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ORIGINAL ARTICLE

Effects of pretreatments and salt concentration on rohu (*Labeo rohita*) roes for preparation of roe pickle

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Abstract In India, fish egg (roes) is the underutilized rich source of protein and essential fatty acids. An attempt was made to utilize the roes for the preparation of pickle product. Roes of rohu (*Labeo rohita*) were subjected to pretreatments such as pasteurization and blanching in varying molar concentrations of salt solutions to improve the texture and sensory quality. Losses in protein content and absorption of salt by roes were assessed in both pasteurized and blanched roes for processing them into roe pickle. The pasteurized roes were very soft coupled with high fishy odour. Roes blanched in 1M NaCl solution were found to be good in taste in terms of salt, minimum loss in protein content and reduced fishy odour. The blanched roes were used to prepare fish roe pickle. The pickle was analyzed for physico-chemical parameters, microbial and sensory quality during storage. The roe pickle packed in glass bottle was shelf-stable and scored good for sensory characteristics of flavour, texture, taste and overall acceptability during a storage period of 6 months at room temperature (28 ± 2°C).

Keywords Roes · Rohu roe pickle · *Labeo rohita* · Pasteurization · Blanching · Storage

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Introduction

Fish eggs (roes) are produced in considerable quantity (25–30% of the total body weight) during spawning season. Roes are rich in essential amino acids and polyunsaturated fatty acids and quality and quantity of roe proteins differ depending on the variety (Mukhopadyay et al. 1981). Recent studies revealed that mature roes of salmon fish had lower moisture content (55.5%) and higher protein (26.16%), lipid (10.19%) and ash (1.42%) content compared to immature roes (Bekhit et al. 2009). The freeze-dried immature roes of marine Pollack fish contained 81.7% protein which constituted 43.4% total essential amino acids. The lipid content was 9.2% which had the following composition: palmitic acid (21.2%), docosahexaenoic acid (21.2%) and eicosapentaenoic acid (19.0%) (Bechtel et al. 2007). Preparation of fish egg protein concentrate from rohu and its physico-chemical and functional properties were studied earlier (Balaswamy et al. 2007). They also studied some physico-chemical and functional properties of roes of 4 common fish species and their use in the preparation of some bakery and extruded foods (Balaswamy et al. 2009).

The fresh roes were found to be more acceptable than frozen or frozen-thawed roes in terms of sensory quality (Miettinen et al. 2003). It was reported that the salt processing (3.5–4.8%, w/w) did not affect the proximate composition, total amino acids and fatty acids composition compared to fresh roes (Basby et al. 1998). The solubility of homogenized roe proteins of 4 common carps were in the range of 40–60% at pH 6.5 (Balaswamy et al. 2009). Ground caviar proteins of salmon and sturgeon when extracted into 5% (w/v) brine solution were soluble to an extent of 84.2 and 86.1% (w/w), respectively (Al-Holy and Rasco 2006). Kopylenko and Rubtsove (2004) reported that pasteurization of salmon roe at pH 6.0–6.4 completely inactivated the proteolytic enzymes, in particular, aspartyl proteinases (Cathepsin D).

In India, pickles and *chutneys* are consumed by a major section of population as food adjuncts, which are made from sour tasting mango, lime, tamarind, gooseberry and tomato. Consumption of pickles made from fish, poultry and meat is also on the rise. These pickles are generally made by pressure cooking, deep fat frying of de-boned meat/bone-in meat and blending with spice mixes and other ingredients (Puttarajappa et al. 1996, Sen and Karim 2003, Khanna et al. 2004, Das et al. 2007).

In Indian fish markets, roes are separated during processing and most of them are underutilized. The roes are highly perishable in nature. However, they can be stored under frozen conditions for further use. Traditionally, roes are deep fat fried and served hot as an appetizer. Roes were made into ready-to-use product namely cutlets and packed in metalized polyester pouches which was stable for 30 days at a storage temperature of 8°C (Balaswamy et al. 2006). Earlier literature reviews are available on caviars, fermented roe products and canned roes from marine fishes (Bledsoe et al. 2003) and processes for the production of cooked roes, smoked roes, canned roes and roe sausages (FAO 2001). However, there was not much work carried out on the methods for preparation of roe pickles, and the effect of various process parameters on the storage life of roe pickles. There is a need to develop value added products for proper utilization of roes from rohu or other related species. The objective of present work was to study the effect of pasteurization and blanching of roes to assess their utility in producing rohu roe pickles and shelf-stability.

Materials and methods

Chemicals used were of laboratory grade and were procured from Sd. Fine Chemicals, Mumbai. Salt, tamarind, chilly powder, acetic acid and spices were purchased from local supermarket.

Fish roes were obtained from freshwater fish, rohu (*Labeo rohita*) at local fish market immediately after dressing of live fish. The roes were packed in polyethylene bags (film thickness 25μm) and stored at 4°C for not more than 2 h prior to experimental work. The stored roes were thoroughly cleaned to remove adhering fat deposits, blood vessels and washed in fresh water. Roes were separated manually from skin (a sac which covers roes). Roes were subjected separately to pretreatments like pasteurization at 65°C for 20 min or blanching at 110°C for 5 min with 0.5, 0.75 and 1M NaCl solutions. Solid to liquid ratio was maintained at 1:4 (w/w). Immediately after treatment, the wet roes were squeezed manually through a triple layered muslin cloth (pore size 250 μm). The filtrate was separated. The above treatments without salt were used as control.

Fresh roes were analyzed initially for moisture and protein content, the treated roes for moisture, protein and salt contents, and the filtrates for protein and salt content (AOAC 1995).

Preparation of fish roe pickle and storage studies: The treated roes were evaluated by 10 panelists for sensory, texture, flavour and taste for suitability in pickle preparation (Peryam and Pilgrims 1957). The highly scored roes among the treated samples were chosen for preparation of fish roe pickle as mentioned in flow chart (Fig. 1). The pickle was prepared in bulk (8 kg) and sodium benzoate 250 ppm was added as preservative. Sodium benzoate is a permitted preservative in roe caviars at < 1000 ppm (Bledsoe et al. 2003). After addition of oil and mixing, the pickle was filled into pre-sterilized 200 g capacity glass bottles up to the brim. Care was taken to cover the surface with previously heated oil. The pickle bottles were kept at room temperature (28 ± 2°C) for storage studies.

The pickle was analyzed for proximate composition (AOAC 1995). The pH was measured by using single electrode of a digital pH meter (Control Dynamics, Bangalore, India). The microbial load was assayed in terms of total plate count (TPC) (APHA 1976). Sensory evaluation for appearance, colour, flavour, texture, taste and overall acceptability of fish roe pickle was carried out with a panel of 10 semi trained judges served with cooked rice using a 9-point Hedonic scale (1 = dislike extremely; 5 = neither like nor dislike and 9 = like extremely) (Peryam and Pilgrims 1957). The control sample was stored in refrigerator (4 ± 1°C).

All analytical determinations were carried out in triplicate and mean values with standard deviation (SD) are presented. Sensory scores were analysed statistically by ANOVA using SPSS 15.0 to ascertain whether differences were significant at $p<0.05$.

Results and discussion

Effect of pre-treatments on fish roes: Fresh roes of rohu had a moisture content of 58.9% and total protein content of 36.3%. Control roes, which were without salt, had ~72% moisture. Moisture content in pasteurized roes and in blanched roes with salt solutions was approximately 65.0 and 60.3%, respectively. The difference in moisture might be due to effect of salt. Increase in moisture content or water absorption capacity was one of the functional properties of proteins present in roes, which gave higher moisture content than the fresh roes.

The pasteurized roes became gelatinized and sticky with unpleasant fishy odour. Hence, these were discarded for further study; however the filtrates were analyzed for protein content to measure their losses. Table 1 indicates higher losses in protein content in pasteurized roes as compared to control and blanched roes. Pasteurization process resulted in significantly higher protein solubility in NaCl solutions. Higher solubility of 40.1%, medium solubility of 35.9% and minimum solubility of 17.1% protein was observed in 0.75, 1.0 and 0.5 M salt solutions respectively.

The blanched roes were undamaged, granular, and easily separable with least fishy odour. The chewability was good

with characteristic mouth feel in case of 1M NaCl blanched roes. In the blanching process, protein losses were found to be 4.2, 5.9 and 7.3% in 0.5, 0.75 and 1M salt solutions, respectively (Table 1).

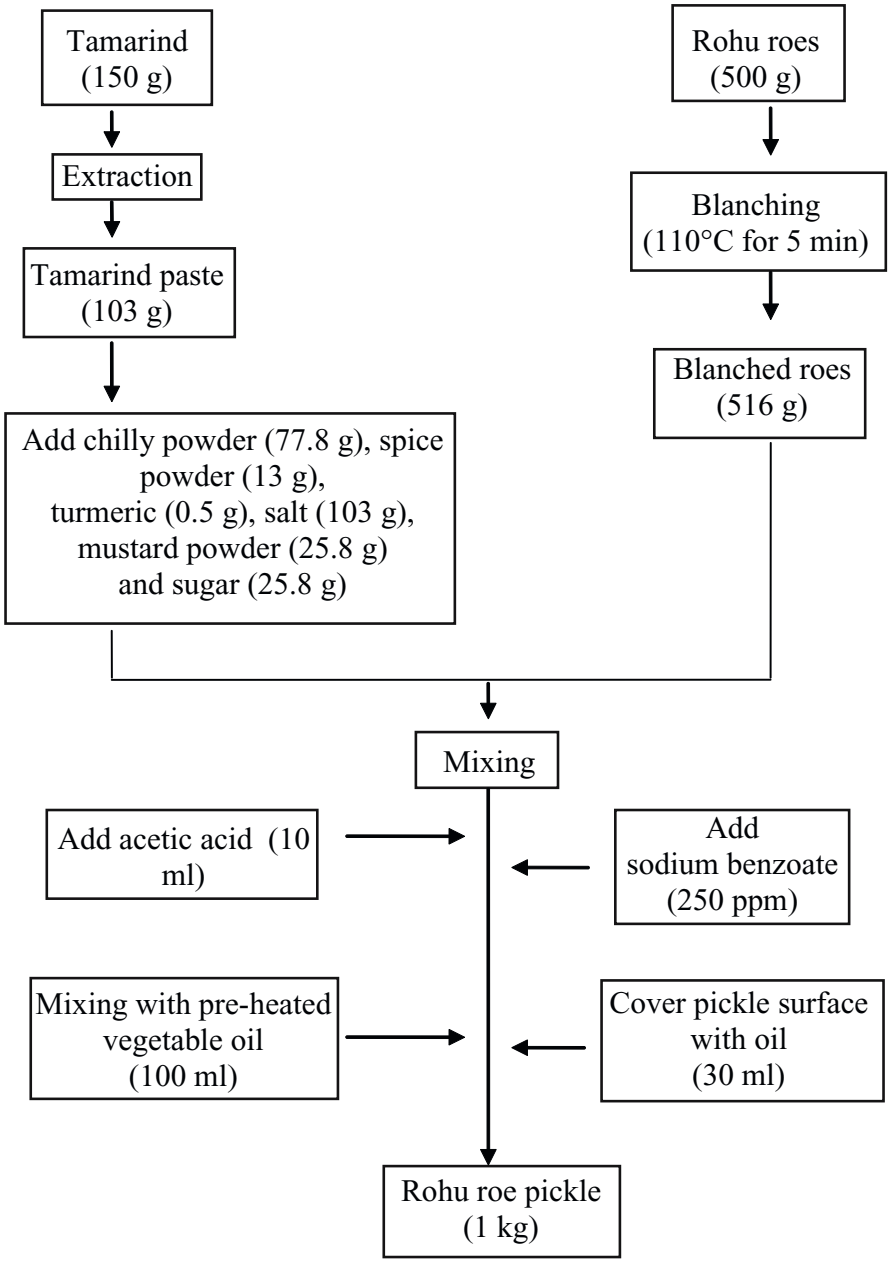

Fig. 1 Flow chart for the preparation of fish roe pickle

Table 1 Changes in protein and salt contents (%) in rohu roes and filtrates after pretreatments in different molar salt solutions

Sample	Parameter	Pasteurization				Blanching			
		C	0.5M	0.75M	1M	C	0.5M	0.75M	1M
Roes	Protein	34.3 ± 0.62	ND	ND	ND	35.3 ± 0.20	35.5 ± 0.06	33.5 ± 0.03	34.3 ± 0.03
	Salt	ND	ND	ND	ND	ND	2.7 ± 0.01	3.9 ± 0.06	3.9 ± 0.03
Filtrate	Protein	2.2 ± 0.10	6.2 ± 0.16 (17.1)	14.6 ± 0.22 (40.1)	13.0 ± 0.57 (35.9)	1.1 ± 0.01	1.5 ± 0.01 (4.2)	2.2 ± 0.02 (5.9)	2.6 ± 0.01 (7.3)
	Salt	ND	2.2 ± 0.02	4.0 ± 0.06	5.5 ± 0.12	ND	2.8 ± 0.03	4.2 ± 0.02	5.1 ± 0.03

ND = Not determined; C = Control, treatment without salt; Values in parentheses indicate % protein solubility, (n = 3)

Table 2 Physico-chemical composition of rohu roe pickle

Moisture, %	48.9 ± 0.20
Acidity, %	0.9 ± 0.01
pH	3.8 ± 0.03
Total ash, %	12.3 ± 0.03
Salt, %	12.0 ± 0.06
Crude fat, %	10.5 ± 0.27
Total protein, %	12.7 ± 0.16
Carbohydrates by diff, %	15.6
Total energy, kcal/100 g	208

(n=3)

logs cfu/g after 6 months storage (data not shown). This is far below the maximum recommended bacterial counts (5.7 log cfu/g) for good quality products (ICMSF 1986). Hsu et al. (1983) reported that intermediate moisture roe (a_w=0.84, salt content = 4%) stored at 5–25°C were acceptable after 30 days in terms of chemical, microbial and sensory characters. Joong-Han Shin and Rasco (2007) studied the growth of *Listeria monocytogenes* by incorporating at log 2.4 and 4.2 cfu/g while curing of salmon roe in 0.22–4.4% (w/v) brine solutions at 7°C. They noticed an increased count of log 5–6 cfu/g during 30 days of storage. In the present study, blanching of roes in salt solution at lower pH and higher acidity was responsible for greater shelf-stability of the product.

Table 3 Changes in sensory score of rohu roe pickle during storage

	Storage period, months			
	0	2	4	6
Appearance	8.1 ± 0.52	8.1 ± 0.40	8.0 ± 0.54	7.5 ± 0.44*
Colour	8.0 ± 0.53	7.8 ± 0.59	7.5 ± 0.47*	7.4 ± 0.26*
Flavour	8.1 ± 0.34	7.8 ± 0.34	7.5 ± 0.44*	7.5 ± 0.41*
Texture	7.5 ± 0.44	7.5 ± 0.44	7.5 ± 0.44	7.3 ± 0.42
Taste	8.1 ± 0.57	8.1 ± 0.50	7.5 ± 0.41*	7.3 ± 0.26*
Overall acceptability	8.0 ± 0.40	8.0 ± 0.40	7.5 ± 0.33*	7.2 ± 0.26*

*Significantly different ($p<0.05$) from 0 day in rows (n=10 panelists)

Pasteurization might have increased the protein solubility due to interaction of surface amino acids with ionic groups in the salt solution. The lower protein solubility in blanched samples might be due to coagulation or denaturation of roe protein. Sternin and Dore (1993) reported that the pasteurization of roe between 50 and 70°C for longer time was suitable for preparation of caviars. However, >70°C the roe protein coagulated and further increase in temperature denatured the protein. Fish muscle myofibrillar proteins were soluble at 0.4-0.6 molar salt concentrations at neutral or low acid pH (6.6) due to depolymerisation of protein filaments (Parsons and Knight 1990).

The study indicated that blanched roes in 1M salt solution containing 60.3% moisture and 3.9% salt were suitable for the preparation of fish roe pickle. Earlier, Wakamaeda et al. (1995) described the processing of Alaska Pollock roe and recommended the processed roes containing a minimum salt of 2.5% and water content of 59% as starting material for preparation of roe products.

Proximate composition of roe pickle and storage studies: Moisture content in the pickle was 48.9% (Table 2) and hence might be classified under the intermediate moisture foods. The composition of freshly prepared pickle is given in Table 2. During storage for 6 months, pH marginally increased from 3.8 to 3.9 and the acidity decreased from 0.9 to 0.8% (data not shown). Sensory scores decreased gradually during storage (Table 3). However, no off-flavours were observed. TPC increased from 4.8 to 5.0

Conclusion

The blanched roes in salt solution were found to be suitable for the preparation of roe pickle. Pasteurization was not useful in preparing base material for pickle production. Rohu roe pickle was acceptable with good sensory characteristics and was microbiologically safe during 6 months of storage. Blanching of roes in salt solution and higher acidity in pickle helped control TPC.

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