on account of their intrinsic problems such as odd flavour, presence of fine bones or the typical taste and texture. However, there are several methods available to improve the texture, consumer acceptability as well as shelf life of fish pickle. These include pretreatments such as salting, partial drying, frying and smoking, and use of suitable food additives. Salting or drying involves removal of part of the moisture content of

fish meat, making it tougher in texture. Frying in oil can further add on to a more acceptable flavour.

Pickles are acidic foods and commonly used acid for pickle making is vinegar that has to be added in sufficient quantities for effective preservation.

Considering the great potential for carp as a suitable raw material for pickle and the problems associated with it, this study was undertaken. The carp, rohu (*Labeo rohita*), was used as the study material as it is commonly available in the market. The effects of preprocess treatments such as salting, drying, frying, baking and smoking on the sensory quality and shelf-life of fish pickle were studied in order to determine a suitable combination of treatments. Attempts were also made to reduce the amount of acid and to select a suitable acid combination for preparing a more acceptable product. Storage studies were conducted to determine the quality variations during storage of the product.

MATERIALS AND METHODS

Procurement of raw material and collection of meat pieces

A base recipe as per George (2012) with slight modification was followed for preparing pickle. Fish meat pieces were marinated using a mixture of salt, chilli powder and garlic-ginger paste in the proportion 100:3:1:1 by weight.

Table 1: Ingredients of fish pickle

Sl. No.	Ingredients	Weight (g)	8	Pepper powder	2.5
1	Fish meat pieces	1000	9	Vinegar	300
2	Gingili oil	200	10	Table salt	60
3	Green chilli sliced	50	11	Cane sugar	10
4	Garlic peeled	100	12	Cardamom powder	0.5
5	Ginger peeled	100	13	Clove pieces	0.5
6	Chilli powder	50	14	Cinnamon pieces	0.5
7	Turmeric powder	2			

They were divided into required number of lots and subjected to various treatments as according to the stages of standardisation explained in Fig. 1. Other ingredients were then weighed out as per Table 1. The oil was heated in a frying pan to a temperature of 180-190°C and the fish pieces were deep fried for 30 sec.

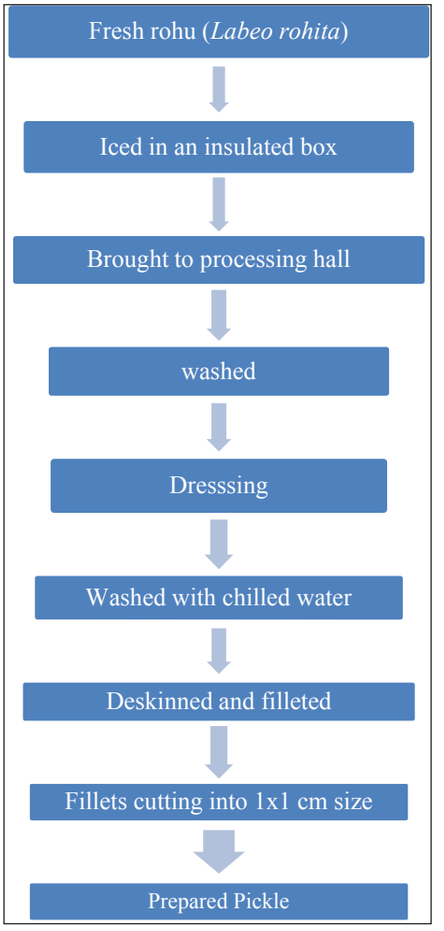

Fig. 1: Base recipe for pickle

In the same oil, mustard, garlic, ginger, and green chillies were fried at the same temperature for two minutes. The pan was then, removed from flame. The fried fish pieces were added to the contents of the pan and mixed well and cooled for a few minutes followed by addition of vinegar and rest of the spices (cardamom, clove and cinnamon) also salt and sugar were added and mixed. Water was then, added taking care to cover the pieces and stirred. The

pH was checked using an electronic pH meter then weighed and packed according to the required net weight and transferred to clean dry glass jars, sealed, labelled and stored.

Standardisation of preprocess treatments

The fish pieces were subjected to different combinations of treatments, *viz.* baking, smoking, frying, drying, salting and marinating, before preparing pickle (Fig. 1). In order to select the most suitable set of preprocess treatments the study was conducted in various stages. Pickles were prepared by adopting a base recipe as given in Table 1. The pH was adjusted to 4.5 using 1:1 mixture of vinegar and tamarind juice. Prepared pickles were subjected to sensory evaluation and most acceptable pickle combination obtained at one stage was used as the control for the next stage of study and so on.

Following were the treatments adopted at various stages:

Stage I

Baking for a period of 15 min. at a temperature 100°C (2) Smoking for 1 h at 70°C (3) Smoking for 1 h at 70°C, followed by frying for 30 sec at 180°C (4) Baking for 15 min at 100°C, followed by smoking for 1 h at 70°C, followed by frying for 30 sec at 180°C (5) Salting @ 3% of meat weight and keeping for 30 min, followed by drying for 2 h at 50°C, followed by frying for 30 sec at 180°C.

Stage II

1. Salting @ 3% of meat weight for a period of 30 min, followed by smoking for 1 h at a temperature 70°C.
2. Marinating with salt, chilli powder and ginger-garlic paste in the proportion 3:1:1 for 15 min, followed by drying for 2 h at 50°C, then frying for 30 sec at 180°C
3. Salting @ 3% of meat weight for 30 min, followed by smoking for 1 h at 70°C, then frying for 30 sec at 180°C
4. Smoking for 1 h at 70°C
5. Smoking for 1 h at 70°C, followed by frying for 30 sec at 180°C (selected from stage I as control)

Stage III

In this stage, fish pieces were marinated with salt, chilli powder and ginger-garlic paste in the proportion 3:1:1 for a period of 15 min. and divide into 4 lot and gave pre-treatments on different time and temperature in lot (1) drying for 2 h at 50°C (2) smoking for 1 h at 70°C (3) drying for 2 h at 50°C (4) smoking for 2 h at 60°C and followed by frying for 30 sec at 180°C for all the above lots.

Stage IV

In this stage, meat pieces were marinated with salt, chilli powder and ginger-garlic paste in the proportion 3:1:1 for 15 min and divided into seven lots. Smoking treatments was tried for a six lots with different time and temperature. In three lots, smoke was given for period of 1 h, 2h, and 3h at a temperature of 70°C and another three were also smoked for the same period but at a temperature of 60°C and the last one lot used for drying for 2 h at 50°C, followed by frying for 30 sec at 180°C.

Stage V

In this stage, smoking was carried out for a period of 3 h at a temperature of 60°C, followed by frying for 30 sec at 180°C and different. Smoking time and temperature was tried i.e. 5 h at temp of 60°C, 3 h at 50°C, 5 h at 50°C followed by frying for 30 sec at 180°C for all.

Stage VI

Two most suitable pretreatment combinations were selected from stage V. Pickles were prepared adopting the two treatment combinations but varying the source of acid. The acids were added to attain a pH 4, 4.5 and 5 of pickle. The combinations studied were as follow:

1. Smoking for three lots for a period of 3 h, at a temperature of 60°C, followed by frying for 30 sec at 180°C, then adding vinegar, tamarind

and 1:1 mixture of vinegar and tamarind juice respectively.

2. Smoking for three lots for a period of 5 h at 60°C, followed by frying for 30 sec at 180°C, followed by adding vinegar, tamarind and 1:1 mixture of vinegar and tamarind juice respectively.

Stage VII

The most acceptable combination of pretreatments and acid source were finally, selected for making pickle for storage study. Fish pieces subjected to the pretreatments were marinated using salt, chilli powder and ginger-garlic paste in the proportion 3:1:1 for a period of 15 min, followed by smoking for 3 h at a temperature of 60°C, then frying for 30 sec at 180°C. The pieces were divided into three lots and pickles were prepared using 1:1 vinegar-tamarind juice mixture as a source of acid. The pH of each lot was adjusted to the following values of pH 4.0, pH 4.5 and pH 5.0.

Storage study

Prepared pickles was packed in clean glass containers and stored at ambient temperature. Storage study was done for a period of 72 days. Sampling was done every twelfth day and subjected to various tests.

Analysis

Proximate composition of raw meat *viz.* Moisture content was determined by the oven drying method of AOAC (1984). The Soxhlet method of AOAC (1990) was followed for fat estimation. The method of AOAC (1984) was followed for ash content estimation. Carbohydrate content was indirectly calculated using the formula: % of carbohydrate = 100 - (%moisture + %protein + % fat + % ash). TVBN content was determined by Conway's microdiffusion method of Beatty and Gibbons (1937). The method of Connell (1975) was followed for peroxide value determination. pH was determined as per the method of Lanier *et al.* (1991). Total plate count and total fungal count were determined according to the method of APHA (1992).

Sensory evaluation

The organoleptic quality parameters, *viz.*, odour, taste, texture and overall acceptability, of the product on storage were evaluated periodically by an expert panel of 6-9judges. A seven point hedonic scale was used for this purpose. Sensory evaluation sheet was provided to the judges for recording their judgments.

Statistical analysis

Data obtained were analyzed using Friedman's Two-way Analysis of Variance by ranks for related samples. The analysis was carried out using SPSS package ver. 20. In cases, where treatment showed significant differences pairwise comparison was performed. For total plate count (TPC) and total fungal count (TFC), ANOVA was performed after applying logarithmic transformation to the data. Comparison of treatments was done using Post Hoc test - Tukey HSD at 5% level of significance. Chemical parameters pH, TVBN, PV variations shown in graphs.

RESULTS AND DISCUSSION

Proximate composition of rohu meat

The moisture, crude protein, fat, ash and carbohydrate content were 76.10, 17.25, 0.81, 3.75% and 2.09%, respectively. The meat of rohu used for the study was found to be lean, containing only 0.81% fat, but with fairly high protein content (17.25%). The results for proximate composition of rohu meat was similar with slight similarly variation to that reported by Sagar (2005) who had observed moisture of 78.80%, crude protein 18.43%, fat 0.80 %, ash 1.02 % and carbohydrate content of 0.95% in rohu meat. Gopal *et al*. (2012) reported that on an average meat of Indian major carps contains 70-80% moisture, 15-19% protein, 3-9% fat and 1-1.4% ash. Variation in proximate composition could be due to size, sex, type of feed and cultural environment.

Dressed yield of rohu

The non-edible portion of rohu such as head, gut, fin and intramuscular bones were removed and

meat was separated. The yield of rohu meat was 42 % based on total weight of fish. Similar result with slight variation in yield 37.50% of rohu meat was obtained by Sagar (2005).

Evaluation of treatments at different stages of rohu pickle preparation

Rohu meat is required to be subjected to various treatments such as smoking, salting, frying and drying, generally in combinations. Muraleedharan *et al.* (1980), followed combinations of various treatments like dried and pickled, fried and pickled and lightly smoked and pickled, before making pickle. If raw meat is directly used, it can result in extensive breakage of pieces, and unacceptable texture and flavour (Dipty *et al*., 2010). In the present study, the effect of the following pretreatments were tested, *viz.,* salting, partial drying, smoking, frying, baking and marinating, either individually or in combinations. The results of studies on the effect of various combinations of treatments have been describe under stages. Sensory quality was adopted as the main criterion for deciding the treatment combinations of the fish pickle.

Scores obtained for various sensory parameters for pickles prepared using rohu meat subjected to various treatments in the stage 1 to 6. Maximum score: 7 are given in Table 2.

Table 2: Sensory evaluation of pickles of various stages

Stage I

Treatment	Average scores			
	Odour	Taste	Texture	Overall quality
1	4.35	4.21	4.71	4.5
2	5.28	5.14	5.14	5.21
3	5.14	5.14	4.92	5.28
4	4.64	3.85	3.71	4.14
5	5.35	4.42	5.28	4.85

Stage II

Treatment	Average scores			
	Odour	Taste	Texture	Overall quality
1	4.41	4.25	4.50	4.08
2	5.58	6.16	5.66	5.83
3	5.25	5.50	5.91	5.33
4	4.75	4.58	3.91	4.16
5	5.08	5.41	5.00	5.41

Stage III

Treatment	Average scores			
	Odour	Taste	Texture	Overall quality
1	5.12	5.12	5.18	5.06
2	5.31	5.00	5.00	5.00
3	5.00	5.00	5.31	5.37
4	5.31	5.87	5.93	5.68

Stage IV

Treatment	Average scores			
	Odour	Taste	Texture	Overall quality
1	5.16	5.41	4.94	5.17
2	5.17	5.11	4.72	4.85
3	5.22	5.55	4.83	5.14
4	5.38	5.55	5.46	5.47
5	5.16	5.33	5.00	5.27
6	5.44	5.94	5.75	5.75
7	4.50	5.27	5.16	5.05

Stage V

Treatment	Average scores			
	Odour	Taste	Texture	Overall quality
1	5.28	5.95	5.35	5.28
2	5.21	5.35	4.85	5.24
3	5.28	5.31	5.14	5.14
4	4.92	5.21	4.97	4.97

Stage VI

Treatment	Average scores			
	Odour	Taste	Texture	Overall quality
1	5.35	5.21	5.21	5.21
2	5.47	5.47	4.84	5.21
3	5.70	5.57	5.25	5.28
4	5.60	5.74	5.61	6.00

5	4.98	5.48	5.50	5.62
6	5.54	5.90	5.78	5.68

Stage I

The sensory evaluation scores obtained in the first stage for pickles preparation using fishes subjected to five different combinations of treatments. The data were subjected to statistical analysis using related samples Friedman's two-way analysis of variance by ranks. The combination of salting, partial drying and frying, which was adopted from George (2012), was run as a control. Although no significant difference in sensory quality was observed between any of the pretreatments the combination 3rd, *i.e.* smoking followed by frying, gave comparatively higher scores particularly for taste and texture. According to Muraleedharan *et al.* (1980) smoking and frying are two effective means of improving the taste of fish pickle. This combination was therefore adopted as the control for the next stage of study.

Stage II

In the second stage another set of five combinations of treatments were adopted including the selected combination from the previous trial. Significant differences between treatments could be obtained with respect to taste, texture and overall quality, but not with respect to odour. The most acceptable combination, 2nd (marinating, drying and frying), gave highest scores for all parameters except for texture. Taste, which is one of the most important quality parameters, was highest and hence, the combination 2nd was selected to serve as control for the next stage of study.

Stage III

The third stage of standardisation involved four combinations of treatments. The analysis showed significant difference in the case of taste only ($p<0.05$). Pair wise comparison showed significant difference between treatments 2nd and 4th and between 3rd and 4th in the case of taste. Combinations of treatments, 2nd and 4th were found more acceptable and selected for the next stage of standardization. By the end of the third stage, it was clear that marinating for a period of 15 min and frying fish pieces for 30 sec at 180°C in vegetable oil resulted in a more acceptable product. Marinating was done by salt: chilli powder: ginger garlic paste in the ratio 3:1:1. According to Sahu *et al.* (2012) during marination diffusion of body water occurs and texture of meat softens. Smoking also was found beneficial, but a suitable time-temperature combination had to be found out.

Stage IV

In this stage fish pieces were subjected to marinating, smoking and frying, but under different smoking conditions, prior to pickling. The effect of various smoking conditions on the sensory quality was tested. The results indicate that there were no significant differences between the treatments for any of the sensory parameters.

However, the combination, marinating fish pieces for 15 min, followed by smoking for 3 h at 180°C, resulted in a slightly better product with respect to all the parameters tested.

Stage V

The effect of marinating, smoking and frying under various conditions were again tested and the results are presented. The treatments showed significant difference only in the case of taste and not with odour, texture and overall quality. Treatments 1st and 4th significantly different and 1st and 3rd are significantly different. The combination of treatments, 1st and 2nd were found to be comparatively more acceptable and was selected as the controls for the next stage of study.

In order to further optimize the conditions of smoking, the next stage of trial was conducted. Significant difference between treatments was noticed only with respect to taste. The combination, A, of marinating for 15 min followed by smoking for 3 h at 60°C and then frying for 30 sec at 180°C, which was selected from the previous stage of study, again gave higher scores for all parameters. Thus, this particular combination was selected for the next stage of study. The combination

2nd, i.e. marinating for 15 min followed by smoking for 5 h at 60°C and then frying for 30 sec at 180°C, was also selected as it was different only in the extent of smoking.

Stage VI

No significant difference between treatments was obtained for any of the sensory parameters tested in the sixth stage of standardization. However, the combination of treatments under 6th, *i*.e. smoking fish pieces for 3 h at 60°C followed by pickling using vinegar-tamarind juice mixture, was found comparatively more acceptable and was selected as control for the storage study.

The sixth stage of treatment involved preparation of pickle using various acid sources. Vinegar (dilute acetic acid of concentration 4% v/v) is probably the most commonly used acid medium for pickles including fish pickle (Behanan *et al.*, 1990). However, studies of Behanan *et al.* (1990, 1992) and Chandrashekar *et al.* (1978) have shown that combinations of acid sources, e.g. vinegar-tamarind juice mixture, could be beneficial to impart better flavour and other characteristics. Therefore, fish pieces subjected to pretreatments selected in the fifth stage were used for preparing three lots of pickles, one using vinegar, one using tamarind juice and one of 1:1 mixture of the two. However, in the present study no significant difference was obtained between treatments for any of the quality parameters. Fairly good scores of 3.8-4.0 out of 7 were received for all the sensory quality parameters for pickle prepared using meat pieces pretreated with combination 6th, i.e. marinating pieces for 15 min, followed by smoking for 3 h at 60°C and followed by frying for 30 sec at 180°C, and pickling using vinegar-tamarind juice mixture. Comparatively higher scores were obtained for taste and texture. Since these two are probably the most important parameters deciding consumer acceptability, this combination of treatments was finally selected for further studies.

Stage VII

The seventh stage of standardisation was about the effect of pH on the sensory quality of the pickle which was studied along with the storage study.

The final (seventh) stage of standardization involved selection of a suitable pH for the rohu pickle. Since pH is an important parameter that decides the shelf life of the pickle, the effects of pH on the sensory quality were studied along with the storage study of the pickle. Generally the pH of fish pickles varies from 3.5 to 4.5 (Behanan *et al*., 1990) therefore the pH values selected were 4.0, 4.5 and 5.0. It is understandable that the pickle must not taste too sour, but at the same time, the product must have a long shelf life. Thus the type acid or acid combination, the amount of acid added and the final pH of the product will have profound influence on the sensory quality and keeping quality.

Many workers (Basu, 1999; Gopal *et al*., 2012; Sehgal and Sehgal, 2002 and Vijayan, 1998) are of the opinion that value addition is required for enhancing the consumer acceptability. A combination of marinating followed by smoking was found to be advantageous for eliminating the muddy flavour and softening the bones. The pretreatments adopted in the present study also could totally mask the muddy flavour as none of the judges could detect any such flavour in the pickle. Studies of Yamprayoon and Noomhorm (2000) revealed that a pre-treatment of Nile tilapia (*Oreochromis niloticus*) in acidified brine followed by smoking, reduced the geosmin content and masked the muddy flavour. Another advantage of smoking is that it improves the texture of meat.

Storage studies

The finally selected combination were used and made three lots of pickles by using vinegar-tamarind juice (1:1) mixture with the pH adjusted to values of 4.0, 4.5 and 5.0.

Pickles are generally stored in glass bottles or suitable laminate pouches at ambient conditions. According to Behanan *et al*. (1990) the product may be stored chilled for a greater keeping quality. The parameters selected for storage studies were intended for assessing quality variations in the products. Quality

decreases on account of various microbiological and biochemical changes. The variations in one parameter can be related to those of another.

Quality assessment of rohu pickle during storage

Variations in the different sensory parameters during storage of rohu pickle are shown in Figs 1 to 4. The scores for odour, texture, taste and overall quality dropped significantly after 24 days of storage in the case of pickle of pH value 5.0, whereas in pickles adjusted to pH values 4.0 and 4.5, an increasing trend in the quality was noticed. Pickle with initial pH value of 5.0 was found unacceptable on the 36th day. As per pair wise comparison, significant difference in pickles was noticed in the case of taste and overall quality on the 0th day, whereas by 36 days, all parameters showed significant difference. Beyond 36 days, only pickles of pH values 4.0 and 4.5 were stored, and the parameters showed no significant difference between the samples.

Pickles initially adjusted to pH values of 4.0 and 4.5 (Figs 1-4) generally showed an increase in sensory evaluation scores for all the four parameters assessed, viz., odour, taste, texture and overall quality, during the first few weeks of storage, after which the scores remained fairly stable till the end of the study period. Pickles generally require a few weeks to mature during which time the components of various ingredients diffuse into the fish pieces as reported by Emilin *et al.* (2012). Similar increasing trend was noticed in green gram pickle by Puranik *et al*. (2011).

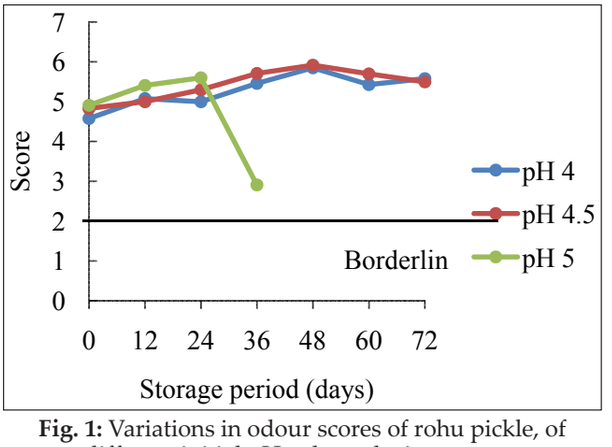

Fig. 1: Variations in odour scores of rohu pickle, of different initial pH values, during storage

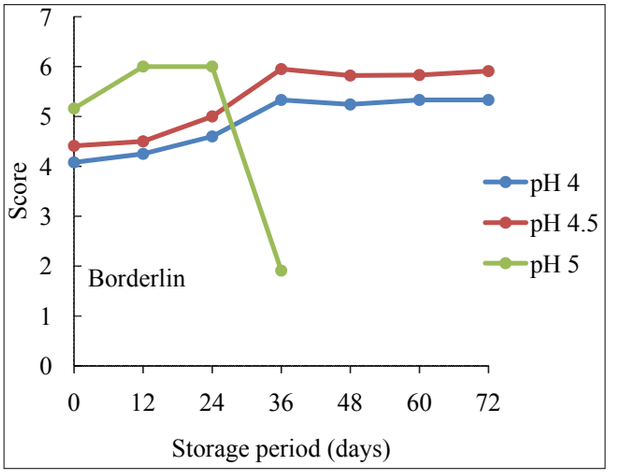

Fig. 2: Variations in odour scores of rohu pickle, of different initial pH values, during storage

Once equilibrium is reached, the product may remain stable for quite some time after which there may be a reduction in the sensory quality on account of biochemical or microbiological changes, resulting in the production of foul smelling and off flavour compounds. The scores for all the parameters for pickles of pH 4.0 and 4.5 reached maximum values in the range 5-6 out of 7 in around four to five weeks' time, indicating that the products became of excellent organoleptic quality after maturation. Muddy flavour was totally absent in the pickles during the entire period of storage and irrespective of the pH, probably on account of the effect of marinating, smoking and spices.

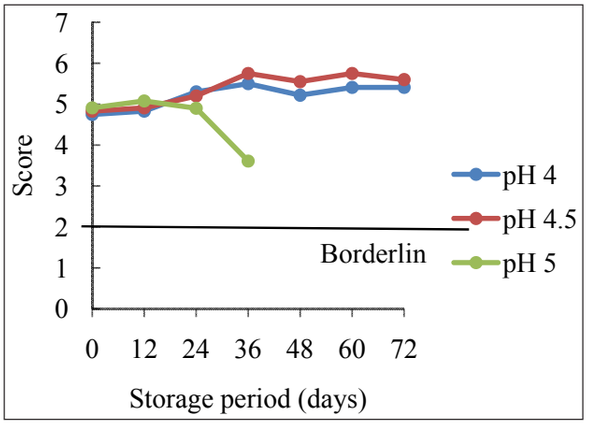

Fig. 3: Variations in texture scores of rohu pickle, of different initial pH values, during storage

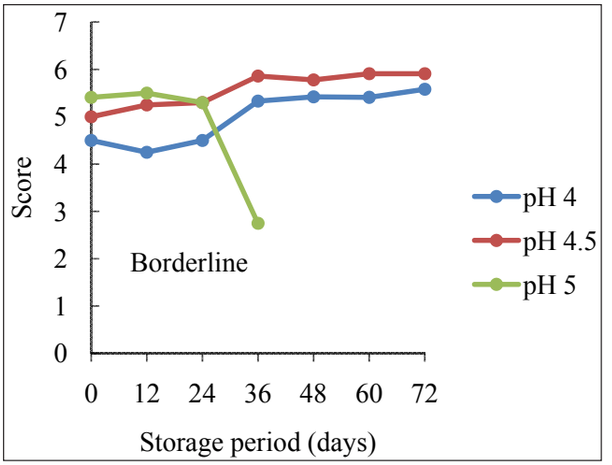

Fig. 4: Variations in overall quality scores of rohu pickle, of different initial pH values, during storage

Variations in Total Plate Count (TPC) during storage of the pickles are shown in Fig. 5. The initial microbial load in all the pickles ranged between 1.33×10^4 and 1.54×10^4 cfu/g. the count remained more or less the same in pickles of pH 4.0 and 4.5, with a slight decreasing trend. In case of pickle of pH 5.0, there was a significant increase in TPC by about 1.5 log cycle in 24- 36 days.

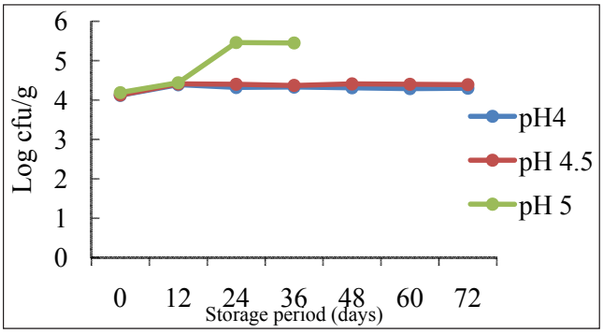

Fig. 5: Variations in Total plate count of rohu pickles, of different pH values, during storage

Pickle with pH adjusted to 5 was judged less sour than the other two during storage. However, the pickle of initial pH 5.0 could be stored only for about three weeks after which there was sharp fall in sensory scores of all parameters, attaining very low values, as may be clearly noticed in Figs 1- 4. In case of taste, the mean score had gone down even below the limit of acceptability. The purpose of adjusting pH to 5.0 was to determine whether pickle of low sourness could be stored well. However, the study clearly indicates that rohu pickle cannot be stored for long time at this pH. Since the product was found unacceptable on the 36th day, no further storage study was conducted for the pH 5.0 pickle. Although judged as more sour, a reduction in the sensory quality of pickles of pH 4.0 and 4.5 was never noticed in the present study. The microbial activity could have been significantly affected by the low pH. Sugumar *et al.* (1995) reported that low pH inhibits most of the bacterial activity. It is well established that organic acids attain greater antimicrobial activity at lower pH values compared to inorganic acids on account of their ability to remain as undissociated molecules at lower pH values (Behanan *et al.*, 1992) and thus, whole molecules can easily enter bacterial cell resulting in their destruction.

The sensory quality variations during storage of fish pickle can be related to either microbial and or biochemical changes. It may be indicated that in addition to acid, partial drying, smoke components and salt also contribute to preservation of pickle (Muraleedharan *et al.*, 1979, 1980). The results of the present study clearly indicate that bacterial activity did not affect the pickles. The slight decreasing trend probably would be caused due to the killing action of organic acid on bacteria. Similar observations have been made by Dhanapal *et al*. (1994). On the other hand, there was a significant rise in the Total plate count of pickle of pH 5.0 and clearly shows that spoilage bacteria could thrive at pH 5.0 or above in rohu pickle.

The initial fungal counts were 3.6×10^3, 4.3×10^3 and 1.12×10^4 cfu/g in pickles of pH 4.0, 4.5 and 5.0, respectively. As can be noticed in Fig. 6, the count remained more or less the same in pickle of pH 5.0, but showed a slight decreasing trend in the other two.

Fungi in general are more tolerant to acid conditions and they generally grow in conditions where bacterial activity is less. Hence, fungal count was also selected as a parameter for the study. Fig. 6 shows that all the three lots of pickles contained fungi.

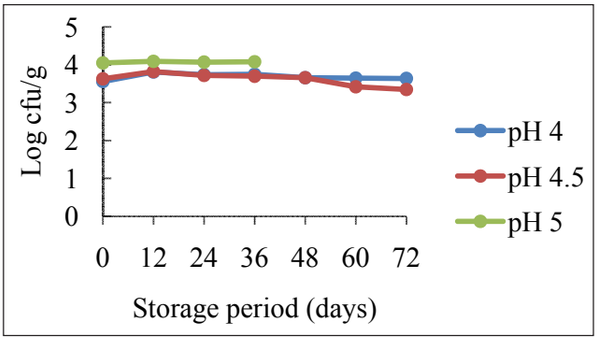

Fig. 6: Variations in total fungal count of rohu pickles, of different initial pH values, during storage

However, the counts were lower in pH 4.0 and 4.5 pickles compared to that of pH 5.0 pickle. This is probably because of greater extent of destruction of fungi as more amount of acid has been used in the former two cases. However, the count remained fairly constant for the first 36 days. A marginal decrease noticed in pH 4.0 and 4.5 pickles towards the end of the storage period, although not significant, may be on account of further destruction of fungi by the acid or other components.

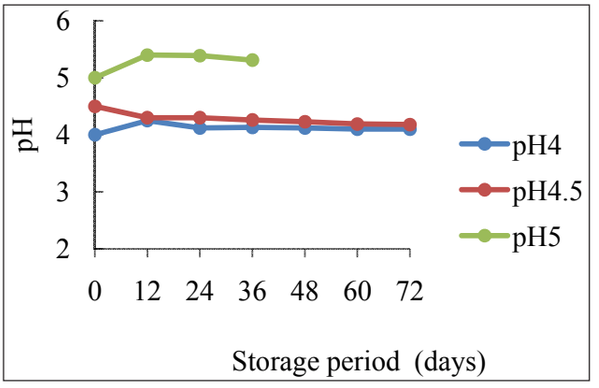

Fig. 7: Variations in pH of rohu pickle, of different initial pH values, during storage

pH values remained fairly constant throughout the storage period of 72 days in the case of pickle of initial pH 4.0 and 4.5. However, a definite increase in pH was noticed in the case of pickles of initial pH value 5.0 in Fig. 7.

Total volatile base nitrogen (TVBN) content (Fig. 8) showed an increasing trend in all the three pickles. However, pickle of pH 5.0 showed a sharp increase to a TVBN content of over 40 mg% in 36 days. In the case of the other two samples, TVBN content increase was more gradual reaching about 20 mg% after 72 days. Pickle of pH 4.0 showed a slightly lower rate of increase compared to that of pH 4.5.

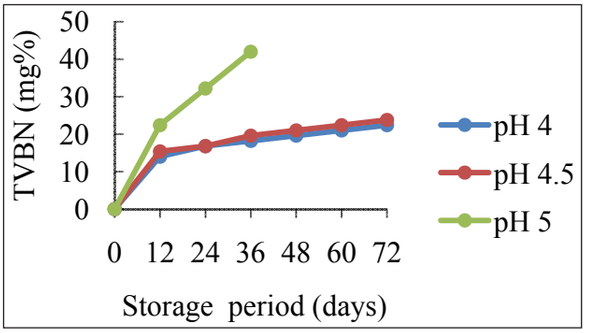

Fig. 8: Variations in TVBN content of rohu pickle, of different pH values, during storage

Variations in Total volatile base nitrogen content and pH are mainly on account of microbial (bacterial) activity (Behanan *et al.*, 1990, Joseph *et al.*, 1998). Thus, an increase in TPC of fish product should result in an increase in TVBN content and pH value. In case of fish pickle of pH 4.0 and 4.5, the count remained more or less the same throughout the 72 days of storage. pH increase in pickle can be an indication of microbial activity. Sugumar *et al.* (1995) reported that when bacterial activity takes place various basic compounds are released into the medium or food.

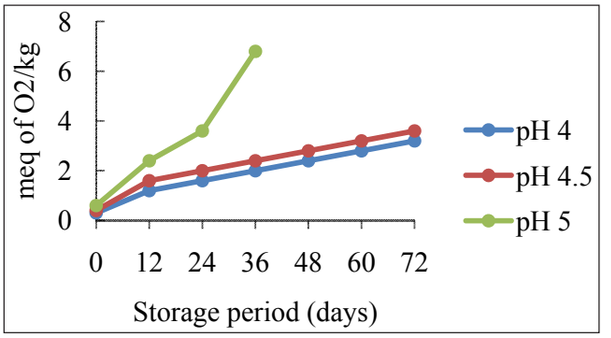

Fig. 9: Variations in peroxide value of rohu pickle, of different pH values, during storage

A similar trend was noticeable in the case of peroxide

value also (Fig 9). Pickle of pH 5.0 showed a sharp increase from the beginning, with the value reaching about 7 meq O_2/kg fat. Pickles of pH 4.0 and 4.5 showed an increase, but reached only around 3 meq in the case of former and 2.7 meq in the case of latter.

Peroxide value is one indicator of the extent of fat oxidation and the latter affects the sensory quality by the development of rancid flavor as reported by Chattopadhyay *et al.* (1985) and Saritha *et al.* (2014). However, the fish rohu used was lean, with a fat content of about 0.8% only. However, considerable amount of oil had been used for frying in the making of the pickle. Therefore, it is possible for an increase in the PV. Fig. 9 shows a steady increase in PV in all the lots of pickles. However, the values remained considerably lower in the cases of pH4.0 and 4.5 compared to pickle of pH 5.0 during the period of study. The components of acid source or pH may have a protective effect on fat, which probably is responsible for the lower PV in high acid pickle.

The sensory evaluation studies on pickles prepared using rohu meat subjected to various preprocess treatments have demonstrated that marinating, smoking and frying are beneficial to the improvement in the consumer acceptability of the product. The treatments also aid in preserving the product. The acid source of vinegar and tamarind juice mixture has also been found most acceptable. According to Behanan *et al.* (1992) a pH of 4.5 would be ideal as the product can be well preserved at ambient conditions, and at the same time the product is not adversely affected. As per the present study, a pretreatment combination of marinating fish meat pieces for 15 min followed by smoking for 3 h at 60°C and frying for 30 sec at 180°C in refined vegetable oil could be selected. Pickle could then be made using the base composition of ingredients (Table 1) with acid source as vinegar-tamarind juice mixture in 1:1 proportion with the pH adjusted to 4.5. The sourness can be expected to less at pH 4.5 compared to that at pH 4.0. A maturation period of about three weeks is suggested before consuming the product.

Average scores were analysed using Related samples Friedman's two way analysis of variance by ranks and pairwise comparison for the 0th day, 12th, 24th and 36th day to check whether there is any significant difference between days for odour, taste, texture and overall quality of the pickles adjusted on pH 4, 4.5 and 5.

For pickle adjusted to pH 4 only taste was significantly different over days. Pair wise comparison was done for taste attribute. Taste scores for 0th and 36th day are significantly different and those for 12th and 36th day are also significantly different.

Between 48th and 60th day samplings the scores for odour, taste, texture and overall quality were not significantly different.

For pickle of pH 4.5 odour, taste and texture scores were found to be significantly different over days. Pair wise comparisons were done for odour, taste and texture. Taste scores for 0th and 36th days were significantly different so, also for 12th and 36th day.

In the case of pickle adjusted to pH 5 texture was not significantly different. Odour, taste and overall quality were significantly different. Pair wise comparisons were done for odour, taste, and overall quality. Odour scores for 0th and 12th day and for 24th and 36th day were also significantly different. In the case of taste scores, significant difference was found between 12th and 35th day as well as between 24th and 36th day. Overall quality between 0th and 36th day was also significantly different.

CONCLUSION

Based on the sensory, biochemical and microbiological studies, it could be concluded that rohu pickle prepared this way and packed in glass containers could be stored at room temperature for at least 72 days.

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