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Shrimp head autolysis and use of protein-rich fraction for salted snacks enrichment

Zo Randriamahatody[1,*], Jean Caude Randriamidosy[1], Mboahangy Ravoninjatovo[1], Christine Ravonizafy[1], Onja Andriambeloson[2], Louisette Razanamparany[3]

[1]National Environmental Research Center, Department Environment and quality of life, Antananarivo, Madagascar
[2]National Environmental Research Center, Department Terrestrial ecosystem, Antananarivo, Madagascar
[3]University of Antananarivo, Sciences Faculty, Department of Fundamental and Applied Biochemistry, Antananarivo, Madagascar

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Corresponding Author
Tel.: +261331411087
E-mail: rzogab@yahoo.fr

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Abstract

In order to contribute to the fight against malnutrition and to limit environmental pollution, shrimp head autolysis was performed. Resulting fractions were characterised to identify the protein-richest one which was used to improve the nutritional quality of wheat flour-based snacks. Supernatant fraction from shrimp head autolysis (SSHA) was found to have the highest protein content (73.56 ± 0.42%). In addition to 5% and 10% flour substitutions with powder SSHA, the direct use of liquid SSHA was performed against a control. As expected, the protein contents increased after the enrichments. They were similar (*P>0.05*) for the 5% flour substitution (10.41±0.17%) and the use of liquid SSHA (10.08±0.21%). The proportion was 13.08±0.07% for the 10% flour substitution. The fat content increased significantly when the snacks were fortified. SSHA and snacks were of good microbiological quality despite the presence of moulds up to 1.0x102 CFU in the snacks. The use of SSHA in the snacks increased their appreciation. The snacks enriched with liquid SSHA were the most appreciated with a likeness score of 7.34. Liquid SSHA is thus a very interesting form of enrichment from a sensory point of view.

Introduction

During shrimp processing, by-products which are potentially valuable are generated. Given the environmental pollution they could generate and their composition, several techniques are used to bring out valuable molecules contained in these by-products: physical ones such as flour production (Brazileiro *et al.*, 2012; Fernandes *et al.*, 2013; Gonclaves and Junior, 2019), chemical such as pH change (Cordova-Murueta *et al.*, 2013; Abreu *et al.*, 2019), and biological such as fermentation and enzymatic hydrolysis (Mao *et al.*, 2017). Enzymatic hydrolysis involves the use of enzymes for solubilisation and extraction of nutrients. In fisheries by-products valorisation, proteases are the most commonly used. This technique requires the addition of commercial enzyme to the by-products and the regulation of the reaction with chemicals such as acid and/or base. However, some by-products like cephalothoracic part of shrimps contain endogenous enzymes composed by the digestive enzymes of the animal (Sriket *et al.*, 2012; Liang *et al.*, 2016; Xu *et al.*, 2018). Sriket *et al.* (2012) reported the richness of shrimp heads in trypsin, chymotrypsin, pepsin and lipases. Liang *et al.* (2016) identified 14 enzymes

in shrimp cephalothorax and Xu *et al.* (2018) more recently highlighted 10 enzymes in these cephalothorax. In these studies, enzyme activities were reported in acidic, neutral and basic media as well as at different temperatures ranging from 30°C to 70°C. The use of such native enzymes minimises the cost of proteolysis. Several studies have been carried out on autolysis, which consists of using native enzymes from the matrices for their hydrolysis. Those of Cao *et al.* (2008) consisted in optimising the conditions for autolysis of shrimp heads and, among other things, showed that it is an effective way for producing high nutritional quality derived products that can be used in human food. Some autolysis requires the adjustment of the initial pH of the reaction medium, thus involving the addition of buffer solution. Some autolysis has been coupled with enzymatic hydrolysis (Sinthusamran *et al.*, 2018), fermentation (Guo *et al.*, 2019) or UV pre-treatment (Cao *et al.*, 2014). The interest of the autolysis technique resides also in the ability to separate the molecules of interest by producing several liquid and solid fractions. In the studies conducted by Cahu *et al.* (2012), autolysis at 40°C for 2h resulted in a protein-rich liquid hydrolysate and the solid phases were found to be sources of chitin, carotenoids and glucosamines. In all cases, the autolysates obtained are of good nutritional and food quality.

A practical way of using autolysis products in human nutrition is food enrichment. Shrimp heads have been studied in this area on carbohydrate matrices. Enrichment of lipids and essential fatty acids with encapsuled oil extracted from shrimp heads has been successfully explored on bread (Takeungwongtrakul *et al.*, 2015) and on biscuits (Takeungwongtrakul and Benjakul, 2017). Shrimp head hydrolysate has also been used after autolysis followed by hydrolysis with Alcalase for protein enrichment of biscuits (Sinthusamran *et al.*, 2019). Several studies have focused on protein enrichment with shrimp head meal such as Fernandes *et al.* (2013) who used the meal as a flavouring in soup and in pasta. More recent study by Gonclaves and Junior (2019) showed the effectiveness of protein enrichment of biscuits with shrimp head meal. In the production of the flour, the whole head was used after heat treatment, drying and grinding.

In our study, the protein-rich fraction from shrimp heads autolysis will be investigated for nutritional, microbiological and sensory aspects in the enrichment of salted snacks, the other autolysis fractions being suitable for other uses.

Materials and Methods

Biological materials

Heads of tropical shrimp *Penaeus indicus* caught in the western coast of Madagascar, kindly provided by the company MANDA S.A. were used. Frozen raw materials were transported in isothermal bags to the laboratory. Aliquots of 150g were formed and stored at -20°C until use.

Proximate composition determination

The composition was determined according to the method described by AOAC (1990). Moisture was determined by oven drying at 103±5 °C for 24 h, ash by incineration at 550 °C until white or grey ash was obtained. Total lipids were determined by extraction with hexane using the soxhlet apparatus and total proteins by the KJELDHAL method using the conversion factor of 6.25. The caloric value was assessed using Atwater's factors of 4 kcal per 100 g for carbohydrates and proteins and 9 kcal per 100 g for lipids.

Mineral elements analysis

After incineration and acid solubilisation of the resulting ash, calcium, magnesium, iron, potassium and heavy metals such as lead, cadmium and nickel were determined by atomic absorption spectrometry (VARIAN Spectra A 20) following the section 965.09 described by AOAC (1990), while phosphorus was determined by UV/Vis spectrometry (Beckmann DU-64) at 430 nm after treatment with a vanado-molibdic reagent as described in section 970.01 of AOAC (1990).

Autolysis

The autolysis technique adopted was that described by Cao *et al.* (2009), with some modifications. 150 g of samples were allowed to thaw for 24 h at 4 °C, then ground and homogenised with distilled water twice their weight. Autolysis was started at the initial pH (7.44) of the reaction medium at 40 °C. Then the temperature was increased by 5 °C every 30 min and a sample was taken in parallel to evaluate the solubilised protein content. During the whole manipulation, the reaction medium was stirred with a magnetic stirrer. The reaction was stopped when the temperature reached 70 °C, i.e. after 3 h of reaction. The reaction medium was then heated in a water bath at 100 °C for 10 min to stop autolysis. The resulting preparation was filtered to separate the liquid from the solid. The filtrate was centrifuged at 4000 rpm for 20 min after which the supernatant (SSHA) and pellet (PSHA) were recovered. In order to separate the substances in the filtration residue, it was washed three times with water 3 times its weight and the washing water (WSHA) was recovered as well as the residue (RSHA). The SSHA, PSHA, WSHA and RSHA were then oven dried at 70 °C for 72 h and reduced to powder. All fractions were characterised and only the most protein-rich fraction (SSHA) was used for snacks enrichment. Enrichments with this fraction in the liquid and powder form were compared.

Thus, a second autolysis under the same conditions was carried out, after which SSHA was recovered and was kept in a liquid state and stored at -20 °C.

The efficiency of autolysis was assessed by the ratio between the solubilised protein content and the total protein content determined according to the KJELDHAL method.

The yield of the different fractions was estimated as: (mass of the powder fraction / mass of the initial raw material) * 100

Salted snacks enrichments

SSHA powder and SSHA in liquid form were used to enrich wheat flour-based salty snacks. Four batches of flour were prepared to produce four types of snacks: two batches enriched with SSHA powder at 10% w/w (S10) and 5% w/w (S5) and two non-enriched batches, one serving as control. For the 2 enriched flour batches and the control (SC), water with a proportion of 1/2, v/w was added. For the last batch of non-enriched flour (SL), SSHA in liquid form was added (1/2, v/w). Salt (4%, w/w), sugar (3%, w/w), oil (20%, w/w), egg (10%, w/w) and baking powder (1%, w/w) were further added to each batch. The resulting doughs were then mashed and allowed to rest for 3 h before being flattened and cut into small flat rectangles of about 1 cm wide, 3 cm long and 0.5 cm thick. The snacks were fried in soybean oil at 700 °C according to the heater, for about 2 min.

Microbiological analysis

The microbiological analysis was carried out to ensure the safety of food, which is of great importance for its consumption and marketing. Currently, heat resistance of microorganisms is a serious problem (Smelt and Brul, 2013) and there were cases where shrimp by-products were contaminated (Samia *et al.*, 2014). The analysis, focused on pathogens and spoilage germs, were carried out on SSHA and snacks ST, S5, S10, and SL according to the microbiological methods described by AOAC (1990). After grinding, diluting, plating the samples on appropriate media and incubating them under favourable conditions for each germ, the Total aerobic mesophilic flora, total coliforms, faecal coliforms, moulds, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Clostridium perfringens*, *Salmonella* and pathogenic *Vibrionaceae* were counted.

Test of preference

The preference analysis for all four types of snacks followed the method used by Takeungwongtrakul and Benjakul (2017) with slight modifications. The test was conducted on 80 untrained students aged from 15 to 18 years, who are regular consumers of such snacks. Samples were presented in monadic way, in identical containers and anonymously. Each sample was marked with a 3-digit code in a different order. Each panel had to test all the products, and a glass of water was provided to rinse the mouth between product tastings. The products were rated from 1 to 9 according to their appreciation, going from 1 which corresponds to not appreciated at all to 9 which is equivalent to very appreciated.

Statistical analysis

Analysis were performed in triplicate. Analysis of variance and comparison of means by Tukey's test were performed with R version 4.1.2.

Results and Discussion

Shrimp heads composition

According to the analysis, *Penaeus indicus* shrimp heads are composed of 77.42±0.45% water. On a dry weight basis, 100 g of shrimp heads contain 50.70±0.2g proteins, 8.94±0.31 g lipids and 23.47±0.03 g mineral matter. These raw materials have less proteins compared to *Penaeus vannamei* heads studied by Cao *et al.* (2014) (60.25% of dry weight) and *Litopenaeus vannamei* heads studied by Brasiliero *et al.* (2012) (58.08% of dry weight). However, they have a higher amount of lipids compared to 7.94% of dry weight for *P. vannamei* (Cao *et al.*, 2014) and 4.11% of dry weight for *L. vannamei* (Brasiliero *et al.*, 2012). Mineral matter is 19.91% of dry weight for *P. vannamei* (Cao *et al.*, 2014) and 30.79% of dry weight for *L. vannamei* (Brasileiro *et al.*, 2012). These differences are probably due to species, seasonality and geography.

Autolysis

The evolution of autolysis kinetics (Figure 1) shows a curve with 2 phases: a rapid phase reflecting high autolytic activity and a gradually latent phase signifying a continuous reduction in activity that may be due to the decrease in the amount of available substrate. The kinetic curve is similar to other shrimp head autolysis and is typical of enzymatic proteolysis, suggesting the involvement of enzymes during autolysis (Cao *et al.*, 2014). Furthermore, the same author reported that endogenous shrimp head enzymes possess broad substrate specificity and numerous sites of action on shrimp head proteins (Cao *et al.*, 2014). Autolysis at progressive temperatures corresponds to the temperatures required for the majority of endogenous shrimp head enzymes (Cao *et al.*, 2014). The duration of 3 h has been reported as optimal for autolysis of shrimp heads by different authors. It corresponds to the fast phase for the studies of Cao *et al.* (2014) and marks the end of autolysis at 50 °C, 23% w/v and pH 8.75 according to Cao *et al.* (2008). The results obtained here are similar, with the beginning of a latent phase after 2h 30 min of autolysis. Regarding pH, according to the studies of Guo *et al.* (2019), a better solubilisation of proteins is observed when the initial pH of the medium is 5 to 8. Sowmya *et*

al. (2011) reported an increase in activity proportional to pH and highest activity at pH 8. Therefore, it is very interesting to work at the natural pH of shrimp heads which is equivalent to 8 according to Guo *et al.* (2019). In our study, the initial pH ranges from 7.44 to 7.84. Performing autolysis at the natural initial pH of shrimp heads will also avoid the addition of chemicals for pH adjustment, resulting in chemical-free biological autolysates and lowering the cost of production by eliminating the chemical-related cost.

The solubilisation of 39.20% of proteins was obtained after 3 h of autolysis. It has been reported that the enzymatic activity of fish viscera varies between species and physiological functions (Khalil *et al.*, 1987). The pH, temperature and duration of autolysis also influence the hydrolysis (Sowmya *et al.*, 2011). All of these factors could be behind the differences in the DH.

Yields

After drying, the yields of SSHA, PSHA, WSHA and RSHA were 8.96%; 3.82%; 2.20% and 8.89% respectively. These results are similar to a larger scale study (20 kg) conducted by Guo *et al.* (2019) who obtained 8% (1.6 kg) of protein fraction powder, which corresponds to SSHA; and 9% (1.8 kg) of chitin equivalent to RSHA, during autolysis followed by fermentation of shrimp heads.

Composition of autolysis fractions

Powdered SSHA is largely composed of protein with a content of 73.56% on a dry weight basis. Ash is present at 10.95%. In the liquid form of SSHA, protein is present at 5.50% and ash at 0.79%. Compared to the 8.43% protein content obtained by Bueno-Solano *et al.* (2009), the protein content of the liquid SSHA is lower. The autolysis liquid fraction obtained by Cahu *et al.* (2012) also has a higher dry matter content (9%) than the 7.58% obtained here. The difference between the methods used could lead to these different concentrations of the soluble fractions.

SSHA is thus a good source of protein that can be used for food enrichment, as found previously by several authors. This is the case for the studies conducted by Brasiliero *et al.* (2012), where a shrimp head protein concentrate and freeze-dried shrimp head meal were

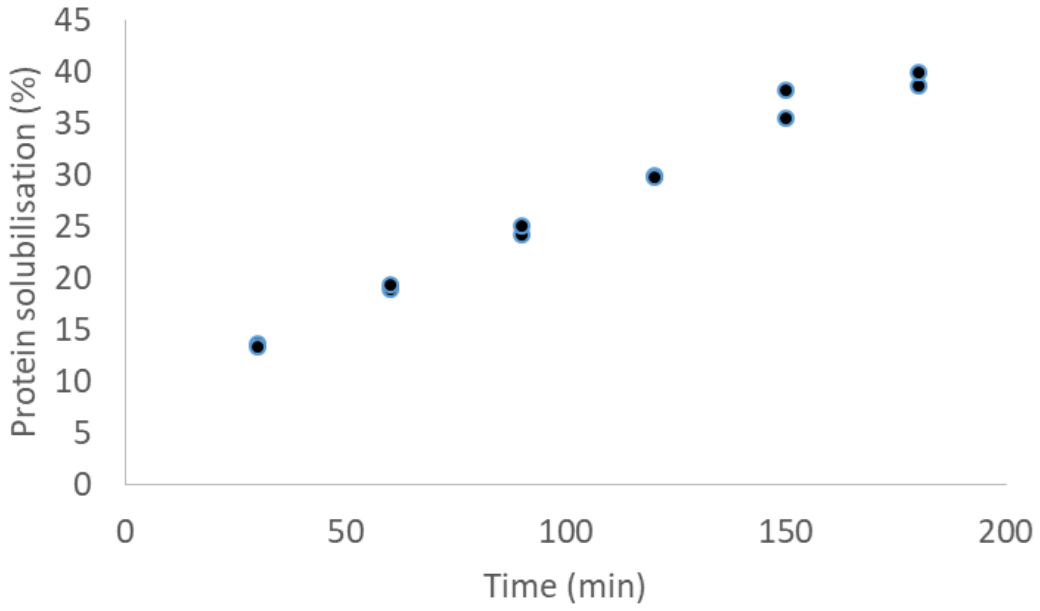

Figure 1. Shrimp heads autolysis evolution (n=2)

Table 1. Composition of shrimp heads fractions recovered after autolysis

Parameter	SSHA	PSHA	WSHA	RSHA
Moisture (g/100g)	6.67±0.09^{a}	4.91±0.12^{b}	4.55±0.22^{b}	4.03±0.21^{c}
Protein (g/100g DM)	73.56±0.42^{a}	22.74±0.39^{b}	44.00±0.53^{c}	30.09±0.45^{d}
Lipid (g/100g DM)	8.07±0.92^{a}	3.02±0.67^{b}	13.62±0.63^{c}	8.92±0.46^{a}
Ash (g/100g DM)	10.95±0.13^{a}	19.83±0.06^{b}	18.25±0.08^{c}	44.81±0.17^{d}
Calcium (g/100g DM)	1.48±0.12^{a}	9.53±0.21^{b}	7.99±0.17^{c}	20.16±0.17^{d}

Values are presented as mean ± SD (n=3). Means followed by the same letter in the same line are not statistically significantly different from each other (*P>0.05*), by the Tukey test at 5% probability. SSHA: Supernatant fraction from Shrimp Head Autolysis; PSHA: Pellet fraction from Shrimp Head Autolysis; WSHA: Washing water fraction from Shrimp Head Autolysis; RSHA: Residue fraction from Shrimp Head Autolysis; DM: Dry Matter

obtained with protein contents of 54.41% and 51.01% respectively. The ash content was 15.75% and 19.19%. A hydrolysate with 46% of protein content was obtained by Bueno-Solano *et al.* (2009) after fermentation of shrimp heads. A shrimp head protein powder with 80.2% protein was also obtained by Guo *et al.* (2019) after autolysis followed by fermentation. The differences among protein contents could be due to the diversity of shrimp head species and the methods used for extraction.

It should be noted that the other fractions recovered after autolysis contain interesting molecules that can also be valorised, among which the high ash and calcium contents of RSHA (Table 1). However, SSHA is the most protein-rich fraction whose use in human food is the main focus of this paper.

Mineral assessment

Shrimp heads contain 9.34% calcium, 0.79% magnesium, 1.19% phosphorus and 0.02% iron on a dry weight basis. Similar predominance of calcium in the mineral composition of shrimp heads was reported several times. Fernandes *et al.* (2013) reported similar amount (10.03%) of calcium in shrimp head meal. Cao *et al.* (2009), Brasiliero *et al.* (2012), and Balogun and Akegbejo-Samsons (1992) found that calcium is the predominant mineral element in shrimp heads with about 1.35 to 4.45%. In the freeze-dried soluble fraction after autolysis, 0.7% calcium was reported by Cao *et al.* (2009) compared to 1.4% by Guo *et al.* (2019). The calcium content of SSHA is similar to that found by Guo *et al.* (2019). An increase in calcium content in protein concentrates was however reported by Brasiliero *et al.* (2012). Initially 3.16% in shrimp heads, these contents reached 6.48% in the protein concentrate obtained with 1% sodium chloride followed by cooled ethanol (5-10°C) 3/1 v/v treatments. It is 6.14% for the flours prepared by freeze-drying heads previously placed in a boiling water bath for 15 minutes. These differences in calcium behaviour may be explained by the different treatments of the raw materials.

Fewer than the amount observed in *P. indicus* heads here, magnesium is present at 0.10 to 0.42% in different parts of 4 shrimp species collected from the Nigerian coast according to the investigations of Balogun and Akegbejo-Samsons (1992). The same authors reported that the heads contain more mineral elements than the shells and muscle. In derived products, 0.2% magnesium in shrimp head protein powders are reported by Guo *et al.* (2019). In the studies of Cao *et al.* (2009), this content is 0.48% in the soluble autolysis fraction. An increase in magnesium and potassium content is reported in the studies of Cao *et al.* (2009), due to their free ion form in the shrimp heads which leads to their concentration during autolysis. The magnesium

a. Snacks S10

b. Snacks S5

c. Snacks SL

Figure 2. Pictures of enriched salted snacks.

content of the 0.75% SSHA did not change significantly compared to the raw material.

Phosphorus is 1.61% of the meal according to Fernandes *et al.* (2013), which is comparable to the content of the shrimp heads in the present study. The content decreased after autolysis and the SSHA contains 0.39% phosphorus. It is present at 0.75% in the shrimp heads and 0.21% in the autolysates according to Cao *et al.* (2009).

Iron is present at 0.12 to 0.37% according to the investigations of Balogun and Akegbejo-Samsons (1992) in the different parts of the shrimp. It is reported to be the trace element present at the highest concentration (0.005%) in shrimp head autolysate meals following the investigations of Guo *et al.* (2019) while it occurs at 0.08% in shrimp head meals according to Fernandes *et al.* (2013). Iron is found at 0.006% in shrimp heads studied by Cao *et al.* (2009) and its content decreased to 0.0014% in autolysates. Iron could not be detected in SSHA probably due to its presence at very low dose.

Lead, cadmium and nickel were present in trace amounts in *P indicus* shrimp heads and the resulting SSHA powder. The absence of cadmium and lead in shrimp head and autolysates has been reported by Cao *et al.* (2009), Guo *et al.* (2019) and Bueno-Solano *et al*. This absence of heavy metals, which are harmful contaminants to health, reinforces the suitability of shrimp heads and their derivatives for food use.

Salted snacks enrichments

Snacks pictures are provided on Figure 2. Snacks made from flours enriched with SSHA powder and by use of liquid SSHA improved the protein content of these feeds (Table 2). An increase of 4.38% is observed for 10% enrichment. Among the studies on the use of shrimp head derivatives in food matrices, those conducted by Sinthusamran *et al.* (2019) reported a protein content of 11.82% in biscuits enriched with 5% shrimp head hydrolysate, equivalent to an increase of 2.49% compared to the initial biscuits. Gonclaves and Junior (2019) reported a protein content of 20.52% in biscuits fortified with shrimp head meal.

The mineral contents also increase with incorporation (Table 2). Shrimp head autolysate contains an interesting amount of ash and the frying method preserves the mineral elements in the feed as they are not soluble in oil (Oke *et al.*, 2017). Authors have even found an increase in mineral element contents during frying of fish, probably due to a concentration effect (Oke *et al.*, 2017).

The lipid contents of the enriched products are higher than that of the control (*P≤0.05*). Compared to the results of Sinthusamran *et al.* (2019) obtained with oven cooking, the snacks are richer in lipids, probably due to the deep frying method of cooking where oil passes into the food. Indeed, during frying, an exchange

Table 2. Composition of control and SSHA-enriched snacks

Parameter	ST	S5	S10	SL
Moisture (g/100g)	3.36±0.25^{a}	3.55±0.13^{a}	3.32±0.13^{a}	2.15±0.08^{b}
Protein (g/100g DM)	8.64±0.23^{a}	10.41±0.17^{b}	13.08±0.07^{c}	10.08±0.21^{b}
Lipid (g/100g DM)	20.58±0.55^{a}	27.68±0.35^{b}	24.38±1.13^{c}	24.34±1.02^{c}
Ash (g/100g MD)	1.48±0.01^{a}	1.69±0.01^{b}	2.39±0.01^{c}	1.95±0.03^{d}
Carbohydrate (g/100g DM)	65.94±0.51^{a}	56.67±0.09^{b}	56.83±1.20^{b}	61.48±1.07^{c}
Energetic value (kcal/100g DM)	483.55±3.65^{a}	517.47±2.24^{b}	499.09±5.18^{c}	505.33±5.34^{c}

Values are presented as mean ± SD (n=3). Means followed by the same letter in the same line are not statistically significantly different from each other (*P>0.05*), by the Tukey test at 5% probability. ST: Control snacks; S5: Snacks enriched with 5% SSHA; S10: Snacks enriched with 10% SSHA; SL: Snacks enriched with liquid SSHA; DM: Dry Matter

Table 3. Microbiological profile of SSHA and snacks

Count and research	SSHA	ST	S5	S10	SL	Criteria (m)
Total aerobic mesophilic flora (CFU/g)	$3.9x10^{5}$	$<10^{3}$	$<10^{3}$	$<10^{3}$	$<10^{3}$	$10^{6*}/5x10^{4**}$
Total coliforms (CFU/g)	-	<1	<1	<1	<1	10^{2**}
Faecal coliforms (CFU/g)	<1	<1	<1	<1	<1	10*
Escherichia coli (CFU/g)	-	<1	<1	<1	<1	10*/10**
Staphylococcus aureus (CFU/g)	<1	<1	<1	<1	<1	10^{2**}
Bacillus cereus (CFU/g)	-	<1	<1	<1	<1	10^{2**}
Clostridium perfringens (CFU/g)	<1	<1	<1	<1	<1	10*/10**
Moulds (CFU/g)	-	$<10^{3}$	$<10^{3}$	$1.0x10^{2}$	$<10^{3}$	10^{4**}
Salmonella	Absence	Absence	Absence	Absence	Absence	Absence/25g
Pathogenic Vibrio	Absence	-	-	-	-	Absence/25g

*: Shrimp criteria according to Fédération du Commerce et de la Distribution 2021; **: Flour criteria according to Fédération du Commerce et de la Distribution 2021; m: satisfactory concentrations of microorganisms; SSHA: Supernatant fraction from Shrimp Head Autolysis; SC: Control Snack; S5: Snack from flour enriched with 5% SSHA powder; S10: Snack from flour enriched with 10% SSHA powder; SL: Snack performed with Liquid SSHA

takes place between the water contained in the food and the frying oil (Martinez-Yusta *et al.*, 2014). The lipid composition of low-fat foods changes significantly after frying. This change occurs both during frying and during cooling where 20% and 64% respectively of the frying oil was found to be absorbed in a fried potatoes study (Oke *et al.*, 2017). The factors that change the nutritional value of fried foods are oil and food composition, texture, size, shape and frying conditions such as temperature and duration (Oke *et al.*, 2017). Degradation of frying oil has also been suggested to be a cause of the high lipid content in fried foods. The change in the lipid content of fried foods can be positive if the frying oil is of good quality and not yet thermo-degraded, hence allowing the food to be enriched with lipids (Martinez-Yusta *et al.*, 2014). In this study, fat contents increased when the snacks were enriched with SSHA. Generally, after hydrocolloids, proteins are used as a coating agent in fried products to reduce oil absorption. Positive results are reported with wheat proteins and soy proteins (Brannan *et al.*, 2014). This property is attributed to their gelatinisation which forms a barrier to the movement of water and oil during the frying process, to changes in surface hydrophobicity during cooling (Brannan *et al.*, 2014). The change of the pore size on the food surface due to heat-induced cross-linking of proteins is also suggested (Oke *et al.*, 2017). On proteins of animal origin, few studies are done.

SSHA is thus an interesting ingredient for enriching wheat-based foods with protein and minerals, and frying is a good way to improve their lipid content, if good nutritional quality frying oil is used.

Microbiological analysis

Microbiological analysis showed the absence of pathogenic germs in the SSHA and the different snacks (Table 3). Total aerobic mesophilic flora is present in SSHA with an amount lower than 10^6 CFU/g required for shrimps (Fédération du Commerce et de la Distribution, 2021). In snacks, this amount is largely lower than that of SSHA. Microorganisms may have been destroyed during snacks preparation, probably during frying.

Moulds are present in the snacks, contrary to SSHA, with amounts lower than 10^4 CFU/g required for flours (Fédération du Commerce et de la Distribution, 2021). They could have been introduced by other ingredients, in particular wheat flour, which is a good substrate for moulds. Indeed, fungal growth has been reported to be the most common cause of microbial spoilage and deterioration of grain and flour quality during storage (Ntuli *et al.*, 2013). These fungi usually infect grains during cultivation and storage, which can lead to mycotoxin contamination and high spore counts in flours (Garcia *et al.*, 2019). Moulds persisted in finished products even after frying. They are microorganisms that can sporulate if conditions are not favourable, like high temperature. Spores could then stay in the treated food and, once at room temperature, return to their normal form. This may lead to the presence of mould in snacks even after deep-frying. Moulds have been found, furthermore, in pasteurised fruit juice (Fereira *et al.*, 2011).

In general, both shrimp heads and their derived products have a satisfactory microbiological quality. This is the case of the flours produced by Fernandes *et al.* (2013), which did not contain *Salmonella* and have total coliform and *Staphylococcus* counts below the standards. The absence of pathogenic germs and enterobacteria was reported by Harrabi *et al.* (2016) in shrimp by-products. In terms of microbiology, shrimp heads are suitable for human consumption. This is confirmed by the study of Filho *et al.* (2019) on shrimp head flavour extracts and the one of Gonclaves and Junior (2019) on shrimp head meal and enriched products.

Preference test

The best likeness score of the snacks (7.34) was obtained with the direct use of the liquid in the dough (SL). Likeness scores above 5 were obtained with ST and S10. The latter were more appreciated (6.68) than the control ST (5.46). Snack S5 have a likeness score of 4.92 lower than 5 although there is no significant statistical difference between S5 and ST (*P>0.05*). Fernandes *et al.* (2013) found a likeness score higher than 7 on pasta flavoured with shrimp head meal, similar to that obtained with snacks made directly with liquid hydrolysate (SL). The investigations of Sinthusamran *et al.* (2019) gave the highest likeness score at 5% incorporation of shrimp head hydrolysate powder in savoury biscuits, unlike the present study. The difference in likeness score may be due to the variation in recipes and shrimp species used. In addition, food preference is conditioned by physiological status, nutritional status, environment and socio-cultural factors, among others (Vink *et al.*, 2020). Trakeungwongtrakul *et al.* (2015) attribute brown colouration of biscuits to the Maillard reaction while Sinthusamran *et al.* (2019) suggest the existence of taste-enhancing amino acids in shrimp heads that influence sensory perception, thus inducing an increase or decrease in their likeness score.

Conclusion

Shrimp head autolysis was performed in a biological and low-cost manner since no chemicals were used. It resulted in a protein-rich supernatant fraction and other fractions that can also be used in other areas. Supernatant introduced into snacks has increased the protein content and in some cases improved the appetence of the snacks, whose likeness score has increased as a result. The incorporation of the supernatant in liquid form is the most appreciated, although the protein enrichment with this method is not the best. Further studies are needed to optimise enrichment with liquid SSHA, which is also the most economical to produce. The resulting feed has good microbiological quality and heavy

metals are present in trace amounts. The use of shrimp heads and their autolysate in food would thereby contribute to fight against malnutrition, to use biological ingredients and to develop the shrimp sector while avoiding environmental pollution by these by-products.

Ethical Statement

Not applicable

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Author Contribution

RZ: Conceptualisation, conduct of autolysis, writing, review; RJC: Conduct of autolysis, conceptualisation and conduct of snack enrichment, review; RM: Physicochemical analysis, review; RC: Mineral analysis, review; A0: Microbiological analysis, review; RL: Supervision, review, validation

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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