CHAPTER - V

SUMMARY AND CONCLUSION

Globalization has greatly influenced the food choices of Indians leading to radical changes in their dietary habits. A direct consequence of this has been an excess intake of mere calories and the concurrent incidence of nutrient deficiencies, especially that of protein, iron, calcium and phosphorus. This leads to the pressing need of advocating food based approaches that are the most natural and safest way of ensuring adequate intake of all nutrients.

One such food based approach would be the value addition of foods. Utilizing animal source foods in food based approaches, offer potentially sustainable solutions to multiple deficiencies. Small fish are an affordable source of animal protein for a wide cross section of the population in developing countries when compared to other animal sources (Hansen et al., 1998). Small fish with bones are excellent sources of highly bioavailable protein and calcium (Odote & Kazungu, 2008). White bait being the most abundantly available small pelagic fish species in India, with very fine brittle bones remains underutilized in terms of the protein and calcium it provides. This calls for innovative methods of processing white bait fish that would result in the formulation of a novel fish based value added product which includes the bones as well as flesh.

Based on the fact that fish wafers have a global impact on nutrition and that variations of this product are well accepted, the present study was attempted to prepare ready-to-cook fish wafers utilizing ‘white bait fish’ with bones without the addition of chemical preservatives.

The objectives of the present study were

1. To prepare white bait fish wafers with and without the incorporation of bones in two proportions of starch to fish (1:1 & 1:1.5).
2. To evaluate the nutrient, biochemical, microbial and sensory qualities of fish wafers.
3. To study the effect of packaging, drying and duration of storage on fish wafers.

4. To determine the invitro bioavailability of calcium and phosphorous from fish wafers prepared with bone.

5. To popularize the preparation of fish wafers among women entrepreneurs.

The research design adopted in the present study was pre test post test experimental research design with control group. Fish wafers were prepared in two proportions of starch to fish, that is, 1:1 and 1:1.5. In both proportions, fish wafers were prepared with and without the inclusion of bones. The starch used was tapioca starch in the form of tapioca pearls. The control was prepared without the inclusion of fish.

The wafers were subjected to two types of drying (solar tent drying and sun drying) and two types of packaging (ordinary packaging and vacuum packaging), to study the effect of different drying and packaging methods on wafer quality.

The prefried white bait fish wafers were stored for a period of 90 days. The prefried wafers were subjected to the following assessments

- Evaluation of nutrient quality (moisture, ash, carbohydrate, protein, fat, calcium and phosphorus) on the 1st and 90th day.

- Evaluation of biochemical quality (pH, TVBN, TMA, FFA & TBA) on the 1st, 45th and 90th day.

- Evaluation of microbial quality (TPC, aw and testing for the presence of pathogenic organisms such as E. coli, Salmonella, Staphylococcus aureus and Pseudomonas) on the 1st, 45th and 90th day.

The wafers were subjected to two cooking methods, that is, frying and puffing it in the microwave. The fried and micro wave puffed wafers were assessed for the following

- Evaluation of sensory quality on the 1st, 45th and 90th day

- Assessment of linear expansion and oil absorption on 1st, 45th and 90th day
A comparison between the control and the experimental groups 1:1 (with bone – WB & without bone - WOB) and 1:1.5 (WB & WOB) was made for each of these parameters. The effect of duration of storage on the above parameters was further evaluated. Besides this, an invitro assessment of the calcium and phosphorous bioavailability from white bait fish wafers prepared with bones was also analyzed.

The following conclusions were arrived from the results of the present study

**EFFECT OF DRYING METHODS ON FISH WAFER QUALITY**

On periodical evaluation of the nutrient, biochemical, microbial and sensory qualities of the fish wafers, it was observed that, though there were differences in the mean values of all the parameters between sun dried and solar tent dried wafers, they were not statistically significant as shown by the t values. Both the drying methods produced similar quality fish wafers. This was attributed to the washing and cooking of the fish prior to drying of the fish wafer gel, use of drying table to dry wafers and the covered environment the wafers were in during the drying period, that greatly reduced the microbial load on both the sun dried and solar tent dried wafers.

Nevertheless, the most noticeable advantage of solar tent drying over sun drying method in the present study, was on the time taken for drying. Wafers that were sundried took two days to dry whereas one and a half days when the solar tent drier was used. There was a maximum temperature difference of about 10 °C between inside and outside the tent.

**EFFECT OF PACKAGING ON FISH WAFER QUALITY**

The effect of the two types of packaging employed in the present study on the nutrient, microbial, biochemical and sensory qualities of fish wafers were assessed at regular intervals. It was observed that the mean values for all the parameters between ordinary and vacuum packed fish wafers showed marginal variations but this was not statistically significant, as shown by the t test values. Both types of packaging offered products that were similar in quality. The reason for this may be the moisture content
being less than 10 per cent and $aw$ being less than 0.6, irrespective of the type of packaging, which to a large extent controlled microbial proliferation.

However, the increase in the cost of production while adopting vacuum packaging, the risk of breakage of the wafers during application of vacuum pressure and the subsequent risk in the development of leakages due to the sharp hard edges of the broken wafers in the vacuum packages besides marring the appearance of the product, led to the conclusion that vacuum packaging may not be the ideal choice for packaging fish wafers when compared to ordinary packaging which is relatively cheaper with no risks involved.

Since the type of drying and the method of packaging adopted did not affect wafer quality, the results of the study were presented and discussed excluding the comparison based on drying and packaging methods.

**EVALUATION OF NUTRIENT QUALITY**

The moisture (%) content of the control and experimental groups was below 10 per cent which was within the ideal range of 8 to 14 per cent as specified by Codex Alimentarius Standard (2001) standard for crackers/wafers from marine fish.

The overall ash (g/100g) content for 1:1 and 1:1.5 groups ranged between 3 to 5 g with the 1:1.5 (WB) group having the highest value which may be because of the higher amount of fish used in the fish wafer preparation.

The carbohydrate (g/100g) content of plain starch wafers, (control), was higher (80 per cent) than the 1:1 and 1:1.5 groups where fish was used. The quantity ranged between 70 to 80 per cent. As the protein content in the form of fish increased, the carbohydrate content reduced.

The protein (g/100g) content increased with an increase in the proportion of fish used in the wafers. The protein content of the wafers prepared with bone was found to be higher than the wafers prepared without bone. This may be due to the proteins present in the fish bones that may have had a cumulative effect on the overall protein content of the fish wafer. All the wafers except the 1:1 WOB (10 per cent) group had an overall mean protein content that was above 12 per cent which according to the Codex Alimentarius
Standard for fish wafers (2001) is classified as grade I protein content. The control group had very negligible quantities of protein ranging between 0.2 to 1.07 g per 100 g.

The fat (g/100g) content in the control, 1:1 and 1:1.5 fish wafers was generally observed to be very low ranging between 0.17 to 0.73 g per 100g. However, the 1:1.5 group had a slightly higher value than 1:1 group which may be due to a higher proportion of fish being used in the preparation. The overall low fat content of the prefried wafers may be attributed to the low fat content of white bait fish that was used in the preparation.

Wafers prepared with bone had high calcium (Ca - mg/100g) and phosphorous (P - mg/100g) content which also increased along with the proportion of fish used in wafer preparation. The Ca provided by 1:1 WB and 1:1.5 WB wafers was approximately around 400 to 600 mg and 600 to 900 mg/100g. The P provided by the same categories of wafers was 220 to 270 mg and 310 to 390 mg. The Ca and P values were almost two times more in 1:1 WB and 1:1.5 WB wafers in comparison to wafers prepared without bone. The Ca:P ratio was also nearer to the ideal 2:1 ratio that has been reported for good bioavailability.

Thus the plain starch wafers were rich in carbohydrate but deficient in protein and calcium when compared to the 1:1 and 1:1.5 wafer groups.

**EVALUATION OF BIOCHEMICAL QUALITY**

The pH of the control ranged between 5.77 to 6.0. The pH of 1:1 and 1:1.5 wafers prepared with bone and without bone varied between 5.45 to 6.3. There was not much difference in the pH between wafers prepared WB and WOB. The values were within the acceptable limits for fresh fish.

TVB-N and TMA (mg/100g) were absent in the control since no fish was used in the preparation. The 1:1.5 group showed slightly higher values in comparison to the 1:1 group for both TVB-N and TMA. Besides this, the total mean for TVB-N and TMA showed that the WB wafers had slightly higher values than WOB wafers for both 1:1 and 1:1.5 groups. This may be because of the higher amount of protein present in WB wafers. However TVB-N and TMA were within the acceptable limits. A low bacterial activity
attributed to low moisture content and $a_w$ in the fish wafers led to a very slow and gradual increase in TVB-N and TMA during the storage period.

The FFA content (% oleic acid) of the control was very negligible and lower than the experimental groups. Very small quantities were present in the experimental groups, with the 1:1.5 group exhibiting marginally higher values than 1:1 group. The FFA content of the 1:1 group prepared WB and WOB fell within a range of 0.2 to 0.3 per cent whereas that of 1:1.5 group ranged between 0.4 to 0.55 approximately. However, there was no difference in FFA between the wafers prepared WB and WOB of the same experimental groups. The FFA increased with increase in the proportion of fish. However the overall low FFA content may be attributed to the low fat content of the fish.

The TBA (µ moles malonaldehyde / kg) in the control was also found in minor quantities. The TBA for 1:1 and 1:1.5 wafers was 0.22 to 0.69 and 0.29 to 0.79 µmoles malonaldehyde/kg with the 1:1.5 group recording a higher value in comparison to the 1:1 group, which is evident from their total mean values for the same. When the over all means were compared, there was no difference in the TBA content between 1:1 WB and 1:1 WOB wafers and like wise between 1:1.5 WB and 1:1.5 WOB wafers.

The pH, TVB-N, TMA, FFA and TBA increased very gradually but steadily across the storage period and were well within the acceptable limits which indicate that the product was very stable during the 90 day storage period.

EVALUATION OF MICROBIAL QUALITY

The $a_w$ of the control and experimental groups was below 0.6 which was not conducive for the growth and proliferation of microorganisms. The level was below the range specified by the Codex Alimentarius Standard (2003) for salted and dry fish (<0.75).

The TPC showed a gradual but very slow increase over the storage period with the lowest and highest recorded values of 2 and $10^2$ cfu/g. The TPC increased with an increase in the proportion of fish used in the wafers and the wafers prepared with bone had lower TPC than without bone wafers. Mold growth was also absent in all the samples.
The prefried wafers were also assessed for the presence of pathogens such as *Staphylococcus aureus*, *E.coli*, *pseudomonas* and *Salmonella* which are the most common pathogenic microorganisms present in fish based foods. In the present investigation, the control and the experimental groups answered negative for the presence of all the mentioned microorganisms.

The good microbial and biochemical stability of the fish wafers prepared in the present study can be credited to the low aw and moisture content of the products.

**EVALUATION OF SENSORY QUALITY**

It was observed that the fried wafers had a slightly higher score for sensory aspects such appearance, odour, taste and texture compared to micro waved wafers. The 1:1 fried and micro waved wafers did not show any variation in the sensory aspects based on the presence or absence of bone in their preparation. A similar trend was observed in 1:1.5 wafers as well. This showed that the presence of bone did not affect the sensory qualities. However, the 1:1 wafers scored higher for appearance, odour, taste and texture when compared to the 1:1.5 wafers for both fried and microwaved wafers.

**LINEAR EXPANSION AND OIL ABSORPTION (LE & OA)**

The LE of the fish wafers was inversely related to protein content (fish content) of the wafers and directly related to the carbohydrate content. LE and OA decreased with an increase in the proportion of the fish used in wafer preparation. Wafers prepared without bone had higher LE and OA than wafers prepared with bone. The OA was higher when the LE was higher. The 1:1 wafer group showed increased LE and OA than the 1:1.5 group. The fried wafers exhibited better LE than micro waved wafers.

**COMPARISON OF CONTROL WITH EXPERIMENTAL GROUPS**

Control Vs 1:1 WB Vs 1:1 WOB wafers and Control Vs 1:1.5 WB Vs 1:1.5 WOB wafers

Nutrient quality

There was a significant difference in the moisture, ash, carbohydrate, protein, fat, calcium and phosphorus contents between the control and experimental group 1:1
prepared with and without bone. The control was found to be concentrated in carbohydrate content but deficient in other nutrients when compared to the two proportions of fish wafers. Thus plain starch wafers were deprived of nutrients whereas the fish wafer groups had significant amounts of protein, calcium and phosphorous.

**Biochemical quality**

There was no significant variation in pH between the control and experimental group 1:1 (WB & WOB). A significant difference was observed when the experimental group was compared with the control for FFA and TBA. A comparison of the 1:1 WB with the 1:1 WOB group, showed that the TVBN and TMA were significantly different. Similar observations were made in 1:1.5 (WB & WOB) group. But the values for all these parameters were within the acceptable range throughout the study period.

**Microbial quality**

There was a significant difference in the \( a_w \) and TPC between control & 1:1 (WB & WOB) and control & 1:1.5 (WB & WOB) groups. However, they were within acceptable limits throughout the storage period.

**Sensory quality**

A comparison of the fried control and 1:1 (WB & WOB) waffer group, showed no significant difference in the appearance, texture and OAC. A comparison of micro waved wafers of the same category showed that, the aspects appearance, texture and taste, showed no significant differences. The differences that were observed for odour and taste were between control and experimental group 1:1 (WB & WOB) and not between 1:1 WB and 1:1 WOB groups. This proves that the presence of bone did not affect the sensory aspects of 1:1 wafer group irrespective of whether they were fried or micro waved. The statistically significant difference in odour aspect observed in both the fried and micro waved wafers were not off odours but the characteristic fish aroma as expressed by the panelists.

The 1:1.5 fried and micro waved wafers (WB & WOB) showed a statistically significant difference in all sensory aspects between the control and the experimental
group 1:1.5 (WB & WOB). However for odour, texture, taste and OAC, no statistically significant differences were observed between 1:1.5 WB & 1:1.5 WOB groups, though a difference was prominent between these groups and the control. This also supports the fact that the presence of bone did not affect the sensory aspects of the 1:1.5 wafers irrespective of them being fried or micro waved.

A three way ANOVA to find out the overall acceptability of the fish wafers based on comparisons made between proportion (1:1 Vs 1:1.5), wafer type (control Vs WB Vs WOB) and method of cooking adopted (frying Vs microwaving) showed that when all the three parameters, were taken together as one unit for comparison, all categories of fish wafers showed similar OAC.

Therefore, the type of cooking method that can be adopted prior to fish wafer consumption may not only be a subject of preference but may also take other aspects like the age, physical condition, health condition, activity level etc., of the individual into consideration.

**Linear expansion and oil absorption**

There was a significant difference in LE between fried and micro waved wafers. The 1:1 wafers had better LE in comparison to 1:1.5 wafers. Fried wafers exhibited higher LE than micro waved wafers. The LE was maximum in the control (plain starch wafers) followed by the 1:1 WOB, 1:1 WB, 1:1.5 WOB and 1:1.5 WB wafer group for both methods of cooking.

Oil absorption decreased with an increase in the proportion of fish used in the preparation. Wafers prepared with bone had lower OA than wafers prepared without bone. Control exhibited highest oil absorption due to its high starch content.

Thus LE and OA were influenced by the presence of bone and the quantity of fish used in the preparation, however, the sensory quality was not affected by the same.
EFFECT OF DURATION OF STORAGE

Nutrient content

Duration of storage did not affect the carbohydrate, calcium and phosphorous content. Nevertheless, significant differences were found in the moisture (control and 1:1 WOB), protein (1:1.5 WB and WOB) and fat values (1:1 WOB and 1:1.5 WB). Though differences in fat and protein were expected, they did not show any sign of deterioration as evidenced by their TVBN and TMA values which were within the acceptable range.

Biochemical quality

The control did not show any statistically significant change in pH during the storage period. However, changes were observed in the FFA and TBA values. Experimental groups 1:1 (WB & WOB) and 1:1.5 (WB & WOB) showed very significant changes in the pH (except 1:1 WB), TVBN, TMA, FFA and TBA. These values increased with increase in storage time. But the increase was very slow and gradual and well within acceptable limits for fish products. Therefore the products were stable during the 90 day storage period. This may be attributed to the low moisture and aw of the wafers.

Microbial quality

The duration of storage did not affect the aw of all samples. The TPC was linearly related to the length of storage period, but the increase was very slow and steady and was well within the acceptable limits. This was once again owing to the low aw.

Sensory quality

Appearance and odour of the 1:1.5 (WB & WOB) group was influenced by the duration of storage. However, this was not due to the deterioration fat and protein but was due to the natural taste of fish that was more distinct in the 1:1.5 proportion. This is supported by the TVBN, TMA, FFA and TBA values of the fish wafers which were well within the acceptable limits throughout the storage period.
Linear expansion and oil absorption

The duration of storage did not affect LE of control, 1:1 (WB & WOB) and 1:1.5 (WB & WOB) subjected to both frying and micro waving. A significant difference in OA was observed in 1:1 WB, 1:1 WOB and 1:1.5 WOB wafers. The rest of the groups did not show any difference.

There was a significant difference in LE and OA when comparisons were made between 1:1 WB with 1:1.5 WB and between 1:1 WOB with 1:1.5 WOB. The results further confirmed that the proportion of fish and the presence of bones in fish wafer preparation influenced LE and OA.

Based on the results of the nutrient, biochemical, microbial and sensory evaluations that were done periodically, it can be concluded that the 1:1 and 1:1.5 fish wafers prepared with and without bone had excellent keeping quality for a period of 90 days.

INVITRO BIOAVAILABILITY OF CALCIUM AND PHOSPHOROUS FROM FISH WAFERS

The invitro bioavailability of Ca and P from fish wafers (1:1 and 1:1.5) prepared with bone was studied using the everted sac model as given by El-Gorab et al. (1975). The results showed that the bioavailability of Ca and P for both fried and micro waved fish wafers were above 90 per cent and were comparable to that of milk powder. The bioavailability of Ca and P from 1:1.5 wafers was higher than the 1:1 wafers for both fried and micro waved cooking. The high bioavailability of Ca and P from fish wafers prepared with bone may be attributed to the ideal Ca:P ratio of 2:1 that was observed in all samples.

Though the Ca and P bioavailability from plain wafers was high, the calcium and phosphorous content of these wafers was very low compared to that of fish wafers prepared with and without the incorporation of bones.
POPULARIZATION OF WHITE BAIT FISH WAFER PROCESSING AMONG WOMEN ENTREPRENEURS

Demonstrations conducted for women entrepreneurs in order to sensitize them on the scope of white bait fish wafer manufacture as a profitable business proposal at home level was well accepted by all the attendents. Fifty percent of them expressed that they would consider pursuing this as a business. They also unanimously agreed that this was a healthy, tasty and nutritious snack that could replace the plain starch Vadaams that are deprived of nutrients.

IMPLICATIONS OF THE STUDY

The results of the present study imply the following

1. White bait fish wafers prepared with bone are not only rich in protein but also in highly bioavailable Ca and P compared to plain tapioca starch wafers which are deficient in protein, calcium and phosphorus. Since the bioavailability of Ca and P is comparable to milk, white bait fish wafers prepared with the incorporation of bones may be used as a ready-to-cook snack or as a meal accompaniment in food based approaches to help improve the protein and calcium intake of all ages, especially amongst populations where milk intake is low, and among individuals who have milk allergy and lactose intolerance.

2. Another important observation was that the calcium and phosphorous content in 100 g of 1:1 white bait fish wafers prepared with bone is sufficient enough to meet the recommended dietary allowances for Indians (RDA) of all age groups.

3. White bait fish wafers prepared with bone can be a suitable alternative for plain tapioca starch wafers or ‘South Indian Vadaams’. The ideal proportion of starch to fish is however related to personal preferences. Though the biochemical and microbial quality of both proportions were good, the higher amount of protein, calcium and phosphorous coupled with the increased bioavailability from 1:1.5 group makes it superior to 1:1 group. However, taking the sensory aspects into account, 1:1 was better preferred since the fish aroma was less pronounced than in the 1:1.5 wafers.
4. The method of cooking that may be adopted for preparing fish wafers, depends on personal choice, physical activity level, age and health requirements of the consumer. Nevertheless, puffing it in the micro wave would be a healthier option.

5. According to the present study, the keeping quality of the product for 1:1 and 1:1.5 was very good. The product was very stable for 90 days without the addition of any synthetic preservatives. The biochemical, microbial and physical stability of this product may be attributed to the low $aw$ and moisture content. Therefore, it is certainly a healthy substitute and a more natural way to cure deficiencies when compared to the present trend among populations of resorting to over the counter supplements, to tackle nutrient deficiencies in forms other than food, in spite of being fully aware that the latter pose a greater risk for toxicity.

6. White bait fish is ideal for the preparation of wafers since they are low in fat and are therefore less susceptible to lipid oxidation during storage. Besides this the bones are very fine and can be cooked and blended very easily into a fine paste to be added to the fish wafer gel. Since no difference was observed in sensory aspects between the wafers prepared with bone and without bone, resorting to the method of incorporating white bait with bone in fish wafer preparation is therefore strongly recommended.

7. Fish wafer processing has the potential for a rewarding business venture especially among women who are willing to make it a livelihood.

8. Since the product was not affected by the drying and packaging methods employed in this study, entrepreneurs who are willing to take this up as a business, may adopt different methods for reducing cost of production, such using cost effective material in constructing the solar tent and by adopting ordinary packaging instead of vacuum packaging.

9. Government can consider introducing fish wafers in mid day meals as a part of ‘fish in food based strategies’ to combat protein and calcium deficiency among children. This can indirectly provide business opportunities for women entrepreneurs.
10. Last but not least, fish wafer processing is an innovative method of utilizing white bait fish during seasons of surplus, instead of just salting and drying them which is what is practiced largely.

**LIMITATIONS OF THE STUDY**

1. Though, vacuum packaging had the advantage of utilizing less space for storage, the cost involved in making the custom made vacuum pouches to resist wear and tear while packaging fish wafers was high, which added to the production cost.

2. Specific standards for fish crackers/wafers (Codex Alimentarius Standard, 2001) were available only for a few parameters used in this study. For eg., moisture and protein. For the other parameters, suitable standards (ISI, PNS) available for alternative fish products (for eg., edible fish powder and dried fish) was used for comparison. There is an urgent need to set standards for all the parameters used in this study for evaluating fish wafers as these are routine analysis that are done for the same.

3. Since this fish wafer preparation was intended for all age groups, the sensory evaluation should have been conducted among males and females of all age groups. But this could not be accomplished in the present study due to financial and time constraints.

**RECOMMENDATIONS FOR FURTHER RESEARCH**

1. The quality of white bait fish wafers may be studied for a period that extends beyond 90 days.

2. White bait fish wafer preparation has all the prospective ingredients needed for the formulation of fish-based homogenized weaning foods. This area needs more research.

3. Less expensive and eco friendly packaging materials can be used for packaging and the product quality can be studied.
4. Other small bony fish that are under utilized may be used in formulating fish wafers with bone.

5. Fish wafers may be made in attractive shapes using extruder technology when considering fish wafer manufacture on a larger scale.

In the present work, keeping the health perspective in mind, white bait fish with bones were used in wafer preparation. Though the Codex Alimentarius Standard (2001) specifies that the presence of fish bones as small as 3 mm diameter and 5mm length will affect wafer quality, a bold attempt was made in the present study to include the bones of white bait fish in fish wafer preparation with the sole objective of enhancing the protein, calcium and phosphorous content of plain starch wafers.

This new concept was welcomed and appreciated by all, when the researcher secured the third place at a poster presentation on ‘Value addition of cassava starch using small fish (Stolephorous spp) with bones in the preparation of a nutritious expanded snack for all ages’ at a National conference on ‘Agro Food Processing Technologies on 4th November 2011 organised by the Department of Food Science and Technology, Pondicherry University, India. This was also published in the book ‘Recent trends in Agro Food Processing Technologies ‘with ISBN no 81-87299-57-6 (Annexure 3).

The researcher made another poster presentation highlighting on the bioavailability of calcium and phosphorous from fish wafers prepared with bones entitled ‘Value addition of tapioca starch wafers with white bait (Stolephorous spp) - an excellent source of highly bioavailable protein, calcium and phosphorus’, at an International work shop on ‘Strategies in Value addition and Aspects pertaining to Dairy and Food Industry’ organised by the Department of Dairy science, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, on March 12th 2012, where white bait fish wafer prepared with bone received favourable responses.

This study was therefore an earnest initiative to include this highly bioavailable calcium from fish bone that is otherwise avoided, in a fish wafer formulation. This effort could be just the first step in paving the way for a new arena of research in the development of fish wafer formulations that are not only rich in protein but also in valuable minerals such as calcium and phosphorus.