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Does drying preserve the nutritional quality of small freshwater fish without excessive concentrations of heavy metals?

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ABSTRACT

The potential of drying to preserve the nutritional quality of a small freshwater fish *Henicorhynchus siamensis* was assessed. Drying time to reach moisture content and water activity of 10 g/ 100g and 0.65 ranged from 55 h at 50 °C to 20 h at 80 °C, respectively. Dried fish powder is rich in macronutrients (protein, lipid and ash) and essential minerals (calcium, phosphorus, iron and zinc) due to water removal and despite lipid loss. It is still rich in polyunsaturated fatty acids although docosahexaenoic acid was reduced except at 60°C. Vitamin A was rapidly degraded and manganese was concentrated at high level. However, mean score for the nutritional adequacy of the 15 nutrients (SAIN) and score of nutrients to limit (LIM) show that the fish powder can be used as a food ingredient for example in the formulation of fish snack or instant soup. With the abundance of *Henicorhynchus siamensis*, dried fish powder from this species could contribute to food security in Cambodia, especially vulnerable people in rural areas.

Keywords: Tonle Sap Lake, nutritional profiling, micronutrients.

1. Introduction

Most low-income developing countries face malnutrition, particularly protein and micronutrient deficiencies, and the people most affected are mainly children and women (Roos et al., 2007b; Santos et al., 2017). In poor rural and urban areas, limited dietary diversity leads to long-term micronutrient deficiencies (Roos et al., 2007a). The most common micronutrient deficiencies among low-income Cambodians are related to low dietary intakes of vitamin A, iron, zinc and calcium (Roos et al., 2007c). The consumption of small fish species eaten whole (i.e. with head, bones and, in some cases, guts) could contribute significantly to the protein and micronutrients requirements of populations (Abbey et al., 2017).

In Cambodia, *Henicorhynchus siamensis* is a dominant small fish species in the annual fish catch from Tonle Sap Lake and is mostly found in southern areas of the lake (Chan et al., 2020). Due to its affordability and abundance during the peak catchment period from November to February, it is consumed daily and also processed for the production of fermented fish, fish sauce and dried fish eventually reduced in powder for children. Due to its small size, the whole body of *Henicorhynchus siamensis* is consumed. Its nutritional profile is interesting because it has a balanced composition (proteins, lipids, minerals), is rich in omega-3 fatty acids and essential micronutrients (iron, zinc, vitamin A) and is low in heavy metals (Roos et al., 2007a; Sroy et al., 2021a).

Although fish shows a good nutritional quality, fresh fish contains up to 80% of water and is thus susceptible to spoilage with a short storage life (Guizani et al., 2008). It is estimated that 35% of the fish harvest is either lost and wasted every year (FAO, 2022). Fish spoils rapidly after fishing especially in hot and warm conditions.

Drying is a common and popular method for fish processing and preservation by inhibiting microbial growth, inactivating enzymatic and chemical reactions due to the removal of moisture. Adding value to fish by drying is a promising transformation for the near future as it would contribute to nutritional and food security at an affordable price (Mohod et al., 2014). Dried fish is considered a good source of many micronutrients of significance such as essential minerals and might contribute to meet the nutritional needs of poor, vulnerable groups, particularly in area with limited dietary diversity (Abbey et al., 2017). Dried fish has many advantages in commerce, such as storage ability at ambient temperature, ease of handling, low distribution costs, little space needed for storage and easiness in mixing with other ingredients (Abbey et al., 2017; Shaviklo et al., 2010). However, the nutritive value of fish may be affected by processing. Most of the researches carried out on this topic have assessed the impact of domestic cooking on some fish nutrients and vitamin losses have been particularly highlighted (Sobral et al., 2018). However, there is little information on the kinetics of vitamin A and fatty acid degradation during fish drying; the effect of drying temperature on nutrients degradation is poorly studied and the literature on small fish processed whole is scarce. Moreover, fish may be a source of heavy metals, which may be inevitably increased as the water is removed during drying.

The aim of this study was to determine the evolution of macronutrients and micronutrients during drying of *Henicorhynchus siamensis*. Particular attention was paid to the degradation of vitamin A, the modifications of fatty acids and the concentration of heavy metals hazardous to human health.

2 Materials and methods

2.1 Chemicals

Solvents, reagents and pure standards (retinol, fatty acid methyl esters, minerals, heavy metals) were obtained from Sigma-Aldrich (Saint Quentin Fallavier, France) and 3,4-didehydroretinol was obtained from Santa Cruz biotechnology (Texas, United States).

2.2 Raw material

Fish was bought directly from the fishermen during the fishing hours at Chhnork Tru (12°30'55.03" N, 104°27'28.29" E; 12°30'37.46" N, 104°26'54.69" E and 12°30'44.91" N, 104°27'10.74" E) in Kompong Chhnang province near the Tonle Sap Lake in Cambodia. Approximately 20 kilograms of *Henicorhynchus siamensis* was collected during dry season in January 2021. Fish were placed in a Ziploc bag to prevent any contamination and they were kept in polystyrene boxes with crushed ice and carried to laboratory. Fish were stored at -18 °C until processing. They were then thawed at 4 °C for 12 h before use.

2.3 Fish processing and sampling

Fish were cleaned with tap water and blotted dry with absorbent paper. The average weight of the individual fish was 21.9 ± 7.3 g with an average length of 12.2 ± 1.3 cm (measured on 15 fish).

Fish were dried in a pilot dryer described in Raffray et al. (2015) at a relative humidity of 50% and an air speed of 1.8 m/s. Drying kinetics were obtained at four temperatures (50 °C, 60 °C, 70 °C and 80 °C). Fish were dried until reaching a moisture content in wet basis (wb) of at least less than 10 g/100 g. Three repetitions were conducted for each temperature. For each replicate, 50 fish (about 1 kg) were arranged on three trays in the dryer perpendicular to the airflow. During processing, temperature and relative humidity were recorded every minute. The weight

of each tray was measured automatically each 15 min during the first 10 h of drying and each 30 min after and weight loss kinetics was determined. Drying kinetics was calculated from the experimental raw material moisture content and the weight of fish. Sampling was performed at seven sampling times along each drying and at final time (Table 1). At each sampling time, five fish were randomly selected. For raw material analysis, samples made from five fish were randomly taken for the first, middle and last drying experiment.

Table 1. Drying condition and sampling times

Temperature (°C)	Relative humidity (%)	Air velocity (m/s)	Sampling times (h)
50	50	1.8	0.25; 0.5; 1; 1.5; 2; 3; 24; 55.84
60	50	1.8	0.25; 0.5; 1; 1.5; 2; 3; 8.5; 30.82
70	50	1.8	0.25; 0.5; 0.75; 1; 1.5; 2; 8.5; 27.32
80	50	1.8	0.25; 0.5; 0.75; 1; 1.5; 2; 8.5; 22.82

2.4 Sample preparation for analysis

Fish was ground using the Original Grinder Moulinex (AR110510, France) and it was stored at -18 °C until further analysis. Analyses were performed in duplicate.

2.5 Determination of pH

Five g of sample was homogenized with distilled water and pH of fish was measured by using a pH meter (Hanna, pH 213, Italy), calibrated using standard solution of pH 4 and 7.

2.6 Determination of water activity

Water activity was determined at 25 ± 1 °C using a dew point hygrometer (Aqualab – Series 3, Decagon Devices Inc., USA), checked using distilled water and sodium chloride saturated solutions. The uncertainty of measurement is ± 0.003 .

2.7 Proximate composition analysis

Moisture content of the sample was measured by drying 5 g of sample at 105 °C for 24 h in an oven (Mettler, UF B 500, Germany) (AOAC, 1990).

The ash content was determined by incinerating 5 g of sample placed in a porcelain crucible in a furnace at 550 °C for 4 h (Nabertherm muffle furnace, L 5/11/B410, Germany) (AOAC, 1990).

Total nitrogen content was determined by using the Dumas method with an element analyzer (FP528-LECO Trumac N, EVISA, Europe) (Edeling, 1968). The crude protein content was calculated using a conversion factor of 6.25. The method is validated using a reference sample. The uncertainty of the measurement is 2.5% for nitrogen content.

The total lipid content was determined using the Folch method in chloroform/methanol (2:1, v/v) with slight modifications as previously described (Sroy et al., 2021a).

2.8 Fatty acids analysis

Fatty acid methyl esters (FAME) were prepared from the extracted lipids under alkaline and acid hydrolysis and fatty acids was separated and quantified using gas chromatography (Varian AC3800, England) equipped with a flame ionization detector as described before (Sroy et al., 2021a). Peaks corresponding to FAME were identified by comparing their retention times with

those of standard mixture. The profiles of fatty acids were presented as a percentage of total FAME according to their relative peak areas.

2.9 Determination of vitamin A content

Total vitamin A (retinol and 3,4-didehydroretinol) was extracted by saponification with KOH 50% (w/v) at 80 °C for 43 min and then extraction with ethanol/hexane (4:3, v/v) (Sroy et al., 2021b). Analysis was performed by HPLC (Agilent System 1200 series, Massy, France) with a UV – visible photodiode array detector (Agilent Technologies 1200 series) at 325 nm. External calibration was realized weekly with standard solutions of the pure chemicals in acetone in the range of 0.5 to 25 mg/L. Total vitamin A (retinol and 3,4-didehydroretinol) activity was expressed in retinol equivalents (RE). The method was validated using a reference sample. The repeatability of vitamin A analysis is 7.4%.

2.10 Determination of mineral contents

Minerals were analyzed by inductively coupled plasma mass spectrometry (Thermo Elemental, X-Series, Germany) after digestion with HNO₃:H₂O₂ (65:35, v/v) in a MARS Xpress microwave system (CEM corporation, Mathews, NC, France) as described before (Sroy et al., 2021a). The limits of detections were in µg per g: 71.0 for calcium (Ca); 6.0 for potassium (K); 3.0 for magnesium (Mg); 7.0 for sodium (Na); 25 for phosphors (P); 15.1 for iron (Fe); 5.6 for zinc (Zn); 1.7 for aluminum (Al); 0.005 for cadmium (Cd); 0.1 for cobalt (Co); 0.03 for chromium (Cr); 0.2 for copper (Cu); 0.1 for manganese (Mn); 0.4 for nickel (Ni); 0.8 for lead (Pb) and 0.003 for total arsenic (tAs). The limit of quantification were in µg per g: 321.0 for Ca; 39.0 for K; 14.0 for Mg; 35.0 for Na; 97 for P; 61.9 for Fe; 30.7 for Zn; 7.7 for Al; 0.021 for Cd; 0.36 for Co; 0.18 for Cr; 2.11 for Cu; 0.4 for Mn; 1.85 for Ni; 8.82 for Pb and 0.031 for tAs.

Total mercury (tHg) was quantified as described before (Sroy et al., 2021a) with a mercury analyzer (Leco, France) after combustion of samples at 750 °C. The limit of detection was 0.005 µg per g for tHg. The limit of quantification were 0.02 µg per g for tHg. The methods were validated using reference samples. The uncertainty of the measurement is about 10%.

2.11 Determination of methyl mercury and inorganic arsenic contents

Methylmercury (MeHg) and inorganic arsenic (iAs) were estimated from the tHg and tAs contents. In fish, MeHg and iAs account for 92% of tHg and 10% of tAs, respectively (Kelly et al., 2018).

2.12 Calculation of energy value

The total energy values were calculated by adding the energy provided by proteins and fats using their respective equivalents in kcal (1g of protein corresponds to 4 Kcal, 1g of fat corresponds to 9 Kcal). The energy values were expressed in kcal per 100 g.

2.13 Vitamin A degradation kinetics modeling

A second order kinetics model was used to describe the degradation of vitamin A during drying as described by equation 1.

$$\frac{d\text{vitA}}{dt} = -k \text{vitA}^2 \quad (1)$$

Where t is the drying time (h), vitA is vitamin A content in dry basis (RE µg/g) and k is the reaction rate constant (g/µg.h).

The estimation of kinetics parameters was performed using a non-linear regression based on the minimization of the sum of the squared residuals (SSr) between the experimental data set and prediction (equation 2).

$$SSr = \sum_{i=1}^n (y_i - \hat{y}_i)^2 \quad (2)$$

Where y_i is the experimental value of vitamin A content and \hat{y}_i is the predicted value of y_i .

The coefficient of determination R^2 was calculated using equation 3.

$$R^2 = 1 - \frac{SSr}{SSt} \quad (3)$$

Where SSt is the total variance between the experimental data and the mean of the data \bar{y} (equation 4).

$$SSt = \sum_{i=1}^n (y_i - \bar{y})^2 \quad (4)$$

The rate constants were assumed to vary with temperature according to the Arrhenius equation (equation 5).

$$k = k_{ref} e^{-\left(\frac{E_a}{RT} \times \left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right)} \quad (5)$$

Where k_{ref} is the rate constant (g/ μ g. h) at the reference temperature (T_{ref} chosen in the middle of the studied temperature (338K), E_a the activation energy (J/mol), T is temperature (K) and R is the gas constant (8.314 J/K/mol).

The kinetics parameters were identified using Excel solver and the uncertainty of parameters was calculated with the macro SolverAid (de Levie, 1999).

2.14 Calculation of SAIN and LIM scores

The SAIN and LIM scores were developed to describe the nutritional profile of food by taking into account the positive and negative nutrients (Darmon et al., 2007). SAIN and LIM nutritional profiling allows the comparison of the nutritional composition of foods and the impact of formulation and processing on the overall nutritional quality to be visualized. These indicators SAIN and LIM were created to evaluate the quality of raw materials but also their derived-products through processing (cooking, drying, fermentation) or formulation. They were calculated in raw fish and three food products in which dried fish powder can be used (snack, instant fish soup and NumTrey ready to use supplementary food (RUSF)).

The SAIN score (equation 6) corresponds to a nutrient density calculated by the arithmetic mean of the percentage adequacy for 15 positive nutrients to assess a global evaluation of the nutritional quality (Darmon et al., 2007). The positive nutrients included in the calculation are protein, fiber, vitamins (C, E, B1, B2, B6, B9), calcium, iron, magnesium, zinc, potassium, alpha linolenic acid and DHA.

$$SAIN = \frac{\sum_{i=1}^{15} \frac{Nut_i}{RV_i}}{15} \times 100 \quad (6)$$

where Nut_i is the quantity (g, mg or μ g) of positive nutrient i in 100 g of food, RV_i is the daily recommended value for nutrient i and E is the energy (in kcal) in 100 g of food. Six vitamins content of freshwater fish (C, E, B1, B2, B6, B9) were obtained from a literature review (Rehbein & Oehlenschläger, 2009).

The LIM score calculates the mean content of disqualifying nutrients in 100 g of foods (equation 7).

$$\text{LIM} = \frac{\sum_{j=1}^3 \frac{\text{Nut}_j}{\text{MRV}_j}}{3} \times 100 \quad (7)$$

where Nut_j is the quantity (g, mg or μg) of disqualifying nutrient j in 100 g of food and MRV_j is the daily maximal recommended value for nutrient j . The LIM3 was calculated based on the saturated fatty acids (SFA) content, sodium and added sugars of the food (Darmon et al., 2007). As it is common to add salt, saturated fats or sugar to formulated products, these nutrients are considered in the calculation of the LIM indicator. Furthermore, they are also responsible for chronic diseases according to the results of epidemiological surveys in several countries. Added sugars were equal to zero in raw material as the fishes were not submitted to any formulation. The number of nutrients involved in calculating the LIM score can be adapted according to the type of food and disqualifying nutrients. A LIM11 was calculated by taking into account in addition eight heavy metals (identified in the fish species of this study (Cr, Cu, Mn, Ni, tAs, Cd, tHg and Pb) and their maximum permissible level (MPL) (Sroy et al., 2021a). The snack is formulated with corn (40%), rice (40%), roasted Bengal gram dal (10%) and dried fish powder (10%) (Kuna et al., 2013). Instant fish soup is made from fish powder (10%), corn flour (34%), tomato powder (17.5%), cauliflower powder (17.5%), salt (5%), sugar (5%), spices powder (5%) and salt (1%) (Rahman et al., 2012). NumTrey RUSF is formulated with dried fish powder (5.9%), mung bean (9.6%), rice (4.2%), soy beans (12.2%), sugar (10.3%), maltodextrin (9.3%), canola oil (3.7%), palm vegetable fat (14%), dried coconut (1.5%), rice bran (2.2%), vitamin and mineral mix (0.9%), rice flour (9%), duck egg (2.5%), refined sugar (7.2%), coconut (7.2%), vanilla (0.1%) and oil for cooking (0.4%) (Sigh et al., 2018). The nutritional profile of all the other ingredients than dried fish powder used in snack, instant fish soup and NumTrey RUSF were obtained from the food composition table of ANSES (French Agency for Food, Environmental and Occupational Health & Safety) (Martin and Issanchou, 2019).

This profiling allows classifying the food in four groups:

- SAIN > 5 and LIM < 7: food recommended for health
- SAIN < 5 and LIM < 7: neutral food
- SAIN > 5 and LIM > 7: food to consume in small quantity
- SAIN < 5 and LIM > 7: food to avoid or limit.

2.15 Statistical analysis

Data were analyzed using one-way analysis of variance (one-way ANOVA) with Statgraphics plus 5.1 (Virginia, USA). Significance was accepted at probability $P \leq 0.05$. Comparison of means was performed using Tukey test.

3. Results

3.1 Drying kinetics, water activity, pH and proximate composition

Drying kinetics are shown in Figure 1A and the kinetics of weight loss, water activity in Figure 1B and 1C respectively. The physico-chemical characteristics of raw material and dried fish powders are shown in Table 2. Obviously, it can be seen from drying and weight loss kinetics that higher drying temperature speeded up the drying process, and thus shortened the drying time. The time required to reach the desired moisture content of 10 g/100 g (wb) at 50 °C, 60 °C, 70 °C and 80 °C were about 55, 28, 24 and 20 h, respectively. Interestingly, the duration to reach this moisture content was divided by almost two at 60 °C compared to 50 °C. In comparison, increasing the temperature to 70 °C and 80 °C was not saving much time (about 4 h each time temperature was increased of 10 °C). The moisture sharply decreased at the initial drying stages and subsequently slowly reduced as the drying proceeded. At the end of drying, the weight loss was 62.3 – 65.4 g/100 g. The water activity decreased from 0.99 to about 0.65

at the end of drying. Its evolution at 60 °C, 70 °C and 80 °C was quite close while the decrease was slowed down at 50 °C.

The pH values in raw material and dried fish powders varied from 6.2 to 6.6 and pH values slightly decreased after drying but there was not significant difference compared to raw material. pH of fish powders dried at 50 °C was significantly lower than pH of fish powders dried at 80 °C.

The protein content of the dried fish powders increased by more than 2.5 times compared to the raw material. This is due to the removal of water as there was no variation in the protein content expressed on a dry basis. In the same way, the higher ash content of *Henicorhynchus siamensis* powder compared to fresh fish was due to the substantial loss of water as shown by the non-variation of ash content when expressed on dry basis.

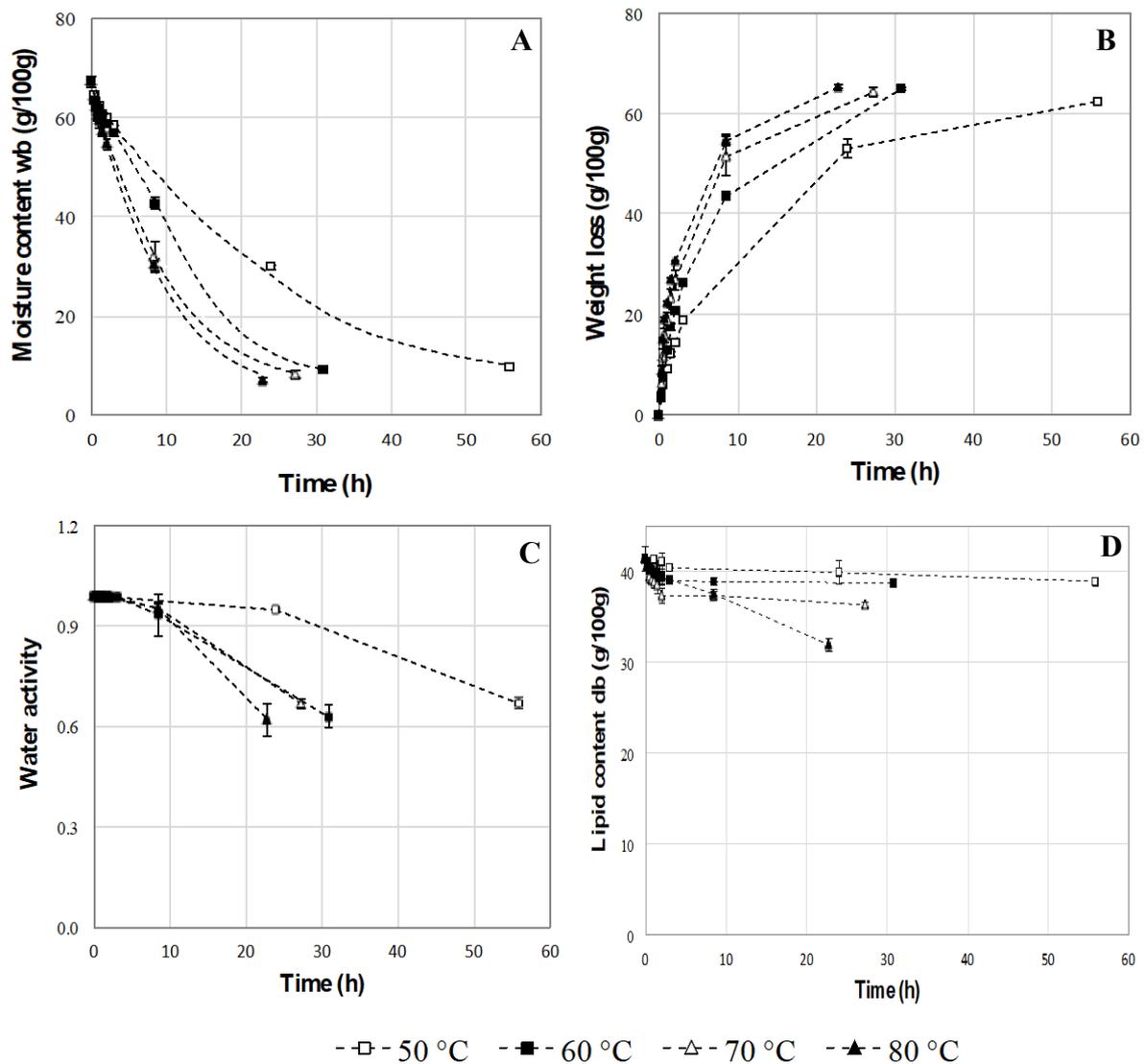


Figure 1. Moisture content (A), weight loss (B), water activity (C) and lipid content in dry basis (db) (D) of fish during drying at four different temperatures (50 °C, 60 °C, 70 °C and 80 °C). Errors bars represent standard deviations (n=3). In (A), lines represent calculated drying kinetics.

The lipid content of the dried fish powders has increased compared to the raw material, but unlike the other nutrients, the increase was not as great as would be expected due to the loss of moisture. In fact it significantly decreased when expressed in dry basis. The decrease was all along the drying (Figure 1D) and was more pronounced at 80 °C and moderate at 70 °C

compared to the other temperatures.

The energy values of the raw material were 184.3 Kcal/100 g, while those of the fish powders increased twice due to the concentration of lipids and proteins by drying. The energy value of the dried fish powder produced at 80°C was significantly lower than the others due to its lower lipid content.

3.2 Fatty acids

The fatty acid profiles of *Henicorhynchus siamensis* and its dried powders are shown in Table 3. Fatty acids representing less than 2.0% on average are not shown. Overall, 35 fatty acids were identified and constituted approximately 90% of total fatty acids. SFA, monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) were in average 50.8%, 23.1% and 16.0% respectively. There was no significant difference in the fatty acid profile between the fish powders dried at different temperatures. However, the fatty acid profile of fresh fish and dried fish powders differed significantly in omega-3 polyunsaturated fatty acids (n-3 PUFA) and more specifically in docosahexaenoic acid (DHA). The latter was in lower proportion in the dried fish powders, except the one produced at 60 °C. Similarly, eicosapentaenoic acid (EPA) decreased slightly during drying, but this decrease was not significant. Some fatty acids (hexanoic acid, caprylic acid), not detected in raw fish were found at low levels in dried fish powders (data not shown).

Table 3. Fatty acid profiles of raw fish and dried fish powders.

Fatty acids (%)	Raw material	Temperature of drying			
		50 °C	60 °C	70 °C	80 °C
C13:0 (tridecanoic acid)	2.3±0.2	2.6±0.5	2.7±0.4	2.8±0.4	2.7±0.2
C14:0 (myristic acid)	5.7±1.0	5.7±1.0	6.2±0.3	5.8±0.6	5.4±0.4
C15:0 (pentadecylic acid)	6.9±0.7	7.5±1.2	7.5±0.5	8.2±1.0	8.0±0.8
C16:0 (palmitic acid)	19.1±0.6	18.6±0.5	19.2±0.1	19.3±0.4	19.8±0.5
C17:0 (heptadecanoic acid)	2.8±0.2	3.2±0.2	2.9±0.4	3.0±0.3	3.2±0.3
C18:0 (stearic acid)	7.0±0.7	6.6±0.3	6.7±0.3	6.8±0.2	6.9±0.1
C21:0 (heneicosanoic acid)	2.3±0.1	2.3±0.1	2.3±0.2	2.3±0.1	2.2±0.1
C16:1n-7 (palmitoleic acid)	4.6±0.6	5.3±1.6	4.9±1.2	4.9±0.7	5.0±0.8
C18:1n-9 cis (oleic acid)	15.0±1.4	14.3±0.7	14.8±0.6	14.7±1.4	15.2±0.5
C18:2n-6 (linoleic acid)	3.1±0.1	2.9±0.5	2.9±0.3	2.9±0.2	3.1±0.1
C18:3n-3 (alpha linolenic acid)	5.3±0.2	5.4±0.2	5.4±0.3	5.4±0.2	5.6±0.2
C20:5n-3 (eicosapentaenoic acid)	3.3±0.6	2.6±0.2	2.8±0.2	2.8±0.4	2.5±0.2
C22:6n-3 (docosahexaenoic acid)	4.6±0.2 ^a	3.2±0.6 ^b	3.7±0.3 ^{ab}	3.1±0.3 ^b	3.0±0.2 ^b
SFA	49.8±1.6	50.0±3.1	51.0±1.2	51.5±1.2	51.6±1.3
MUFA	22.7±2.1	22.9±2.4	23.1±1.0	22.8±1.6	23.8±1.2
PUFA	17.2±1.1	15.4±0.4	16.0±0.4	15.5±0.9	15.6±0.2
PUFA/SFA	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0
n-6 PUFA	3.9±0.1	4.1±0.6	3.9±0.3	4.0±0.4	4.2±0.2
n-3 PUFA	13.3±1.0 ^a	11.3±0.7 ^b	12.1±0.2 ^{ab}	11.5±0.7 ^b	11.4±0.2 ^b
n-6/n-3	0.3±0.0	0.4±0.1	0.3±0.0	0.3±0.0	0.4±0.0
Unidentified FA	10.2±1.4	11.7±1.6	9.9±0.4	10.2±0.8	9.0±0.8

P_≥0.050; *P<0.050; **P<0.010; ***P<0.001. Data are presented as mean ± standard deviation, expressed as percentage (%). FA, fatty acids; SFA, total saturated fatty acids; MUFA, total monounsaturated fatty acids; PUFA, total polyunsaturated fatty acids. Only mean of FA representing more than 2% is shown. EPA: eicosapentaenoic acid. DHA: docosahexaenoic acid. n-6/n-3, omega-6 (sum of C18:2n-6, C18:3n-6, C20:2n-6, C20:4n-6 and C22:2n-6) to omega-3 (sum of C18:3n-3, C20:3n-6, C20:5n-3 and C22:6n-3) ratio.

3.3 Minerals and heavy metals

The mineral contents (main and trace elements) and the heavy metals in the raw material and dried fish powders of *Henicorhynchus siamensis* are presented in Table 2. They are rich in calcium, phosphorus and potassium as they contain more than 15% of the nutrient reference values (NRV) (European Parliament and Council, 2011). Calcium was the most abundant element. Iron, zinc and manganese were the predominant trace elements. Whatever the drying temperature and whatever the element considered, minerals and heavy metals are significantly concentrated by the treatment; with the exception of aluminium, whose increase was not significant, probably due to the variability of its content in the fish, and elements present at very low levels (nickel, lead, chromium). This is due to water removal as shown by the non-variation of contents when expressed on dry basis. Only the manganese content was above the MPL.

Table 2. Final mass loss, pH, water activity, proximate composition, vitamin A, minerals and heavy metals of raw fish and dried fish powders.

Parameters	Raw material	End product			
		50 °C	60 °C	70 °C	80 °C
Mass loss (g)	NA	62.3±0.3 ^b	65.0±0.2 ^a	64.4±1.0 ^a	65.4±0.5 ^a
pH	6.6±0.0 ^{ab}	6.2±0.1 ^b	6.3±0.1 ^{ab}	6.4±0.3 ^{ab}	6.6±0.0 ^a
Water activity	0.99±0.0 ^a	0.67±0.0 ^b	0.63±0.0 ^b	0.67±0.0 ^b	0.62±0.0 ^b
Moisture wb (g/100 g)	67.3±1.1	9.7±0.3 ^a	9.2±0.7 ^a	8.3±0.9 ^{ab}	7.0±0.5 ^b
Protein wb (g/100 g)	13.9±0.8	38.9±1.8 ^a	40.8±0.8 ^a	41.3±2.0 ^a	42.0±2.9 ^a
Protein db (g/100 g)	42.4±1.3 ^a	42.0±2.2 ^a	45.1±0.9 ^a	44.7±2.3 ^a	43.9±3.0 ^a
Lipid wb (g/100 g)	13.5±0.1	35.2±0.3 ^a	35.2±0.2 ^a	33.4±0.6 ^b	29.7±0.5 ^c
Lipid db (g/100 g)	41.4±1.4 ^a	38.9±0.5 ^b	38.8±0.0 ^b	36.4±0.3 ^c	31.9±0.6 ^d
Ash wb (g/100 g)	3.6±0.1	9.9±0.7 ^a	11.1±1.4 ^a	11.2±1.2 ^a	10.6±0.4 ^a
Ash db (g/100 g)	10.9±0.4 ^a	11.0±0.8 ^a	12.2±1.5 ^a	12.2±1.3 ^a	11.3±0.5 ^a
Energy wb (Kcal/100 g)	184.3±2.0	467.9±9.7 ^a	480.9±3.9 ^a	464.3±8.9 ^a	430.6±6.7 ^b
Energy db (Kcal/100 g)	542.1±7.9 ^a	518.3±12.6 ^{ab}	529.3±3.5 ^a	506.1±8.4 ^b	462.6±7.2 ^c
Vitamin A wb (RE µg/100 g)	555.5±10.8	71.5±17.3 ^b	123.9±11.2 ^a	97.9±7.3 ^{ab}	79.9±11.3 ^b
Vitamin A db (RE µg/100 g)	1698.5±81.8 ^a	79.1±19.0 ^b	136.4±12.9 ^b	106.7±6.9 ^b	85.9±12.6 ^b
Major elements (mg/100 g)					
Ca wb	1227.3±172.1 ^b	2915.6±494.4 ^a	3127.6±346.6 ^a	3510.1±528.0 ^a	3593.5±624.0 ^a
Ca db	3325.5±410.6 ^a	3229.2±549.5 ^a	3440.7±359.1 ^a	3822.8±540.5 ^a	3862.1±659.9 ^a
K wb	249.9±11.5 ^b	653.4±35.1 ^a	686.4±34.8 ^a	691.7±56.7 ^a	700.9±25.1 ^a
K db	679.2±51.1 ^a	723.8±41.5 ^a	755.4±36.7 ^a	753.6±54.3 ^a	753.5±28.6 ^a
Mg wb	42.1±2.7 ^b	107.4±8.4 ^a	113.3±8.0 ^a	119.2±12.4 ^a	121.8±8.0 ^a
Mg db	114.2±5.7 ^a	119.0±9.5 ^a	124.7±8.0 ^a	129.8±12.2 ^a	130.9±8.2 ^a
Na wb	52.0±3.6 ^b	134.7±2.4 ^a	152.6±12.7 ^a	154.4±14.9 ^a	142.6±4.5 ^a
Na db	141.2±12.7 ^a	149.2±2.1 ^a	167.9±13.1 ^a	168.3±16.6 ^a	153.3±5.0 ^a
P wb	750.4±83.5 ^b	1852.3±235.1 ^a	1981.2±201.2 ^a	2170.5±297.0 ^a	2218.0±295.7 ^a
P db	2034.1±188.9 ^a	2051.6±261.8 ^a	2179.7±207.6 ^a	2364.0±301.4 ^a	2383.9±310.9 ^a
Trace elements (µg/100 g)					
Fe wb (MPL 10000 µg/100 g)	2370.3±601.7 ^b	4690.0±535.1 ^a	5136.7±15.3 ^a	4550.0±50.0 ^a	6116.7±1493.6 ^a
Fe db	6390.0±1325.3 ^a	5194.7±597.9 ^a	5653.6±27.1 ^a	4959.9±78.6 ^a	6572.0±1581.6 ^a
Zn wb (MPL 10000 µg/100 g)	2023.5±37.5 ^b	5530.0±348.7 ^a	5903.3±647.0 ^a	6223.3±618.5 ^a	6190.0±255.1 ^a
Zn db	5493.3±145.7 ^a	6124.2±375.3 ^a	6494.5±671.0 ^a	6785.6±706.5 ^a	6654.1±254.2 ^a

Al wb	490.0±513.2 ^a	646.7±208.4 ^a	780.0±260.6 ^a	560.0±262.3 ^a	1160.0±525.7 ^a
Al db	1290.0±1301.5 ^a	716.7±232.8 ^a	858.0±283.5 ^a	608.9±279.7 ^a	1245.8±560.6 ^a
Cd wb (MPL 100 µg/100 g)*	0.8±0.1 ^a	1.2±1.1 ^a	1.0±0.3 ^a	0.5±0.1 ^a	0.8±0.4 ^a
Cd db*	2.0±0.4 ^a	1.3±1.2 ^a	1.1±0.3 ^a	0.5±0.1 ^a	0.8±0.4 ^a
Co wb	4.8±1.5 ^b	12.3±3.2 ^a	11.3±0.6 ^a	10.7±1.2 ^a	10.0±1.0 ^a
Co db	13.0±4.4 ^a	13.7±3.6 ^a	12.5±0.7 ^a	11.6±1.3 ^a	10.7±1.0 ^a
Cr wb (MPL 5000 µg/100 g)	5.4±0.4 ^b	13.3±5.9 ^{ab}	13.3±2.3 ^{ab}	11.3±3.1 ^{ab}	16.0±3.5 ^a
Cr db	14.7±0.6 ^a	14.8±6.5 ^a	14.7±2.6 ^a	12.4±3.4 ^a	17.2±3.6 ^a
Cu wb (MPL 3000 µg/100 g)	61.5±7.9 ^b	138.3±24.0 ^a	148.3±30.0 ^a	126.0±5.2 ^a	121.3±6.4 ^a
Cu db	167.3±25.8 ^a	153.2±26.9 ^a	163.3±33.5 ^a	137.3±4.3 ^a	130.5±7.1 ^a
Mn wb (MPL 100 µg/100 g)	820.9±18.9 ^b	2243.3±341.5 ^a	2676.7±185.8 ^a	2840.0±500.3 ^a	2653.3±516.9 ^a
Mn db	2230.0±121.2 ^a	2485.4±385.8 ^a	2945.3±188.0 ^a	3095.3±545.1 ^a	2851.6±547.8 ^a
Ni wb (MPL 50-100 µg/100 g)*	34.2±50.8 ^a	7.7±4.5 ^a	5.0±2.6 ^a	9.3±2.3 ^a	13.3±12.7 ^a
Ni db*	94.3±140.2 ^a	8.5±5.0 ^a	5.5±2.9 ^a	10.2±2.4 ^a	14.4±13.8 ^a
Pb wb (MPL 50-200 µg/100 g)*	11.6±5.9 ^a	38.0±23.4 ^a	44.3±13.4 ^a	46.3±6.1 ^a	50.0±36.4 ^a
Pb db*	32.0±17.1 ^a	42.1±25.9 ^a	48.7±14.5 ^a	50.5±6.2 ^a	53.7±39.0 ^a
Arsenic and Mercury (µg/100 g)					
tAs wb (MPL 140 µg/100 g)	39.4±15.6 ^b	83.0±14.4 ^a	87.3±1.5 ^a	84.7±9.6 ^a	68.3±11.2 ^{ab}
tAs db	106.0±37.6 ^a	91.9±15.6 ^a	96.1±1.9 ^a	92.3±10.8 ^a	73.5±12.4 ^a
iAs wb	3.9±1.6 ^b	8.3±1.4 ^a	8.7±0.1 ^a	8.5±1.0 ^a	6.8±1.1 ^{ab}
iAs db	10.6±3.8 ^a	9.2±1.6 ^a	9.6±0.2 ^a	9.2±1.1 ^a	7.4±1.2 ^a
tHg wb (MPL 50 µg/100 g)	0.9±0.1 ^b	2.1±0.3 ^a	2.4±0.5 ^a	2.0±0.4 ^a	1.8±0.2 ^a
tHg db	2.4±0.2 ^a	2.3±0.3 ^a	2.6±0.5 ^a	2.2±0.4 ^a	1.9±0.2 ^a
MeHg wb	0.8±0.1 ^b	1.9±0.2 ^a	2.2±0.4 ^a	1.9±0.3 ^a	1.6±0.1 ^a
MeHg db	2.2±0.2 ^a	2.1±0.3 ^a	2.4±0.5 ^a	2.0±0.3 ^a	1.7±0.2 ^a

Data are presented as mean ± standard deviation. Contents are expressed in wet basis (wb) and dry basis (db). tAs: total arsenic. iAs: inorganic arsenic. tHg: total mercury. MeHg: methyl mercury. MPL (Maximum Permissible Level) are indicated in bracket after each element. RE: Retinol equivalent.- NA : not appropriate, *: data is lower than limit of detection. The Maximum Permissible Level (MPL) in µg per 100 g fish (wet basis) were obtained from several authors (Agusa et al., 2005; Kelly et al., 2018; Moustafa et al., 2019; Nargis et al., 2020).

3.4 Kinetics of vitamin A degradation

The vitamin A content of raw material and dried fish powders are shown in Table 2. The vitamin A contents of the dried fish powders were significantly lower than that of the raw material. The kinetics of vitamin A degradation during drying at 50 °C, 60 °C, 70 °C and 80 °C are presented orderly in Figure 2A, 2B, 2C and 2D, respectively. Vitamin A was highly degraded at the few first hours of drying. The mathematical model that best described the kinetics of vitamin A degradation in fish during drying was the second order model with correlation coefficients ranging from 0.91 to 0.96. The resulting reaction rate constants (k) were 9.1 ± 0.7 , 11.4 ± 1.1 , 10.6 ± 0.9 and $14.2 \pm 1.6 \times 10^{-2} \mu\text{g/g} \cdot \text{h}$ at 50 °C, 60 °C, 70 °C and 80 °C, respectively. Degradation rate constant were similar at 50 °C, 60 °C and 70 °C and higher at 80 °C which is the temperature known in the literature for generating complete and rapid degradation of vitamin A (Ribeiro et al., 2020). Vitamin A degradation followed the Arrhenius temperature-dependency pattern ($R^2 = 0.98$). The rate constant at the reference temperature of 65 °C (k_{ref}) was $11.7 \pm 0.5 \times 10^{-2} \mu\text{g/g} \cdot \text{h}$ and the activation energy (E_a) was $11.1 \pm 3.6 \text{ kJ/mol}$.

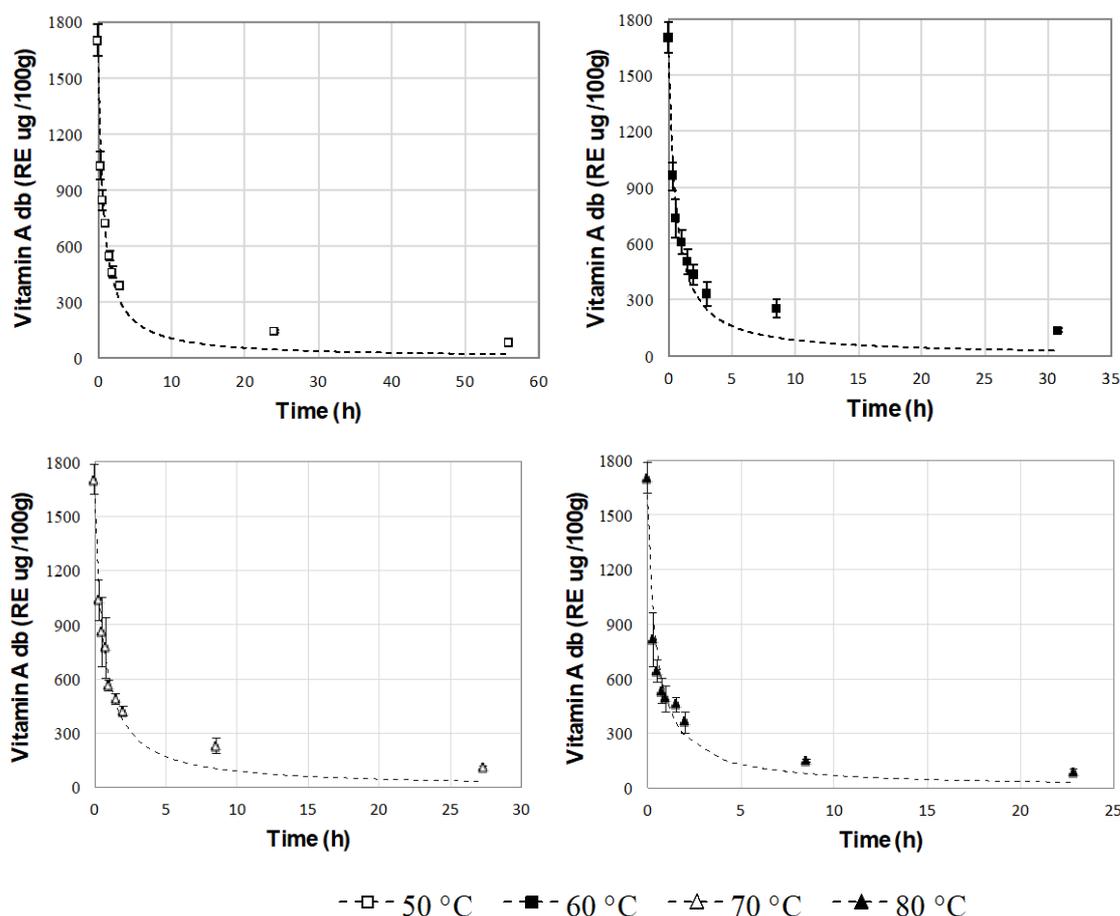


Figure 2. Vitamin A content in dry basis (db) of fish during drying at four different temperatures (50 °C, 60 °C, 70 °C and 80 °C). Lines represent modeled data. Errors bars represent standard deviations (n=3).

3.5 SAIN and LIM nutritional scores

SAIN and LIM scores of raw material, dried fish powder mixed in snack, instant fish soup and NumTrey RUSF are shown in Figure 3. The average value of SAIN, LIM3 and LIM11 of raw material were 33.9, 9.6 and 13.1, respectively. Fresh *Henicorhynchus siamensis* is classified as food to be consumed in small quantity. When dried fish powder was used in snack and instant fish soup, SAIN and LIM3 were classified in recommended food to consume, while SAIN and

LIM11 of snack and instant fish soup were classed in food consumed in small quantity. Interestingly, LIM3 and LIM11 of NumTrey RUSF were higher than in snack and instant fish soup because of their dense nutritional profile in macronutrients.

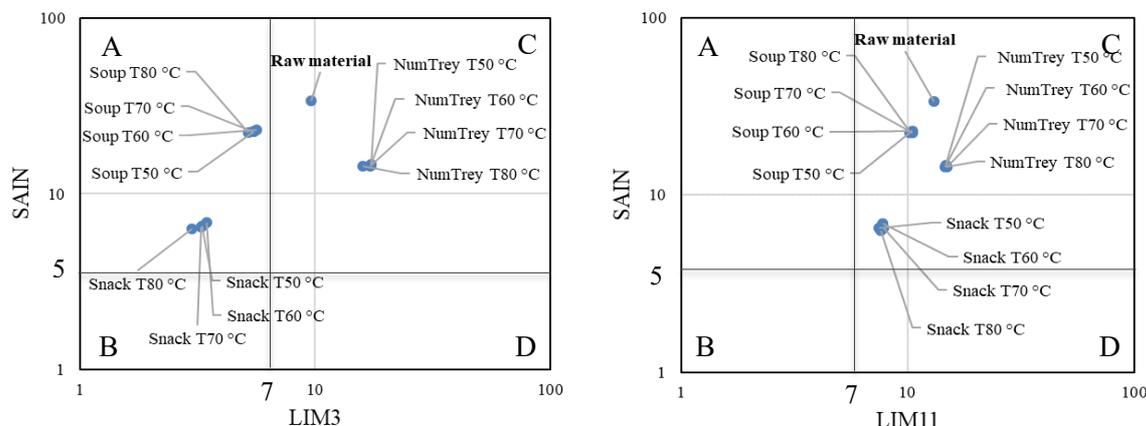


Figure 3. Classification of *Henicorhynchus siamensis* according to SAIN and LIM scores: (A) SAIN LIM 3 of raw material, dried fish powder mixed in snack, soup and NumTrey; (B) SAIN LIM 11 of raw material, dried fish powder mix in snack, soup and NumTrey. Quadrant A food recommended for health; quadrant B : neutral food; quadrant C : food to consume in small quantity; quadrant D : food to avoid or limit.

4. Discussion

Moisture content and more specifically water activity in a dried fish product is very critical because it gives information on the safety and stability of the product with respect to microbial growth, chemical and biochemical reactions and physical properties (Shaviklo et al., 2010). Growth of most of the spoilage and pathogenic bacteria is inhibited when water activity is below 0.8 while yeasts and molds can grow until 0.6 (Mujaffar & Sankat, 2005). Moisture content below 10 g/100 g seems the standard requirement for dried fish powder products (Abbey et al., 2017; Kasozi et al., 2018; Mahmud et al., 2019) and corresponds to water activity values close to 0.65 which is in accordance with shelf stability requirements from a microbial point of view. The slower drying at 50 °C may explain the lower pH obtained for dried fish powder dried at 50 °C compared to 80 °C, even if all powders showed a pH between 6.0 and 6.9 which is considered of very good quality (Zhang et al., 2013). Praveen Kumar et al. (2017) also reported a lower pH for the fish dried at 50 °C – 70 °C compared to the raw material which they attributed to lactic acid fermentation. In fact some lactic acid bacteria are able to grow over wide temperature ranges from 0 °C to 50 °C (Lübeck & Lübeck, 2019). Furthermore, the time needed to reach the desired water activity is quite long at 50°C and this time is compatible with bacterial growth initiation.

Beside the removal of water during drying, results indicated that lipid also melted and were lost. Lipids melt due to heat treatment and melting increase with the increase of temperature. As noticed during processing, most fish had their bellies broken, especially at high drying temperatures. It is known that lipids are located in this area and breaking the viscera allows for lipid loss. This loss of lipids has already been observed in studies on catfish (eviscerated and beheaded) dried at 60 °C – 70 °C (Chukwu & Shaba, 2009). The powder retained much of the lipid content of the fresh fish but the fatty acid profile was altered. Comparison of the fatty acid profiles of fresh fish and dried fish powder shows that n-3 PUFA and more particularly DHA were in lower proportion in dried fish powders except the one produced at 60°C. These changes could be due to losses through degradation reactions (oxidation) and fat melting. In fact, the reactivity of PUFA is higher compared to SFA and MUFA since they have more double bonds. Regarding melting, it is well known that the more triglycerides are unsaturated the lower is

their melting point due to difference of melting point of fatty acids. For example, the melting point of palmitic acid is 65.5 °C while that of DHA is 29.8 °C and that of EPA is 10.1 °C (Knothe & Dunn, 2009). The decrease of DHA during drying has also been reported by Ortiz et al. (2013) during the drying of salmon fillets. The PUFA, such as EPA and DHA, are considered to be especially susceptible to oxidation during heating and other culinary treatments (Weber et al., 2008). Drying at 60°C protected DHA somewhat from degradation compared to drying at 50°C, because the duration of the treatment at 60 °C is quicker. Fish lipids, in particular n-3 PUFA (EPA+DHA), play a very important role in humans. EPA and DHA have been shown to be effective in the prevention and treatment of many diseases (Zhang et al., 2020 ; Valenzuela et al., 2020). They are particularly important for child growth and development. The relevance of DHA during the first months of life is widely accepted due to its fundamental role in neural development (Sambra et al., 2021). Despite this loss, n-6 PUFA/n-3 PUFA ratios were still below four which is the recommended ratio to prevent several diseases and contribute to human health and improved nutrition throughout life (Zhang et al., 2020). Finally, the incorporation of these dried fish powders into everyday food products will certainly improve the coverage of daily human requirements, particularly in terms of lipids and essential fatty acids as also noted by Mahmud et al. (2019) on fish powders from Bangladeshi indigenous fish species.

The vitamin A in fresh *Henicorhynchus siamensis* (555.5 RE µg/100 g) was in accordance to value previously reported (Sroy et al., 2021a). Vitamin A content can vary in different parts of the same tissues, and among animals collected at different times and locations. Indeed, geographic availability, seasonality, and physiological state/maturity are known to affect variability in nutrient composition, particularly for vitamins (Ersoy & Özeren, 2009). Results show that drying is a critical step for the preservation of vitamin A in dried fish powders although vitamin A is known to be less heat-labile (Ersoy & Özeren, 2009; Kasozi et al., 2018) and less sensible to oxygen (Sachdeva et al., 2021) than the water-soluble vitamins. Degradation rate constants of vitamin A is scarce in literature compared to vitamin A precursors. First-order reaction rates were calculated for the degradation of trans-retinol in beef liver puree heated in capillary tubes at five temperatures between 103 °C and 127 °C and varied from 17.9×10^{-5} to $162.3 \times 10^{-5} \text{ s}^{-1}$ (Wilkinson et al., 1981). The activation energy of vitamin A degradation was more than ten times higher (112 kJ/mol) than the activation energy reported in our study. This might be due to the use of very high temperatures in their study (over 100 °C), a different composition of the raw material and the effect of moisture content.

The increase in protein, ash, minerals and heavy metals contents is due to their concentration through the removal of water molecules during drying. Macronutrients and ash contents is close to the results reported on dried fish powders made from degutted *Brycinus nurse* degutted (Kasozi et al., 2018). *Henicorhynchus siamensis* is a small fatty fish rich in bones (Sroy et al., 2021a). Therefore, dried fish powder made from *Henicorhynchus siamensis* contains not only proteins but also lipids and minerals.

The ash content in dried fish powder is likely related to the inclusion of bones, viscera, and fins as edible parts during the production (Kasozi et al., 2018). As the ash content of dried fish powders is high, they are an obvious source of minerals whose use in human nutrition will increase mineral intakes and may reduce micronutrient deficiencies (Mahmud et al., 2019).

Minerals in fish is depending on how fish absorb the minerals from its diet or from ambient water (Kalantarian et al., 2013). *Henicorhynchus siamensis* is a fish species which shows high concentration of essential main elements (calcium, phosphorus and potassium) and trace elements (iron, zinc and manganese) (Sroy et al., 2021a). The literature presents controversial results on the increase and/or decrease of mineral content in fish samples during cooking (Sobral et al., 2018). The gain observed by some authors might only result from the water loss occurring during cooking which can not be assessed as it is not always clear if the contents are

expressed or not on a dry basis. The gain may also result from the migration of such elements from the container used to cook the fish. In the case of decreased values, boiling is the method that most contributes to the loss of minerals by diffusion (Sobral et al., 2018).

Phosphorus and potassium both are important minerals in human physiology (Kalantarian et al., 2013; Mahmud et al., 2019). Children require calcium, iron, and zinc for their body growth to prevent stunting. Iron is one of the elements needed in the process of forming red blood cells. Red blood cells contain hemoglobin, which carries oxygen from the lungs to all parts of the body. Calcium is a mineral that plays an essential role in the bone growth of children and zinc is needed for many enzymes involved in several metabolisms (Putri et al., 2020). Calcium is one of the most abundant cations in the body of a fish and it is readily derived from the water and occurs in adequate amounts in most diets consumed by fish (Kalantarian et al., 2013). Calcium content is higher in species in which bones are consumed and included in the edible parts (Kasozi et al., 2018).

Manganese is an essential element necessary for the growth, development and maintenance of health in humans, animals and plants; however, it is toxic if exposed or consumed in excess. Indeed, excessive intake is harmful and can lead to mental confusion, kidney failure, neurological problems, reduced immune function, increased demand for vitamin C and copper (Santamaria, 2008). Manganese can be present in the environment as suspended particles resulting from industrial emissions, volcanic emissions or in the soil (Sigel et al., 2013). A high content of manganese was found in raw material and dried fish powders in our study. This should be taken into account depending of the amount of fish powder used in dishes/formulations especially if fish powders are to be used in nutrition programs for fragile individuals such as pregnant women or young children.

High quality proteins and omega-3 fatty acids are easily found in fish, which protect against coronary heart disease and stroke (Johnston and Snow, 2007). It was reported that fish intake reduced mortality risk from heart disease by 36% (Thilsted et al., 2016). In particular, it contributes to the neurological development of the foetus during gestation (Johnston and Snow, 2007). For those pregnant whose intake of long chain omega-3 fatty acids is only based on fish, it helps to reduce the risk of early preterm delivery (Thilsted et al., 2016). Agricultural residues are polluting the ecosystems and several heavy metals (arsenic, manganese and mercury) were detected in water and in fish (Kelly et al., 2018). The variation in the amounts of heavy metals in fish depends on the species, metabolism, size, life cycle, habitat, environmental characteristics and feeding habits. In addition, the efficiency of heavy metal uptake by fish from polluted water and food depends on metabolism (Bawuro et al., 2018).

According to SAIN and LIM scores, the raw material was classified in food to be consumed in small quantity as in a previous study (Sroy et al., 2021a). Indeed, LIM3 and LIM11 were higher than 7 due to the high content of manganese. Therefore, special attention should be paid to the amounts of dried fish powders included in food formulations especially if they are intended for vulnerable groups such as pregnant women and young children who have micronutrient deficiencies. Snack and instant fish soup are made mostly from healthy ingredients, while NumTrey RUSF are made from ingredients rich in lipid and with sugar and lipid added. Indeed, RUSF are formulated to help micronutrient deficient or malnourished people to quickly recover a normal nutritional status. Their caloric density is therefore increased to ensure their biological effectiveness. The SAIN and LIM indicators reflect the nutritional quality and nutritional risks incurred by normal consumers and not by sick individuals. Thus, snack and instant fish soup scores were better than those of NumTrey RUSF. Although manganese is concentrated in fish powder, it is not included in large amounts in instant soup recipes and snacks, so these foods are still classified as recommended foods for nutrition and health. It is obvious that the dried fish powder is not the only factor that determines the indicators of SAIN and LIM of the food product, they are also determined by the other ingredients in the recipe.

5. Conclusion

After drying to moisture content close to 10 g/100 g wb or water activity about 0.65, and whatever the drying temperature between 50 °C and 80 °C, protein and mineral contents of powders made from *Henicorhynchus siamensis* increased due to the water removal. Lipid content also increased despite lipid loss especially at 70 °C and 80 °C. Dried fish powder is rich in protein and several minerals (calcium, iron, zinc). A decrease in vitamin A content was observed during drying as well as a slight change in the fatty acid profile (lower proportion of n-3 PUFA and DHA) except for the drying at 60 °C. High drying temperatures (70 °C and 80 °C) and long drying times at 50 °C affect vitamin A content in a larger extent compared to drying at 60 °C. Dried fish powder can however have an interesting nutritional profile if properly processed and can meet the nutritional requirements of specific formulations such as therapeutic foods. On the other hand, the quantities of powders to be added in the formulations must be studied with precaution because the powders are rich in manganese and their incorporation in too great proportion in formulations could make the products dangerous taking into account the potential harmful effect of this element.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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CRedit authorship contribution statement

Sengly SROY: Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing - original draft, Writing – review & editing. **Sylvie AVALLONE:** Conceptualization, Investigation, Methodology, Supervision, Validation, Writing – review & editing. **Adrien SERVENT:** Methodology. **Sokneang IN:** Writing – review & editing. **Elodie ARNAUD:** Conceptualization, Investigation, Data curation, Methodology, Software Supervision, Validation, Writing – review & editing.

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