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EVALUATION OF HISTAMINE CONTENTS DURING THE FISH MEAL PRODUCTION PROCESS

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ABSTRACT

Evaluation of histamine levels at various stages of manufacturing fish meal made from European pilchard *Sardina pilchardus* by-products was conducted on two batches which are distinguished by the quality of raw materials expressed through total volatile basic nitrogen (TVBN) content. The goal was also to assess the impact of the reuse of the concentrated stickwater, the liquid from the pressing process of fish meal. The results revealed that the level of raw material deterioration influenced the level of histamine in the finished product. When the freshness of the raw material is acceptable (TVBN content: 30 mg/100 g), histamine contents recorded in the finished product remain clearly below the limit value of 500 ppm. In this case, incorporation of concentrated stickwater into press cake before drying did not affect the concentration of histamine accumulated in the finished product. When the raw material was spoiled (TVBN content: 120 mg/100 g), the recorded histamine content was twice the limit value (975.1 ± 33.9 ppm).

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INTRODUCTION

It was estimated that 50% of world fish production was rejected as a by-product during processing operations (Kristinsson and Rasco, 2000); in most cases, these by-products were processed into fishmeal, which accounts for 30% of products derived from fishery by-products (Valdimarsson and James, 2001). The exploitation of fishery by-products has become an important sector in some countries (FAO, 2018). For example, in Morocco fish meal manufacturing is an important activity of the fishing sector. In 2018, the country produced 138 000 tons of fish meal, of which 116 938 tons were exported (DPM, 2019). This production required the use of nearly one-third of the annual fish catch and is mainly exported as aquafeeds or included locally in poultry feeds.

The presence of histamine in fishmeal at high levels poses a serious problem to animal health and sometimes even public health. For example, some authors found that the fish meal prepared from a raw material rich in histamine led to a disease called “black vomit” in broiler chicks (Tucker et al., 1975; Harry et Tucker, 1976; Stuart et al., 1986; Džaja, et al., 1996). Histamine plays a key role (precursor) in the formation of gizzerosine (derivative of histamine: 2-amino-9-(4-imidazolyl)-7-azanonanoic acid), a molecule identified by Japanese authors as being responsible for

promoting hydrochloric acid production in poultry with black vomit (Okazaki et al., 1983; Masamura et al., 1985; Sugahara et al., 1988; Sugahara et al., 1992) and causing gizzard erosion and ulcerations. Džaja et al. (1996) found that Hybro broiler chickens treated intragastrically with histamine at a dose of 10 mg/kg of body weight had a decrease of antibody titre after vaccination for Newcastle disease, and Barnes et al. (2001) found that the feed conversion ratio was decreased by addition of 500 ppm of histamine in the feed.

In aquaculture, the disease was accompanied by a delay in growth and a decreased weight gain in shrimp (Tapia-Salazar et al., 2001) and salmon (Opstvedt et al., 2000). In yellow catfish *Pelteobagrus fulvidraco*, diet containing high levels of histamine caused bowel injury and liver damage (Li et al., 2018). It should be noted that the histamine in fish meal also constitutes a danger for people who come into contact with highly contaminated fish meal. Macan et al. (2006) reported that in 1998, a batch of fish meal containing a high-level histamine caused intoxication among workers at the port of Rijeka in Croatia. This intoxication is either due to inhalation or skin contact with fish meal; symptoms began to appear from the histamine level of 2000 ppm in fish meal (Macan et al., 2006) and became acute (allergic eye, skin, gastrointestinal, cardiac and respiratory symptoms in less than 30 minutes) from 5100 ppm (Macan et al., 2000).

Due to this risk, fish meal manufacturers are increasingly confronted with safety and quality requirements in terms of histamine content in raw material and final product.

In this context, our study was carried out to trace the evolution of histamine concentrations throughout the chain of fish meal manufacturing, and to assess the impact of the reuse of the concentrated stickwater, the liquid from pressing process of fish meal, on histamine contents in the finished product in order to make available critical limits of histamine to professionals and risk managers.

MATERIALS AND METHODS

The study was carried out in a factory manufacturing fish meal and fish oil from European pilchard *Sardina pilchardus* by-products supplied by a canning factory. The two facilities are located in Agadir (Morocco) and are approved by the competent authority. It was noted that the processing facilities of fishmeal (place of study) practice recycling of the concentrated stickwater before drying, which leads to a high level of histamine (water-soluble molecule).

Fish meal technology aims to separate solid, oily and aqueous fractions. It involves the following successive stages: reception, cooking, pressing, decantation, stickwater evaporation, drying, grinding, cooling and bagging (Fig. 1).

Preparation of batches

Being a water-soluble amine, histamine will not accumulate in the oily fraction. Therefore, various treatments that the oil undergoes will not be considered in the paper. In order to obtain reliable results, two different batches in terms of freshness were chosen, which is affected by conditions and length of storage before processing.

The first batch concerns a very fresh raw material (assumed to contain a minimum content of histamine) collected from the cannery and processed immediately in the fish meal processing plant.

The second batch corresponds to raw material collected from the cannery and stored at ambient temperature for 36 hours before processing, which constituted the minimum requirements established by the sanitary legislation when the plant lacks refrigerated rooms (DPM, 2010).

Analytical parameters

In the stages where the product is in a solid state, it has been found that it is necessary to check the moisture because histamine is water-soluble. Moisture content was determined by oven-drying the samples at 103°C until their weight became stable. The percent weight of water was calculated from the difference between the beginning and ending mass of fish meal.

The TVBN (total volatile basic nitrogen) content was checked in the raw material to evaluate its freshness (Haaland and Njaa, 1989). TVBN was determined using the reference procedure as described in Chapter III (Section II) of Annex II to Commission Regulation (EC) N° 2074/2005 of 5 December 2005 (Commission Regulation, 2005).

To evaluate histamine content during the various stages of manufacture of fish meal, the spectrofluorimetric method of Lerk and Bell (1976) was adopted. This method consists of extracting histamine with trichloroacetic acid (TCA) and then fixing it on a column filled with an ion exchange resin and diluted with chloridic acid. The assay is carried out by fluorimetry after addition of ophthaldehyde by measuring the intensity fluorescence of three solutions (sample, white, standard).

Sampling

Samples were taken from each batch three successive times at 15 min intervals for each following stages: at the reception (just before cooking); after cooking; after pressing (in both press cake and press liquor); after drying (with and without the addition of concentrated stickwater to the press cake); after decantation (solid phase and liquid phase); after separation (in the stickwater); after evaporation of stickwater (concentrated stickwater); finally finished product (with and without the inclusion of concentrated stickwater).

The TVBN was used as a quality criterion for raw material freshness. A sample was taken from both batches before processing (at the reception stage).

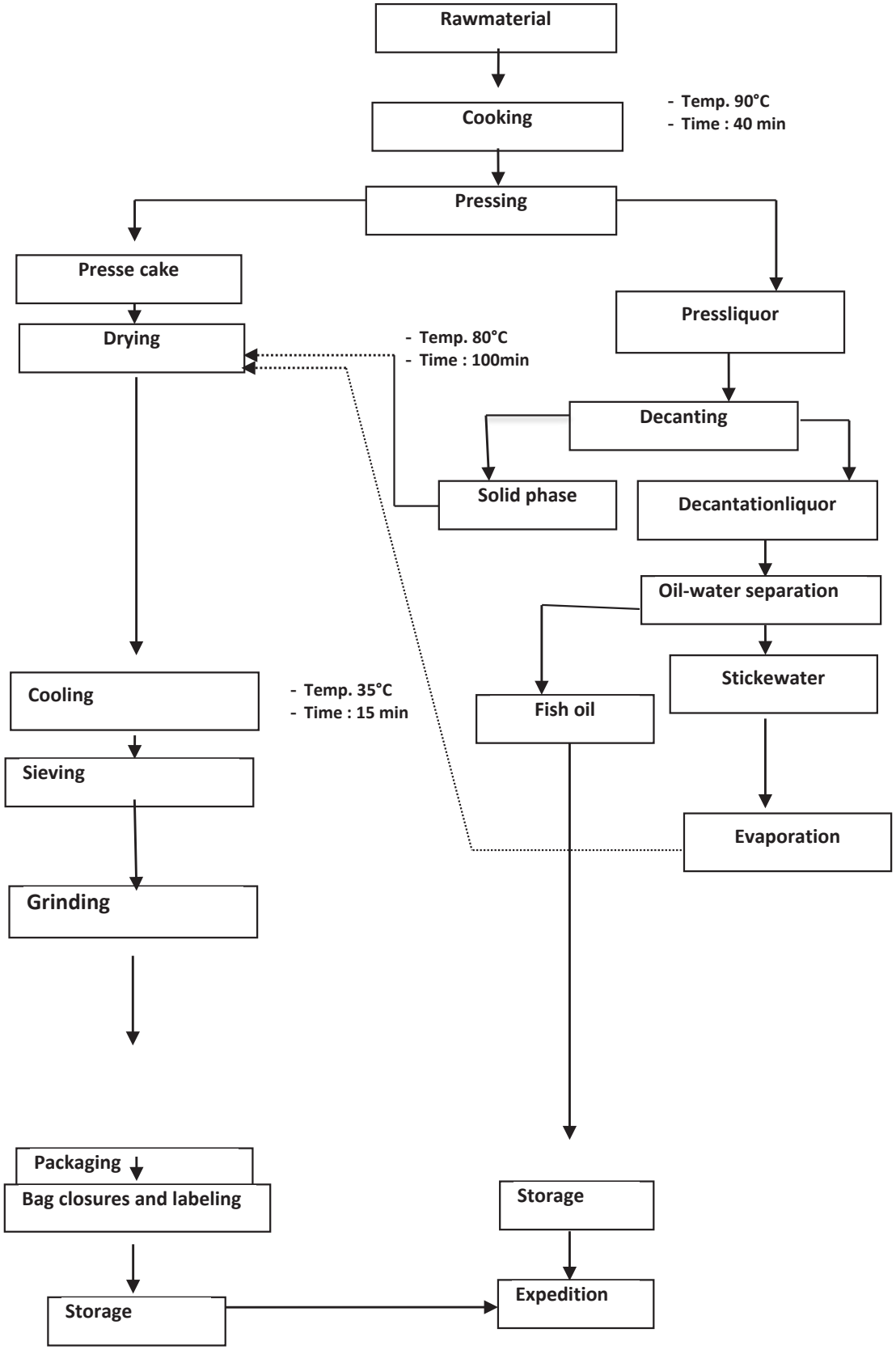

Fig 1. Stages and parameters of fish meal production

RESULTS AND DISCUSSION

Histamine levels

The evolution of histamine levels at the various stages of processing is given in Table 1. It highlights the effect of two factors: the quality of raw material upon receipt and the addition of the concentrated stickwater to press cake prior to drying.

The tested batches are distinguished by their TVBN levels recorded on receipt: "batch 1" had a TVBN content of 30 mg/100 g and may still be characterized as "acceptable" and "batch 2" with a TVBN content of 120 mg/100 g, corresponding to a very advanced state of raw material deterioration (Table 1). Regardless of TBVN levels, histamine levels increase during processing of fishmeal for both lots. However, the ultimate contents depend on the raw material alteration degree and they are in the finished product with concentrated stickwater: 185.6 ppm and 975.1 ppm respectively for batches 1 and 2. Concerning the spoiled batch, histamine contents in raw material and finished product remain much lower than those obtained by Bayrakli (2015) in a fish meal prepared from anchovies with 2359.2 ppm of histamine and 123.25 mg / 100 g of TVBN. The author obtained a value of histamine exceeding 7000 ppm with the reuse of the concentrated stickwater. Considering a pivotal histamine content of 500 ppm (Harry et al., 1975; Masamura et al., 1985; Sugahara et al., 1988), which is a safety limit required to avoid occurrence of certain pathologies as it had been previously reported. Concentrated stickwater addition contributed significantly to increasing histamine level in the finished product even if production was made from an acceptable raw material. This content reached the value of 185.6 ppm, compared to the contents recorded in the finished product without concentrated stickwater addition, which was 110.6 ppm. Contribution of stickwater addition to increase histamine level in the finished product was also demonstrated by Toyama et al. (1981) and Kose et al. (2003). In stickwater, histamine accumulation is even clearer, especially in the spoiled batch. This accumulation is explained by the fact that histamine is a thermostable molecule, not volatile amine and water-soluble substance (accumulates in aqueous fractions). Also, the use of moderate temperatures, which did not favor the transformation histamine into gizzerosine, favored this accumulation. According to Toyama et al. (1982), a significant decrease in histamine level was observed with excessive use of temperatures in each heating stage involved in fish meal processing, especially cooking and drying the press cake with or without concentrated stickwater addition. It is known that histamine is produced by bacterial decarboxylation of free histidine resulting from enzymatic action of certain bacteria genus such as *Proteus, Klebsiella, Escherichia, Shigella, Salmonella* (Taylor and Speckhard, 1983; Subburaj et al., 1984; Geornaras et al., 1995), *Pseudomonas, Staphylococcus aureus, Aeromonas, Lactobacillus* (Middlebrooks et al., 1988), *Phytobacterium* (Morii et al., 1988; Okuzumi et al., 1990) and *Vibrio* (Yoshinaga and Frank, 1982; Middlebrooks et al., 1988; Okuzumi et al., 1990). In the present study, the increase in histamine level cannot be related to any microbial activity since most of histaminogenic bacteria are destroyed by cooking and drying temperatures (Kose et al., 2003).

Table 1. Histamine levels (ppm) during fish meal processing (mean ±SD)

Stages of production	Batch 1 (TVB-N = 30 mg / 100 g)	Batch 2 (TVB-N = 120 mg / 100 g)
Reception	24.1 ± 2.9	361.16 ± 71.3
Cooking	25.6 ± 1.1	1023.3 ±131.7
Press cake	93.7 ± 15	371.6 ± 23.5
Press liquor	19.5 ± 1	387.4 ± 113
Decantation sludge	42.1 ± 9	46.4 ± 3.2
Decanting liquor	18.2 ± 0.3	229.1 ± 69
Stick water	27.4 ± 2.9	438 ± 55
Concentrated stick water	229.7 ± 22.1	1903.9± 220
Dried product without concentrated stickwater	95.6 ± 6.7	836 ± 72
Dried product with concentrated stickwater	174.3 ± 7.4	1022.5± 36
Finished product without concentrated stickwater	110.6 ± 10.5	842 ± 51.5
Finished product with concentrated stickwater	185.6 ± 8.1	975.1±33.9

The results represent the average of three samples taken at 15 min intervals for each stage

Moisture levels

Raw material moisture levels are 75.3± 0.6% in"batch 1" and 71± 1% in "batch 2" (Table 2).

The difference between the two values is attributed to the physiological state of post-mortem fish flesh, characterized by its pH. The latter influenced the water retention capacity of muscle tissue. It is well known that an immediate post-mortem muscle has a high retention power of water, which is the case for "batch 1".

The amount of water lost in raw material during cooking is about 13%. In the press cake, moisture contents for the two batches are about 60%, which constitutes the objective sought by fish meal producers. The finished product prepared with or without concentrated stickwater addition was characterized by moisture content of 11%, which corresponds to a water activity of less than 0.6. The latter gives the finished product a microbiological stability except for molds which are capable of multiplying at this water activity. The most remarkable fact is the water content of concentrated stickwater, which was on average 37± 1%. Its incorporation into press cake before drying is entirely justified in terms of solid material yield of the finished product.

Thus, at the end of this study, some solutions can be recommended to control the histamine level in the finished product, namely stopping stickwater recovery when using a degraded or spoiled raw material, and dividing concentrated stickwater over several dryers to reduce histamine level in the finished product. It should be noted that the distribution of concentrated stickwater on dryers must be done with a reasonable flow rate so as not to reduce the efficiency of dryers and consequently increase moisture in the finished product.

CONCLUSION

The increased histamine content in raw material leads to increased histamine content in the final product. Concentrated stickwater had the highest level of histamine compared to products from other stages. To this end, its reintegration into the processing chain must be done by assessing the risk of obtaining a fish meal with a high level of histamine in the finished product exceeding the required safety limits. Histamine water-solubility property has made it possible to obtain the least concentration in decantation sludge; therefore, it is strongly recommended to exploit this property to decrease histamine level in the finished product.

SAŽETAK

PROCJENA SADRŽAJA HISTAMINA TIJEKOM PROIZVODNJE RIBLJEG BRAŠNA

Provedena je procjena razine histamina u različitim fazama proizvodnje ribljeg brašna od nusproizvoda proizvodnje i prerade srdele *Sardina pilchardus*. Procjena je provedena u dvije serije koje se razlikuju kvalitetom sirovine izraženom udjelom ukupnog hlapivog baznog dušika (TVBN). Također, cilj je bio procijeniti utjecaj ponovne upotrebe koncentrirane „ljepljive" vode - tekućine izdvojene iz procesa prešanja ribljeg brašna. Rezultati su ukazali da je razina kvarenja sirovine utjecala na razinu histamina u gotovom proizvodu. Kada je svježina sirovine prihvatljiva (sadržaj TVBN: 30 mg/100 g), sadržaj histamina zabilježen u gotovom proizvodu ostaje ispod granične

Table 2. Moisture percentage (%) during fish meal processing (mean ±SD)

Stages of production	Batch 1 (TVB-N = 30mg / 100g)	Batch 2 (TVB-N = 120mg / 100g)
Reception	75.3 ± 0.6	71 ± 1
Cooking	62.6± 0.7	58.6 ± 3.2
Press cake	55 ±4.4	45 ± 4
Decantationsludge	45± 1	41± 2
Concentrated stickwater	37± 1	37.3± 1
Dried product without concentrated stickwater	9.3± 0.6	11.1± 0.3
Dried product with concentrated stickwater	10.5± 0.7	11.3± 0.6
Finished product without concentrated stickwater	9.6 ± 1.1	11 ± 0.3
Finished product with concentrated stickwater	11 ± 1	11± 1

The results represent the average of three samples taken at 15 min intervals for each stage

vrijednosti od 500 ppm. U ovom istraživanju, uključivanje koncentrirane „ljepljive“ vode u prešanu pogaču ribljeg brašna prije sušenja, nije utjecalo na koncentraciju histamina akumuliranog u gotovom proizvodu. Kada je sirovina u pokusu bila pokvarena (sadržaj TVBN: 120 mg / 100 g), zabilježeni sadržaj histamina bio je dvostruko veći od granične vrijednosti (975,1 ± 33,9 ppm).

Ključne riječi: Nusproizvod, vlaga, *Sardina pilchardus*, ljepljiva voda, TVBN

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