

# New Approaches to Value Addition

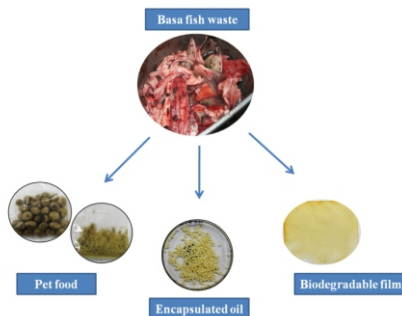
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## Valorization of Fish Waste

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### ABSTRACT

Fish and fishery waste are nutritionally adequate and valorization of this waste can be done by adapting new approaches for proper utilization. Deployment of fish waste has been demonstrated by using three different approaches viz. pet food, encapsulated oil and biodegradable film. Nutritionally adequate and microbiologically safe pet food in kibble and powder form was prepared with an extended shelf life of 65 days using gamma irradiation. Encapsulation of fish oil extracted from fish waste was carried out in calcium alginate beads, which increased the shelf life and storage quality up to 3 times as compared to the non-encapsulated oil. Biodegradable films were synthesised using gamma irradiated mince dispersion where, film prepared from 10 kGy-treated sample exhibited enhanced physical properties compared to the non-irradiated one. These results indicate that fish waste can be utilized for development of value-added products, which not only reduces environmental hazards, but also results in the upliftment of the socio-economic conditions.

**KEYWORDS:** Fish waste, Circular economy, Pet Food, Fish oil, Encapsulation, Biodegradable film.

### Introduction

Fish and other types of seafoods are highly nutritious, and can be an important part of healthy and well-balanced diet. They are also considered as unique sources of natural drugs for deadly diseases like cancer, AIDS, coronary disease etc. It is known to be rich in protein, fat, omega-3 fatty acids, vitamins and minerals. Fisheries and its allied industries are the major economic sectors in the world. Consumption of fishery products has seen a huge increase in the last few years. In 2020, the world's total fisheries and aquaculture production reached a record 214 million tonnes. India is the 3<sup>rd</sup> largest fish producer (16.25 million tonnes), and this contributes to 1.1% of the country's GDP [1].

Unfortunately, commercial processing (beheading, de-shelling, degutting, removal of fin and scales, filleting) of fish results in a huge amount of solid waste, offal or by-products, which are discarded either at sea or in landfills. Waste disposal in the open environment creates pollution and hazards, which has potential to become the source of many infectious diseases [2].

However, fishery waste is an enriched source of nutrients and bioactive compounds that can be utilized in production of several value-added products. The head, bone, and intestine are rich in lipids with high polyunsaturated fatty acids (PUFAs) content and the skin is a rich source of protein [3]. These wastes can be gainfully utilized for several purposes such as preparation of biodegradable/edible films, fish oil, low-cost nutraceuticals, pet food/animal feeds, silage & organic fertilizers and industrial products such as bio-active compounds (gelatin, chitin, chitosan, carotenoids, glucosamine hydrochloride, squalene) etc. Fish oil from waste contains beneficial bioactive compounds (eicosapentaenoic acid, EPA, C20:5 n-3 and docosahexaenoic acid, DHA, C22:6 n-3) that can be encapsulated, thus providing a specific and

controlled release of these bioactives. Encapsulation of bioactives and fish oil is of great pharmaceutical significance due to its growing demand in the market [4].

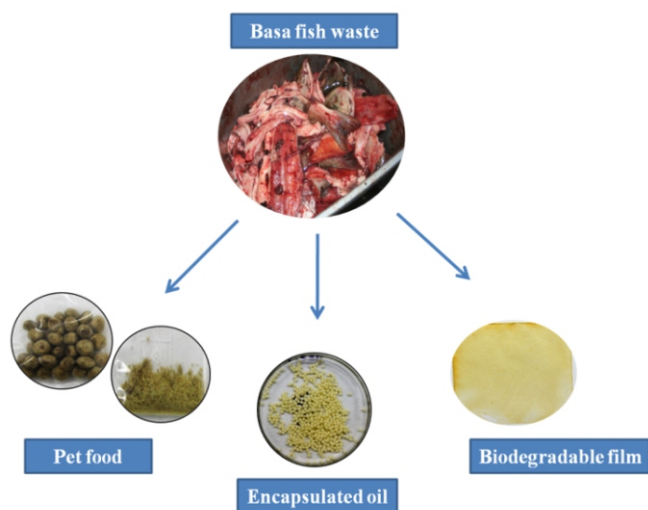
Fish and shellfish proteins from waste offer a wide variety of substrate material for the extraction of new peptides with specialized or multi-functional bioactivity because of their considerable structural diversity. The skin of the larger fish provides leather that could be utilized in the manufacture of handbags, belts, clothing, wallets, belts, etc [5]. Basa fish offal is a good source of potential lipids and can be utilized for preparation of aquatic feed for commercially important fishes [6]. Utilization of fish waste will provide an important tool for lowering the problem of malnutrition and food shortage in developing countries. In addition, it will help in generation of employment opportunities and as a result, turn out to be advantageous both for the environment and the economy.

Basa (*Pangasius bocourti*), an Asian freshwater catfish, is one of the most popular fish worldwide due to its desirable qualities such as white flesh, high PUFA content and digestible protein. It contains high quality balanced essential amino acids and fatty acids with adequate  $\omega$ -6/ $\omega$ -3 ratio [7]. During processing of Basa into fish fillet, fingers, steaks etc., 50-70% of the biomass ends up as solid or liquid wastes. The waste tissues/organs such as viscera, head, bones, skins and fins, generated in the filleting industry, are a boon in direction of fish-waste utilization.

Circular economy is the need of the hour for efficient and sustainable utilization of fish and fishery wastes to recourse, reallocate and reduce the negative environmental effects. An improved and efficient technique will help in utilization of fish-processing discards to generate superior quality co-products.

The present article shows three different ways by which seafood processing waste can be utilized for production of valuable commodities i.e. (i) pet food (ii) encapsulated fish oil and (iii) biodegradable films, which could find various applications in food and feed industries.

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### Materials and Methods

#### Oil and mince extraction

The oil from head, bone and viscera was extracted separately by solvent extraction method as described earlier using chloroform: methanol; ratio of 2:1 [8]. Mince was extracted from the bones and spines using deboning machine. The protein content in the mince was analysed by the Kjeldahl method.

#### GC-MS analysis of oil

This fish oil was esterified with methanol and 2N potassium hydroxide [8]. These esterified methyl esters of fatty acid were analysed using the GC-MS technique.

#### Preparation of pet food

Initially fish waste (100 g) was mixed with 50 ml of potable water and homogenised to make a thick paste; rice and wheat flour (1:1 w/w), 1% turmeric powder and salt 3% were then mixed properly. For powder preparation, this thick paste was dried directly in Infra-red dryer at 50°C for 3 h to a moisture content of 5 - 8%. For kibble preparation, 20 ml water was slowly added to the mixture and round kibbles were made from the prepared dough. Kibbles were dried to a moisture content of 5 - 8%. These pet foods were packed and irradiated at 2.5 and 5 kGy and quality parameters such as proximate, microbial load and lipid peroxidation (in terms of thiobarbituric reactive substances, TBARS) was studied during the storage.

#### Encapsulation of oil

Oil was mixed with various concentrations of sodium alginate to prepare loading concentrations of 10%, 20%, 30%, 40% and 50%. Stability of emulsion was determined by keeping 50 ml of alginate-oil emulsion undisturbed for 1 h at room temperature. To prepare beads, simple extrusion process was used for the encapsulation of fish oil as per the previous report [9]. The size, surface and cross-sectional view of beads was observed with the scanning electron microscope at 20 kV. The quality parameter of beads such proximate analysis, acid value (AV), peroxide value (PV) and free fatty acid (FFA) was done as per the methodology already published [10].

#### Preparation of biodegradable films

The washed and minced meat was homogenized with one volume of chilled distilled water using a homogenizer. Later, 0.5% glacial acetic acid was added slowly to reduce pH with stirring to induce gelation. The gel was homogenized with

three volumes of a chilled solution of 3% glycerol and 0.75% glacial acetic acid to obtain film-forming dispersion. The obtained film-forming dispersion was divided into three parts, irradiated at 0, 10 and 25 kGy and change in viscosity as well as protein content was studied. 100 ml of each sample was poured and spread evenly on 21 cm × 21 cm polystyrene plate and kept at 50°C in a dry air oven for 12 h and further conditioning of films was done at 25°C at 50% relative humidity for 5 days. After conditioning, films were peeled and analysed for different properties [11].

### Results and Discussion

Fish waste is a source of various nutraceuticals and bioactive compounds such as polyunsaturated fatty acids (PUFAs), peptides, vitamins and minerals. The fish waste can be effectively utilized for development of value-added products, thus, reducing environmental pollution and generating economic benefits. This waste can be utilised for the extraction of these bioactives for preparation of different value-added products for e.g. nutrient supplements, packaging materials, animal feeds etc. In the present study, fish oil was extracted from total waste (bones, head and viscera) whereas, fish mince was extracted only from bone waste of Basa (*Pangasius bocourti*). The Basa fish waste contains about 12% oil and 49% mince with 15% protein. Total fatty acid composition of the extracted oil (Table 1) showed that most monounsaturated fatty acid ( $48.27 \pm 1$ ) predominated, followed by saturated fatty acid ( $35.93 \pm 1.13\%$ ) and polyunsaturated fatty acid ( $15.88 \pm 0.26\%$ ). These results suggest that Basa fish waste is nutritionally adequate with substantial amount of essential fatty acids and protein [12]. Hence, this waste was utilized to develop three value-added products namely (i) pet-food (ii) encapsulated oil and (iii) biodegradable film.

Table 1: Total fatty acids (%) of fish waste.

Sr. No.	Fatty Acid Composition	Total fatty acid (%)
1.	Myristic Acid (14:0)	2.27 ± 0.04
2.	Palmitic Acid (16:0)	22.47 ± 1.02
3.	Stearic Acid (18:0)	11.19 ± 0.07
4.	Oleic Acid (18:1, n-9)	48.27 ± 1
5.	Alpha Linolenic Acid (18:3, n-3)	0.55 ± 0.09
6.	EPA (20:5, n-3)	0.29 ± 0.1
7.	DHA (22:6, n-3)	2.1 ± 0.01
8.	Linoleic Acid (18:2, n-6)	12.1 ± 0.02
9.	Gamma-Linolenic Acid (20:3, n-6)	0.45 ± 0.02
10.	Arachidonic Acid (20:4, n-6)	0.39 ± 0.02
11.	n-3/n-6	0.23 ± 0.014
12.	∑ SFA	35.93 ± 1.13
13.	∑ MUFA	48.27 ± 1
14.	∑ PUFA	15.88 ± 0.26

The results shown are mean ± standard deviation of three independent experiments in triplicate (n= 9).



Fig.1: Different forms of pet foods before and after irradiation.

**Pet food preparation**

Valorization of Basa fish to prepare safe pet foods i.e. kibble or in powder form (Fig.1) using gamma irradiation (for extending its shelf life) was attempted. Microbiological (Fig.2) and lipid peroxidation analyses (Fig.3), showed an extended shelf life of 65 days for both the pet foods when irradiated at 2.5 and 5 kGy whereas, non-irradiated kibble and powder samples were spoilt within 28 and 35 days respectively. No significant changes in the proximate composition (Table 2) of both these pet foods were observed on irradiation, indicating that both were nutritionally adequate and microbiologically safe [13-15].

**Encapsulated fish oil**

Fish oil was extracted from total waste and encapsulated

with alginate as a coating material using simple extrusion process. The total fatty acid composition of encapsulated and unencapsulated oil was analysed where no significant difference was observed. Alginate concentration (2.5%) with oil loading of 30% showed higher emulsion stability (Fig.4) with least pore space, hence, was considered optimum for encapsulation of oil [16]. The encapsulated beads were  $2.39 \pm 0.10$  mm in size, with smooth surface (Fig.5) and had 91% encapsulation efficiency. The proximate analysis depicted, ash, moisture, fat, protein and carbohydrate content of oil beads to be  $2.95 \pm 0.07$ ,  $2.26 \pm 0.06$ ,  $91.07 \pm 0.02$ ,  $0.08 \pm 0.01$  and  $3.64 \pm 0.01$  respectively (Table 3). Beads were stable up to 12 days as determined by peroxide value ( $18.67 \pm 1.15$  meq/Kg), acid value ( $14.13 \pm 0.35$  mg KOH/g) and free fatty acid value ( $8.42 \pm 0.51\%$ ) where as the un-encapsulated oil got rancid on day 4

Table 2: Proximate analysis of prepared pet foods before and after irradiation.

Sr. No		Kibble			Powder		
		Control	2.5 kGy	5.0 kGy	Control	2.5 kGy	5.0 kGy
1	Moisture	$6.60 \pm 0.14^a$	$6.92 \pm 0.87^a$	$6.98 \pm 0.81^a$	$6.12 \pm 0.04^a$	$5.90 \pm 0.29^a$	$5.84 \pm 0.01^a$
2	Protein	$20.69 \pm 1.92^a$	$16.16 \pm 0.53^a$	$19.66 \pm 4.36^a$	$13.59 \pm 0.38^b$	$12.87 \pm 0.35^a$	$14.37 \pm 0.32^c$
3	Fat	$14.99 \pm 2.35^a$	$16.48 \pm 2.59^a$	$14.65 \pm 1.91^a$	$15.36 \pm 0.06^a$	$16.57 \pm 2.79^a$	$15.3 \pm 1.06^a$
4	Ash	$7.0 \pm 0.07^b$	$6.8 \pm 0.42^b$	$4.48 \pm 0.01^a$	$5.05 \pm 0.21^a$	$4.95 \pm 0.49^a$	$4.95 \pm 0.64^a$
5	Carbohydrate	$50.72 \pm 0.02^a$	$53.64 \pm 0.55^{ab}$	$54.23 \pm 0.07^b$	$59.88 \pm 0.15^a$	$59.71 \pm 0.05^a$	$59.54 \pm 0.08^a$

The results shown are mean  $\pm$  standard deviation of three independent experiments in triplicate (n= 9). Different letters in the row/column indicate significant differences (p < 0.05).

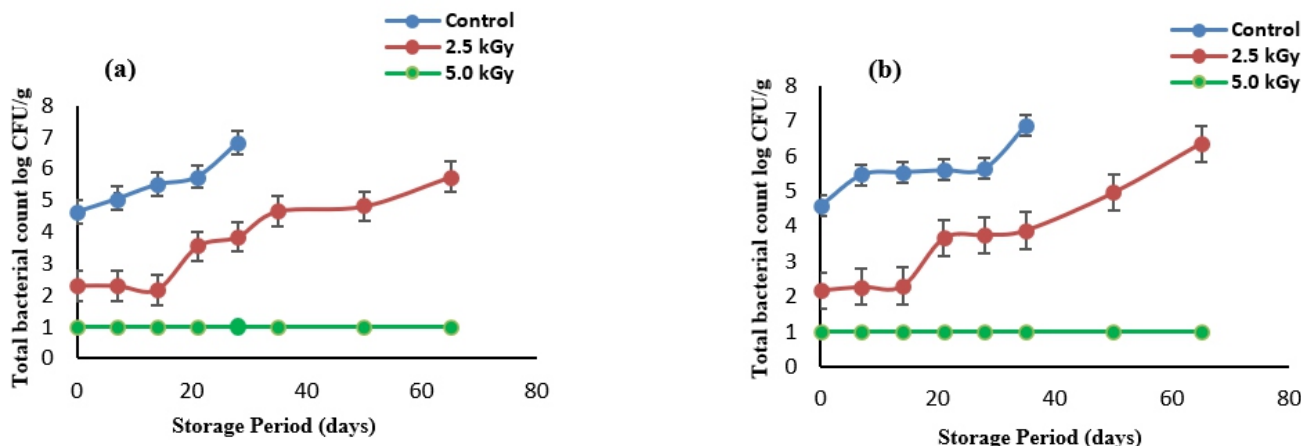


Fig.2: Total bacterial load of developed pet foods on irradiation and storage (a) Kibble form and (b) Powder form.



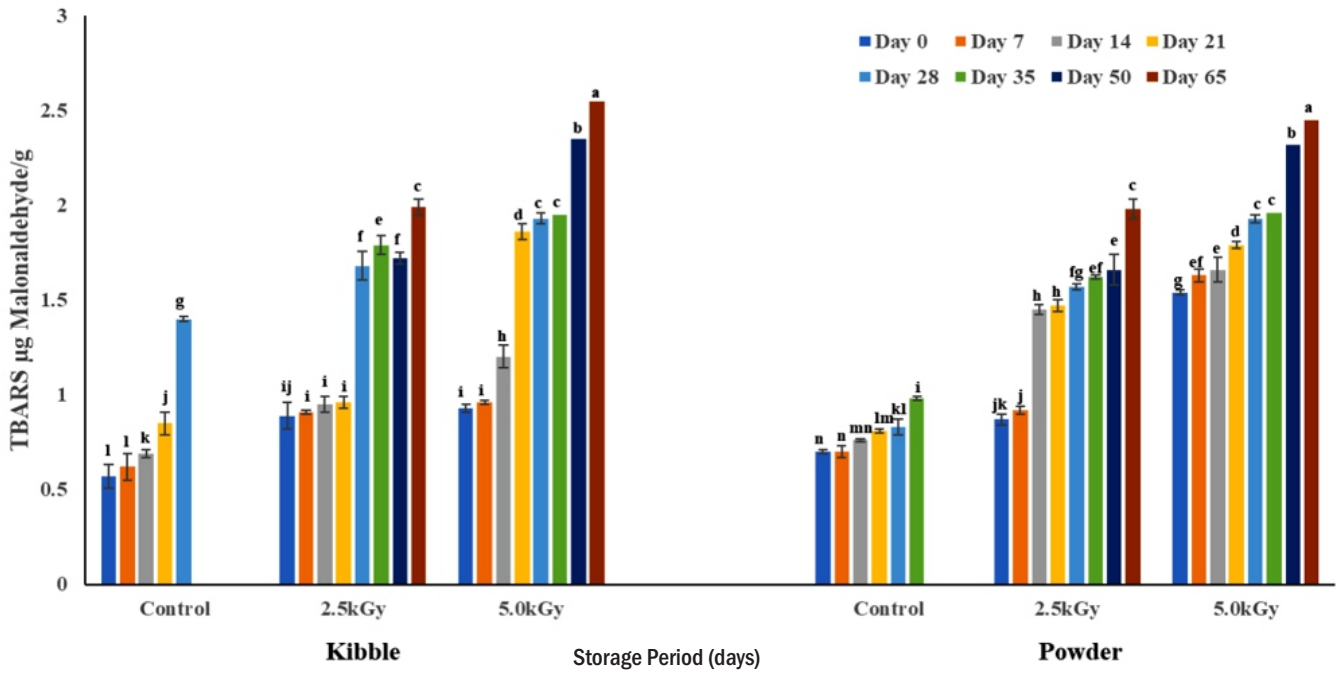


Fig.3: Lipid peroxidation in terms of TBARS for different pet foods on irradiation and storage.

The values are mean  $\pm$  standard deviation of three independent experiments in triplicates (n=9). Different letters on the error bar indicate significant differences ( $p < 0.05$ ).

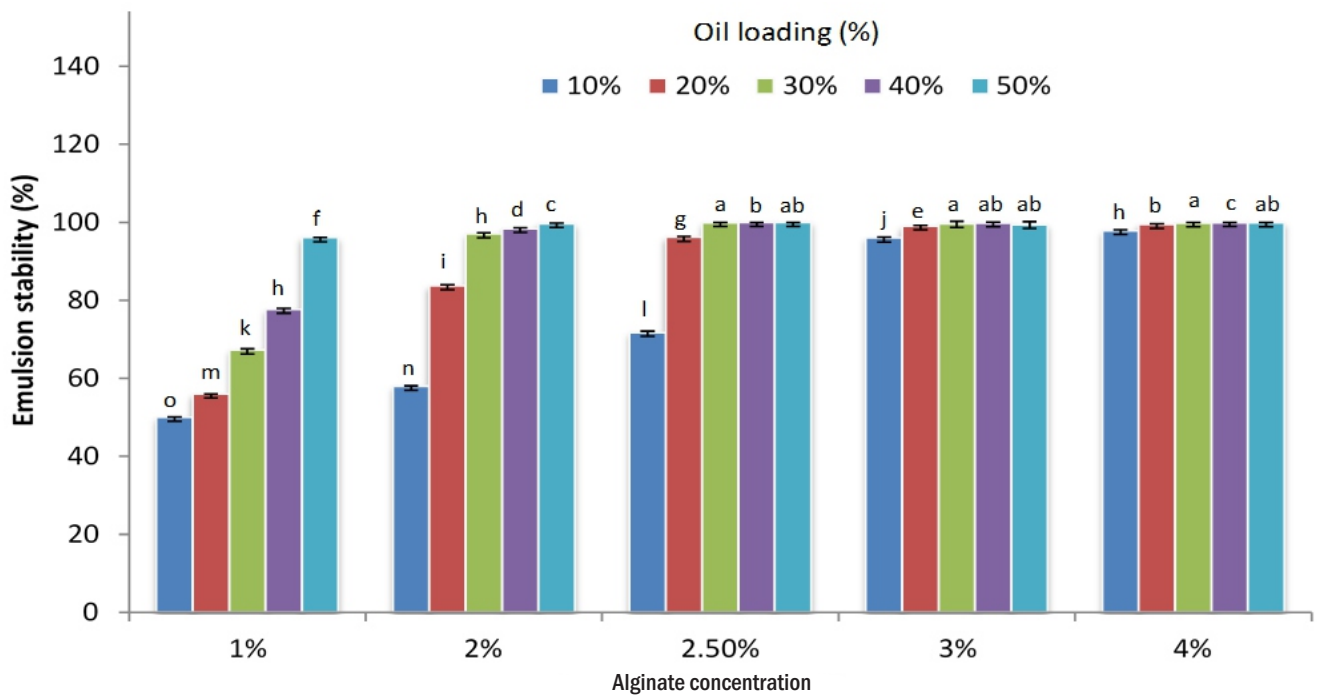


Fig.4: Emulsion stability (ES) at different alginate concentration and oil loading %.

The results shown are mean  $\pm$  standard deviation of three independent experiments in triplicate (n= 9). Different letters in error bars indicate significant differences ( $p < 0.05$ ).

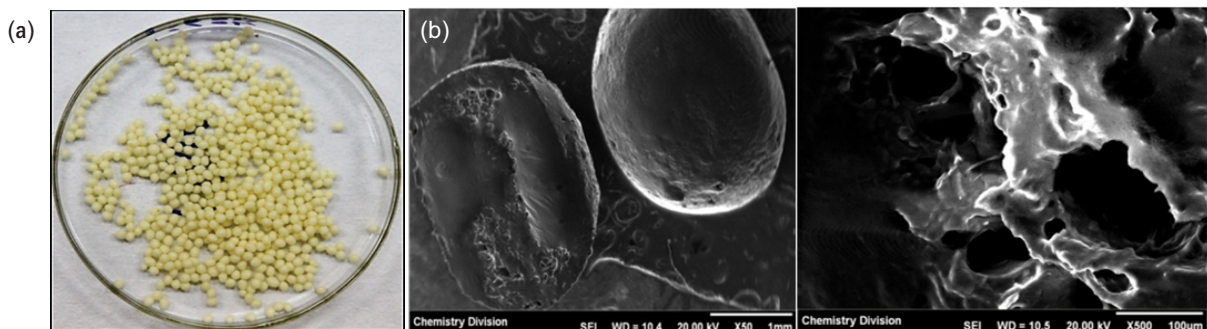


Fig.5: Characteristics of oil beads of 2.5% alginate concentration at 30% oil loading: (a) Physical appearance (b) SEM image of Surface and Cross-sectional view.

Table 3: Proximate analysis of per 100 g of encapsulated oil beads.

Sr. No.	Proximate analysis	Content (%)
1.	Ash	2.95 ± 0.07
2.	Moisture	2.26 ± 0.06
3.	Fat	91.07 ± 0.02
4.	Protein	0.08 ± 0.01
5.	Carbohydrate	3.64 ± 0.01

The results shown are mean ± standard deviation of three independent experiments in triplicate (n= 9).

Table 5: Viscosity and protein content of non-irradiated and irradiated film forming dispersions

Sr. No.	Sample	Viscosity (cp)	Protein content (%)
1.	Control	506 ± 2.3 <sup>a</sup>	1.06 ± 0.02 <sup>a</sup>
2.	Dispersion 10 kGy	365 ± 2.2 <sup>b</sup>	1.19 ± 0.02 <sup>a</sup>
3.	Dispersion 25 kGy	20 ± 0.6 <sup>c</sup>	1.23 ± 0.03 <sup>a</sup>

The results shown are mean ± standard deviation of three independent experiments in triplicate (n= 9). Different letters in the row/column indicate significant differences (p < 0.05).

Table 4: Peroxide value (PV), Acid value (AV) and Free fatty acid (FFA) values of oil beads.

Sr. No	Storage period (days)	PV (meq/kg)		AV (mg KOH/g)		FFA (% wt)	
		Fish oil	Encapsulated	Fish oil	Encapsulated	Fish oil	Encapsulated
1	0	2.33 ± 0.32 <sup>f</sup>	2.67 ± 0.15 <sup>f</sup>	4.7 ± 0.3 <sup>e</sup>	5.46 ± 0.41 <sup>f</sup>	1.86 ± 0.08 <sup>e</sup>	2.73 ± 0.45 <sup>f</sup>
2	4	28 ± 2.65 <sup>b</sup>	5 ± 0.26 <sup>e</sup>	17.20 ± 0.7 <sup>b</sup>	10.02 ± 0.56 <sup>e</sup>	7.87 ± 0.65 <sup>c</sup>	5.01 ± 0.28 <sup>e</sup>
3	8	ND	6.67±0.15 <sup>d</sup>	ND	12.84 ± 0.69 <sup>d</sup>	ND	6.92 ± 0.34 <sup>d</sup>
4	12	ND	18.67±1.15 <sup>c</sup>	ND	14.13 ± 0.35 <sup>c</sup>	ND	8.42 ± 0.51 <sup>b</sup>
5	16	ND	43.33±2.89 <sup>a</sup>	ND	34.03 ± 0.47 <sup>a</sup>	ND	17.02 ± 0.23 <sup>a</sup>

The results shown are mean ± standard deviation of three independent experiments in triplicate (n= 9). Different letters in the row/column indicate significant differences (p < 0.05). ND- Not determined

when stored in amber coloured glass bottle in dark at room temperature (25°C) with relative humidity of 65% (Table 4). Encapsulation of fish oil increases the shelf life and storage quality for longer period by reducing oxidation and bio dehydrogenation [17].

**Biodegradable film**

Biodegradable packaging material from mince dispersion of fish waste was synthesized to serve as “Wealth from Waste”- an eco-friendly initiative. Film dispersions were gamma-irradiated at a dose of 10 and 25 kGy before casting. A significant decrease in viscosity (Table 5) and increase in

yellowness (Fig.6) was observed with irradiation which can be linked to higher oxidative fragmentation of proteins at 25 kGy as compared to 10 kGy. The appearance of different films is shown in Fig.7. The physical properties of prepared films were tested (Table 6), where film prepared after irradiation at 10 kGy showed better Tensile strength, Young’s Modulus with lower water solubility and elongation at break as compared to the control, which had minimum tensile strength and maximum elongation at break. The 25 kGy irradiated film had highest opacity with yellowness and water vapour permeability. Gamma-irradiation affects the physical properties of the film, hence, irradiation of protein dispersion from waste is an effective tool to develop films with improved quality [18,19].

**Conclusion**

Basa fish waste is nutritionally adequate and valorization of this waste can be done by adapting new approaches for value addition. In the present study, three different approaches have been demonstrated for sustainable management of waste and resource reallocation by developing commercially important commodities such as pet food, encapsulated oil and biodegradable films. The fish waste can be effectively utilized for development of value-added products, thus, reducing environmental hazards and generating economic benefits.

**Acknowledgment**

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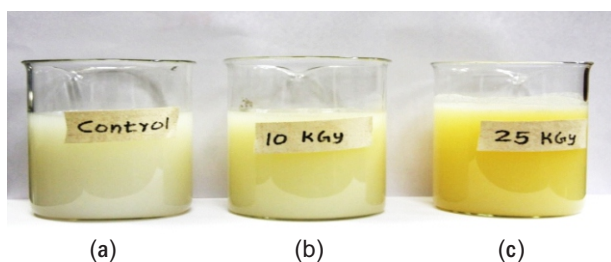


Fig.6: Dispersions treated with different doses of γ- irradiation (a) Control (b) 10 kGy (c) 25 kGy .

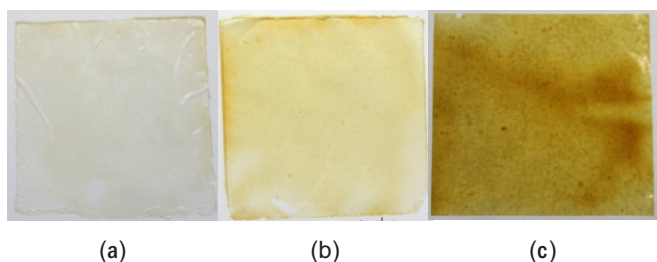


Fig.7: Film prepared from control and irradiated dispersion (a) Control (b) 10 kGy (c) 25 kGy .

Table 6: Physical properties of films obtained from control and irradiated dispersion.

Sr. No	Sample (film)	Control	10 kGy	25 kGy
1	Moisture (%) <sup>#</sup>	14.07 ± 0.29 <sup>a</sup>	14.06 ± 0.03 <sup>a</sup>	13.76 ± 0.07 <sup>a</sup>
2	Protein (%) <sup>#</sup>	30.93 ± 0.5 <sup>a</sup>	29.88 ± 0.71 <sup>a</sup>	29.97 ± 1.01 <sup>a</sup>
3	Water solubility (%) <sup>#</sup>	61.9 ± 4.76 <sup>a</sup>	15.47 ± 1.19 <sup>b</sup>	61.04 ± 5.7 <sup>a</sup>
4	Water vapour permeability (g mm h <sup>-1</sup> cm <sup>-2</sup> Pa <sup>-1</sup> )x 10 <sup>-8#</sup>	7.08 ± 1.02 <sup>a</sup>	7.74 ± 1.27 <sup>a</sup>	11.42 ± 0.09 <sup>b</sup>
5	Thickness (mm) <sup>##</sup>	0.16 ± 0.009 <sup>a</sup>	0.17 ± 0.0 <sup>a</sup>	0.29 ± 0.037 <sup>b</sup>
6	Tensile strength (N/mm <sup>2</sup> ) <sup>###</sup>	0.76 ± 0.13 <sup>a</sup>	1.27 ± 0.26 <sup>b</sup>	1.11 ± 0.22 <sup>b</sup>
7	Young's Modulus (N/mm <sup>2</sup> ) <sup>###</sup>	3.82 ± 1.28 <sup>a</sup>	8.39 ± 2.32 <sup>b</sup>	9.77 ± 1.98 <sup>b</sup>
8	Elongation at break (%) <sup>###</sup>	162.94 ± 19.64 <sup>a</sup>	87.64 ± 15.23 <sup>b</sup>	65.43 ± 6.96 <sup>b</sup>
9	L* (black to white) <sup>##</sup>	38.37 ± 1.0 <sup>a</sup>	35.63 ± 0.28 <sup>b</sup>	33.32 ± 0.78 <sup>c</sup>
10	a* (green to red) <sup>##</sup>	7.86 ± 0.74 <sup>a</sup>	9.38 ± 0.48 <sup>b</sup>	12.77 ± 1.36 <sup>c</sup>
11	b* (blue to yellow) <sup>##</sup>	3.84 ± 0.78 <sup>a</sup>	10.38 ± 1.02 <sup>b</sup>	14.11 ± 0.43 <sup>c</sup>
12	ΔE* (total colour change) <sup>##</sup>	59.19 ± 1.03 <sup>a</sup>	62.92 ± 0.43 <sup>b</sup>	66.26 ± 0.96 <sup>c</sup>
13	Opacity (%) <sup>##</sup>	13.59 ± 0.38 <sup>a</sup>	14.75 ± 0.58 <sup>b</sup>	17.91 ± 1.25 <sup>c</sup>

# (n=3), ## (n=6), ### (n=4), Different letters in the same row indicate significant differences (p < 0.05).

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