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The Nutritional Quality of Dried Salted Cod: the Effect of Processing and Polyphosphates Addition

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Abstract This work investigated the effect of the industrial and domestic processing, including the use of polyphosphates, in the amino acids profile of dried salted cod. Amino acids contents fluctuated throughout the processing. The loss of nutritional quality (*e.g.* decrease of glutamic acid and taurine) could be due to solubilization of free amino acids, peptides, and proteins in brine, in drained water during dry-salting, and also in the desalting water. Changes in the amino acids contents were also related with variations in moisture and salt contents of cod products. The use of polyphosphates also affected amino acids contents in wet salted cod, dried salted cod, and desalted cod. In general, fresh cod and desalted cod presented similar and well-balanced amino acids compositions, with high amounts of glutamic acid (2.5-2.8 g/100g ww), aspartic acid (1.6-1.8 g/100g ww), leucine (1.2-1.4 g/100g ww), and lysine (1.1-1.2 g/100g ww), while histidine had the lowest amino acid score. The data obtained can be used by nutritionists for more informed diet recommendations in response to consumer demand for healthy habits.

Keywords: *amino acids, protein quality, seafood, bacalhau, salting, drying, phosphates*

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1. Introduction

Atlantic cod (*Gadus morhua*) is the most important fish species for the production of the traditional wet salted and dried salted cod (*bacalhau*), and cod products are highly appreciated mostly in Mediterranean countries, but also in Latin America and United States [1]. Accordingly to the most recent information, captures of Atlantic cod accounted for 1.3 million tonnes in 2014 [2]. Particularly in the European Union, cod is the second most consumed seafood product, with a per capita consumption of 2.4 kg in 2014, being mostly imported from Norway and Iceland, with fresh and frozen cod products representing 65 % in terms of economic value, while dried and salted cod products represent 32 % [3].

Salted cod products has long been appreciated due to specific sensory properties (texture, color, flavor) imparted by the processing method [1]. In general, either fresh or thawed cod is butterfly split and then dried salted, with skin side down in stacks alternated with layers of dry salt. During the salt curing process, the resultant brine is allowed to drain away continuously, as salted cod matures. Then, the cod is dried in controlled humidity conditions and the final dried salted cod is stable for several months when stored in appropriate conditions. Before cooking, both wet salted and dried salted cod need to be desalted/rehydrated between 24-48 h and this time-consuming step used to be done only in domestic environment. The industrial desalting process, followed by frozen storage, was introduced as a result of consumer demand for ready to prepare products.

Information on food nutritional composition is important to consumers and nutritionists to establish diets in reply to lifestyle needs and demands to accomplish healthy diet habits. The nutritional composition of fresh cod was characterized in terms of amino acids and reported results evidenced its high protein quality [4]. In what concerns rehydrated dried salted cod, results reported by Bandarra *et al.* [5] show that total amino acids accounted for *ca*. 30 g/100 g, however results are not in accordance with the protein content.

Processing, such as brining with low salt concentration (up to 5 % of NaCl), cooking, and storage, was reported to affect the free amino acids composition of cod [4,6,7,8]. It is known that brining, dry-salting, and rehydration affect the protein fraction of cod muscle [9]. Drying is also believed to have negative effects on the nutritional properties of food products as protein denaturation/aggregation, that takes place during the drying process, leads to changes in amino acid compositions, protein solubility, and protein digestibility [10].

The use of polyphosphates in wet salted fish was requested by producers countries, especially Nordic, allegedly to obtain better quality (avoid oxidation reactions) and appearance (lighter colour), and reduce flavours intensity [11,12]. Polyphosphates use was recently approved in the European Union (Regulation 1068/2013/EU) at the Standing Committee on the Food Chain and Animal Health meeting, and it is applied since January 1, 2014 [13]. Phosphates may enhance water retention of muscle by effects on pH, ionic strength, and specific interactions of phosphates with divalent cations and myofibrillar proteins [14]. Several studies evaluated the changes in added polyphosphates in cod during processing, and their effects on some quality parameters such as protein content, pH, and water holding capacity were also evaluated [11,15,16,17,18].

The amino acids concentration and the nutritional quality of cod products needs to be determined. Moreover, no information is available regarding the effects of cod industrial processing and of polyphosphates on the profile of total amino acids throughout the production of dried salted cod. In this sense, the aim of this work is to study the effect of processing and polyphosphates addition in the amino acids profile and associated nutritional quality throughout the industrial and domestic processing of dried salted cod.

2. Material and Methods

2.1. Biological Material and Experimental Design

Atlantic cod (*Gadus morhua*), captured in the FAO 27 Atlantic northeast area, was used in the trials developed in an industrial dried salted cod processing facility (Riberalves, S.A., Turcifal, Portugal). Gutted and beheaded cod weight ranged between 2 and 3.5 kg. To reduce the variability between individuals each cod was divided in two parts, one was used in the control group and the other in the treatment with polyphosphates. The phosphate blend (BRIFISOL®512 and 750, ICS Food Specialities, Ladenburg, BK Giulini GmbH) used was a mixture of several sodium and potassium polyphosphates (E450-452) with a total amount of 57.6 g P_2O_5/100 g. Salt (NaCl) of food grade (SALDOMAR, Salexpor, Brancanes-Olhão, Portugal) was used for the preparation of the brine solutions, and also for the dry-salting process. In the control group, cod was immersed in a brine solution of 18 g/100 mL of NaCl. The polyphosphates concentration used was 6 %, also prepared in a brine solution of 18 g/100 mL of NaCl. Cod was immersed in the brine solutions at a ratio of cod to brine of 1:2 during 24 h in refrigerated conditions (7 ± 1 °C).

Dried salted cod was produced accordingly to the traditional Portuguese processing methods, and the dried salted cod was desalted/rehydrated following the industrial and domestic processes as described in Teixeira *et al*. [17].

2.2. Sampling

For each treatment, six samples of raw material were collected and six more samples in the end of each processing step. Regarding the desalting step, three samples were desalted following the industrial process and three other samples were desalted according to the domestic procedure. Each sample was homogenized individually, frozen, freeze-dried for 48 h at -50°C and low pressure (approximately 10^{-1} atm; Heto power dry LL 3000, Thermo Fisher Scientific, Waltham, Massachusetts, USA), and stored at -20 °C until analysis.

2.3. Analysis of Amino Acids

The quantification of amino acids in cod samples was done using the hydrolysis method described in AOAC [19]. For total amino acids extraction, 20-40 mg of sample (1.5-2.0 mg nitrogen) were acid hydrolysed in 10 mL Nalgene Oak Ridge Teflon Fep tubes (Nalge Nunc International Corp., Rochester, NY, USA), with 3 mL of 6 M HCl (Merck, Kenilworth, NJ, USA) containing 0.1% phenol (Merck, Kenilworth, NJ, USA). Norvaline (99%, Sigma-Aldrich Corp., St Louis, MI, USA) and sarcosine (98%, Sigma-Aldrich Corp., St. Louis, MI, USA) were further added to samples (final concentration 500 pmol/µL) before hydrolysis and used as internal standards, respectively, for quantification of primary amino acids (OPA derivatized) and secondary amino acids (FMOC derivatized). Nalgene tubes were vacuumed, fluxed, and capped under nitrogen atmosphere, and samples hydrolysed at 110-115 °C for 24h. After the hydrolysis, samples were neutralised with 6 M NaOH, quantitatively transferred into 20 mL volumetric flasks with ultrapure water, filtered using 0.2 µm pore size cellulose membrane syringe filters and stored at -80°C until analysis.

The chromatographic conditions used were in accordance with the Agilent method [20] and amino acids separation was performed by high-performance liquid chromatography (Agilent 1100 HPLC, Agilent Technologies, Palo Alto, CA, USA) in a Phenomenex Gemini ODS C18 guard column (4 × 3 mm), and a Phenomenex Gemini ODS C18 110 Å column (4.6 × 150 mm, 5 µm) (Phenomenex Inc., Torrence, CA, USA) using pre-column derivatization with *o*-phthalaldehyde and 3-mercaptopropionic acid in borate buffer (OPA, Agilent Technologies, Palo alto, CA, USA) for primary amino acids detection and 9-fluorenylmethyl chloroformate in acetonitrile (FMOC, Agilent Technologies, Palo Alto, CA, USA), for secondary amino acids detection. Separation was performed at a flow of 2 mL/min using a gradient of solvents 40 mM Na_2HPO_4 pH 7.8 and acetonitrile: methanol : water (45:45:10, v:v:v) and detection wavelengths set by fluorescence (340/450 nm and 266/305 nm).

Amino acids identification and quantification was assessed by comparison to the retention times and peak areas of standard amino acids (Sigma, Missouri, USA) within the range 9-900 pmol/µL (r^2=0.9999) with the software Agilent ChemStation for LC (Agilent Technologies, Palo Alto, CA, USA). All determinations were carried in triplicate (repeatability 0.28-2.6 % RSD; recovery 93-110 %). Cysteine and tryptophan were not determined due to its destruction during acid hydrolysis.

2.4. Data Analysis

Protein quality was evaluated by means of the essential amino acid scores, ratio of essential amino acids to non-essential

amino acids, rat protein efficiency ratio (rat-PER), biological value (BV), and essential amino acid index (EAAI).

Amino acids scores were determined with respect to reference amino acid requirements for adults [21]. Scores determine the effectiveness with which absorbed dietary nitrogen can meet the indispensable amino acid requirement at the safe level of protein intake [21]. This is achieved by comparing the content of each essential amino acid in the protein/diet with its content in the requirement pattern:

$$amino\ acid\ score(\%) = \frac{mg\ of\ amino\ acid\ per\ g\ of\ test\ protein}{mg\ of\ amino\ acid\ requirement} \times 100. \quad (1)$$

The ratio of essential amino acids to non-essential amino acids was calculated. Proteins with about the same percentage of essential and non-essential amino acids are considered to be of high quality [22].

The rat-PER is defined by the weight gain in g per g of protein consumed and it was estimated according to the linear regression obtained by Lee *et al*. [23] based on seven essential amino acids (expressed in g per 100 g of total amino acids) as follows:

$$rat-PER = 0.08084 \times \begin{pmatrix} ILE + LEU + LYS + MET \\ + PHE + THR + VAL \end{pmatrix} - 0.1094. \quad (2)$$

The BV is defined by the proportion of absorbed nitrogen retained for utilization by the animal organism and it was estimated according to the linear regression obtained by Oser [24] based on the EAAI.

$$BV = 1.09 \times EAAI - 11.73. \quad (3)$$

The EAAI is calculated as the geometric mean of the ratios of the essential amino acids in a protein relative to their respective amounts in whole egg protein, and it is expressed in percentage [24].

2.5. Statistical Analysis

The effect of processing on amino acids composition was tested with a one-way analysis of variance followed by Tukey HSD to identify the differences. The effect of polyphosphates treatments (0 % - control *vs* 6 % of polyphosphates) on amino acids composition was tested using a t-test for independent samples. All statistical analyses were tested at a 0.05 significance level with the software STATISTICA © from StatSoft Inc. (Tulsa, OK, USA, version 7.0, 2004).

3. Results and Discussion

3.1. Effect of Processing in the Amino Acids Composition of Cod

3.1.1. Brining

In general, the contents of amino acids decreased after brining (Table 1). Still, some exceptions were observed, being the contents of histidine, methionine, and lysine not significantly different from those in the raw material. The highest reduction was observed for glutamic acid, from 2.5 to 2.0 g/100 g ww. The decrease in amino acids contents can be due to the solubilization of free amino acids, peptides, and proteins in the brine. A protein content of *ca*. 0.4-0.5 % was reported in brines after the cod brining (fish:brine ratio of 1:1.4; 18 % NaCl; 36 hours) [25]. Moreover, the considerable penetration of salt into the muscle, which was reported to increase from 0.4 to 7.2 % of salt in cod fillets with the brining (17.5 % NaCl) [9], might be responsible for an apparent decrease in amino acids composition.

Table 1. Total Amino Acids Composition (g/100 g wet weight) and Protein Quality Indexes of Cod Throughout the Processing of Dried Salted Cod. Values are expressed as average ± standard deviation. Different letters (a-e) in the same row denote significant differences ($p < 0.05$) among the different processing steps. Hydroxyproline contents were bellow detection limit. Abbreviations: <DL - below detection limit; rat-PER - rat protein efficiency ratio; EAAI - essential amino acid index; BV - biological value

Amino acids	Raw material	After brining	After dry-salting	After drying	After domestic desalting	After industrial desalting
Essential amino acids (EAA)						
HIS	0.26±0.04^{cd}	0.23±0.02^{d}	0.35±0.03^{b}	0.42±0.01^{a}	0.34±0.03^{b}	0.32±0.02^{bc}
THR	0.74±0.04^{c}	0.59±0.04^{d}	0.98±0.03^{b}	1.14±0.06^{a}	0.86±0.08^{b}	0.87±0.03^{b}
TYR	0.53±0.03^{d}	0.45±0.03^{e}	0.73±0.02^{b}	0.87±0.02^{a}	0.62±0.04^{c}	0.63±0.02^{c}
VAL	0.73±0.05^{c}	0.61±0.04^{d}	0.95±0.02^{b}	1.13±0.03^{a}	0.82±0.05^{c}	0.82±0.02^{c}
MET	0.45±0.08^{bc}	0.38±0.04^{c}	0.57±0.02^{b}	0.69±0.08^{a}	0.49±0.09^{bc}	0.43±0.02^{bc}
PHE	0.60±0.05^{c}	0.50±0.04^{d}	0.77±0.01^{b}	0.92±0.03^{a}	0.65±0.04^{c}	0.65±0.02^{c}
ILE	0.67±0.05^{c}	0.56±0.04^{d}	0.88±0.02^{b}	1.04±0.03^{a}	0.75±0.05^{c}	0.75±0.02^{c}
LEU	1.20±0.08^{d}	1.00±0.07^{e}	1.59±0.04^{b}	1.89±0.05^{a}	1.37±0.10^{c}	1.36±0.04^{c}
LYS	1.11±0.17^{ab}	0.87±0.06^{b}	1.22±0.08^{a}	1.13±0.25^{ab}	1.21±0.25^{ab}	1.19±0.13^{ab}
Σ EAA	6.28±0.38^{c}	5.21±0.34^{d}	8.02±0.23^{b}	9.24±0.31^{a}	7.11±0.61^{c}	7.01±0.31^{c}
Non-essential amino acids (NEAA)						
ASP	1.57±0.10^{c}	1.26±0.09^{d}	2.02±0.06^{b}	2.38±0.06^{a}	1.75±0.12^{c}	1.71±0.05^{c}
GLU	2.51±0.15^{c}	2.00±0.14^{d}	3.23±0.11^{b}	3.80±0.11^{a}	2.80±0.21^{c}	2.72±0.07^{c}
SER	0.73±0.06^{c}	0.56±0.03^{d}	0.90±0.03^{b}	1.05±0.03^{a}	0.82±0.07^{bc}	0.75±0.02^{c}
GLY	0.91±0.21^{a}	0.56±0.02^{b}	0.91±0.12^{a}	0.94±0.05^{a}	0.97±0.14^{a}	0.68±0.04^{ab}
ARG	0.99±0.08^{c}	0.74±0.06^{d}	1.25±0.06^{b}	1.44±0.04^{a}	1.12±0.09^{bc}	1.04±0.03^{c}
ALA	0.97±0.09^{c}	0.73±0.04^{d}	1.15±0.05^{b}	1.33±0.04^{a}	1.05±0.09^{bc}	0.95±0.03^{c}
TAU	0.14±0.04^{a}	0.08±0.01^{b}	0.06±0.01^{b}	0.07±0.01^{b}	<DL	<DL
PRO	0.62±0.14^{b}	0.43±0.03^{c}	0.71±0.06^{ab}	0.82±0.06^{a}	0.74±0.11^{ab}	0.57±0.02^{bc}
Σ NEAA	8.44±0.80^{c}	6.36±0.38^{d}	10.23±0.47^{b}	11.81±0.35^{a}	9.26±0.81^{bc}	8.42±0.21^{c}
Σ AA	14.73±1.12^{c}	11.57±0.72^{d}	18.25±0.69^{b}	21.05±0.62^{a}	16.37±1.39^{bc}	15.43±0.52^{c}
EAA/NEAA	0.75±0.05^{b}	0.82±0.01^{a}	0.78±0.02^{ab}	0.78±0.02^{ab}	0.77±0.03^{ab}	0.83±0.02^{a}
rat-PER	2.91±0.12^{a}	3.05±0.04^{a}	2.97±0.04^{a}	2.94±0.05^{a}	2.93±0.07^{a}	3.07±0.04^{a}
EAAI (%)	70.7±2.6^{c}	80.2±2.6^{a}	84.3±3.5^{a}	74.5±2.6^{bc}	77.9±1.3^{ab}	78.1±2.6^{ab}
BV (%)	65.4±2.8^{c}	75.7±2.9^{a}	80.2±3.8^{a}	69.5±2.9^{bc}	73.2±1.5^{ab}	73.5±2.8^{ab}

Amino acids contents expressed in a dry basis (Table 2) revealed a decreased for all amino acids after brining. A similar decrease was observed for free amino acids taurine, glycine, and alanine, in previous studies [6,7]. The reported contents of free taurine by Larsen *et al*. [7] in fresh cod (0.786 g/100 g dw) were comparable to those obtained in the current study, reflecting the free character of taurine, which explains its release from cod muscle in the brine. The losses of amino acids with the brining step represent a reduction of nutritional quality. In particular, taurine losses are critical as this amino acid is important during the development of the central nervous system and muscle in humans, among other physiological functions [26].

Amino acids contents were also expressed per 100 g of amino acids to understand if the variations of their contents resulted in a differential release during each processing step (Table 3). After brining, only glycine and taurine contents decreased and their loss amounted to *ca*. 1.4 g/100 g of amino acids, indicating that these amino acids were released to the brine in a higher proportion than the remaining amino acids. In its turn, the proportion of tyrosine, phenylalanine, and leucine increased, possible due to its location in the muscle proteins. In particular, amino acids with a hydrophobic side chain, like phenylalanine and leucine, are normally located on the interior of the protein due to the lower propensity to be in contact with water [27].

3.1.2. Dry-Salting

Amino acids contents (wet weight) increased with the dry-salting procedure, being the highest increase observed for glutamic acid (*ca*. 1.2 g/100 g ww) (Table 1). Only taurine contents did not follow the same trend, being the levels not significantly different from those before dry-salting.

Lakerda, a Turkish traditional salted fish product produced from Atlantic bonito (*Sarda sarda*) which was salted by brining and wet-salting, experienced a similar trend as observed for cod, (i) increase of valine and glutamic acid + glutamine contents, and (ii) no changes in the contents of proline, lysine, and glycine with the salting processing (brining and wet-salting) [28].

The increase observed in amino acids contents of cod is explained due to a considerable water loss (*ca*. 20 %) characteristic of this processing step. However, results expressed in a dry basis (Table 2) indicate that amino acids might have been lost with the water drained during dry-salting. Specifically, the contents of glycine and proline were not changed with the dry-salting, but for the remaining amino acids values decreased significantly. The highest change was observed for glutamic acid, with the values dropping 2.2 g/100 g dw. For comparison purposes, a previous study showed that free amino acids in Chilled round scad *Decapterus maruadsi* decreased with the dry-salting process [29]. Moreover, wastewater from the dry-salting process of cod presented mainly glutamic acid, aspartic acid, taurine, and tryptophan, and their contents reached a total of *ca*. 2.3 g/L after 5 days [30].

During the 5 months period of the dry-salting step of cod, proteolysis by endogenous enzymes might have occurred, contributing for the degradation of proteins, and the release of amino acids from the muscle by means of drip loss. In fact, in the end of dry-salting process of Chilled round scad, several enzymes, such as cathepsins (B, L, and H), dipeptidyl peptidases (I and IV), and aminopeptidases (alanyl aminopeptidase, arginine aminopeptidase, and leucyl aminopeptidase), demonstrated activity [29].

The decrease of some amino acids contents, such as tyrosine, histidine, lysine, and arginine, might also be due to the decarboxylation of these amino acids producing biogenic amines [31]. Ferraro *et al*. [30] determined a concentration of biogenic amines of 124 mg/L in wastewater from the dry-salting process of cod, and related these to proteolysis and release from codfish muscle before the salting process.

Furthermore, the amount of salt in wet salted cod should not be lower than 16 % of NaCl, accordingly to the legislation [32]. This further increase in salt content during the dry-salting step contributes for an increase of the dry matter of cod muscle, and apparent decrease of amino acids contents.

After dry-salting, the proportion of the different amino acids was kept for almost all amino acids (Table 3). Only the contents of taurine decreased (*ca*. 0.4 g/100 g of amino acids), and in its turn those of arginine increased approximately the same amount.

3.1.3. Drying

In dried salted cod, lysine, glycine, taurine, and proline contents were not significantly different from those observed before this processing step (Table 1). However, for the remaining amino acids, the contents increased significantly after drying, reaching the highest values observed during the processing of dried salted cod. Such increase can be the result of the further decrease in water content. In fact, considering the results expressed in dry weight or in terms of amino acids proportion, drying did not cause significant differences for all amino acids contents (Table 2 and 3), indicating that amino acids were not lost during this processing step.

In comparison, previous studies reported changes in the amino acids composition of shrimps after sun-drying: (i) arginine, phenylalanine, lysine, and leucine concentrations increased in smoked black giant tiger shrimp (*Penaeus monodon*) and (ii) arginine contents increased in smoked Southern pink shrimp (*Penaeus notialis*) [33,34]. For other seafood products also subjected to drying, reported results evidenced changes in amino acids composition of fish flakes prepared with stingray (*Himantura gerrardi*) [35] and also in processed squid (*Todarodes pacificus*) [36], but such differences account for several processing steps.

3.1.4. Desalting

In general, after desalting, the contents of amino acids decreased, especially for glutamic acid (Table 1). As exceptions, glycine and lysine contents after domestic and industrial desalting, and proline contents after domestic desalting were not significantly different from those before desalting. Chilled round scad also experienced losses in the contents of free amino acids, especially histidine and methionine, during desalting [29].

Table 2. Total Amino Acids Composition (g/100 g dry weight) of Cod Throughout the Processing of Dried Salted Cod. Values are expressed as average ± standard deviation. Different letters (a-e) in the same row denote significant differences ($p < 0.05$) among the different processing steps. Hydroxyproline contents were bellow detection limit. Abbreviations: <DL - below detection limit

Amino acids	Raw material	After brining	After dry-salting	After drying	After domestic desalting	After industrial desalting
Essential amino acids (EAA)						
HIS	1.46±0.24^{a}	1.15±0.08^{b}	0.85±0.07^{c}	0.91±0.01^{c}	1.59±0.09^{a}	1.58±0.04^{a}
THR	4.15±0.23^{a}	3.00±0.18^{b}	2.41±0.06^{c}	2.45±0.14^{c}	4.03±0.20^{a}	4.34±0.12^{a}
TYR	2.99±0.08^{a}	2.31±0.20^{b}	1.79±0.04^{c}	1.87±0.04^{c}	2.91±0.09^{a}	3.13±0.09^{a}
VAL	4.08±0.12^{a}	3.10±0.29^{b}	2.34±0.03^{c}	2.43±0.06^{c}	3.84±0.17^{a}	4.06±0.14^{a}
MET	2.50±0.36^{a}	1.96±0.26^{b}	1.40±0.05^{c}	1.48±0.16^{c}	2.29±0.46^{ab}	2.14±0.13^{ab}
PHE	3.36±0.15^{a}	2.55±0.25^{b}	1.89±0.02^{c}	1.98±0.04^{c}	3.06±0.11^{a}	3.23±0.13^{a}
ILE	3.76±0.12^{a}	2.86±0.28^{b}	2.16±0.03^{c}	2.25±0.07^{c}	3.52±0.19^{a}	3.74±0.12^{a}
LEU	6.73±0.19^{a}	5.11±0.47^{b}	3.90±0.07^{c}	4.07±0.09^{c}	6.42±0.22^{a}	6.75±0.31^{a}
LYS	6.24±0.95^{a}	4.45±0.38^{b}	3.02±0.19^{c}	2.43±0.53^{c}	5.61±0.88^{ab}	5.91±0.48^{ab}
Σ EAA	35.27±1.07^{a}	26.49±2.31^{b}	19.75±0.43^{c}	19.87±0.54^{c}	33.27±1.54^{a}	34.88±1.24^{a}
Non-essential amino acids (NEAA)						
ASP	8.80±0.23^{a}	6.39±0.46^{b}	4.97±0.12^{c}	5.12±0.12^{c}	8.21±0.38^{a}	8.51±0.28^{a}
GLU	14.11±0.26^{a}	10.16±0.79^{b}	7.96±0.23^{c}	8.17±0.25^{c}	13.13±0.77^{a}	13.51±0.43^{a}
SER	4.08±0.21^{a}	2.83±0.19^{b}	2.22±0.07^{c}	2.25±0.06^{c}	3.86±0.27^{a}	3.73±0.16^{a}
GLY	5.10±1.02^{a}	2.87±0.27^{cd}	2.23±0.29^{cd}	2.01±0.08^{d}	4.54±0.56^{ab}	3.41±0.31^{bc}
ARG	5.58±0.27^{a}	3.76±0.14^{b}	3.09±0.13^{c}	3.09±0.08^{c}	5.24±0.29^{a}	5.18±0.21^{a}
ALA	5.42±0.34^{a}	3.70±0.31^{c}	2.83±0.10^{d}	2.86±0.07^{d}	4.89±0.32^{ab}	4.72±0.21^{b}
TAU	0.80±0.19^{a}	0.40±0.11^{b}	0.14±0.02^{c}	0.15±0.01^{c}	<DL	<DL
PRO	3.46±0.67^{a}	2.21±0.22^{bc}	1.76±0.14^{c}	1.76±0.12^{c}	3.45±0.49^{a}	2.84±0.19^{ab}
Σ NEAA	47.35±2.85^{a}	32.33±2.40^{c}	25.19±1.00^{d}	25.40±0.67^{d}	43.33±3.06^{ab}	41.90±1.78^{b}
Σ AA	82.61±3.20^{a}	58.82±4.70^{b}	44.94±1.39^{c}	45.28±1.08^{c}	76.60±4.45^{a}	76.78±2.93^{a}

Table 3. Total Amino Acids Composition (g/100 g of amino acids) of Cod Throughout the Processing of Dried Salted Cod. Values are expressed as average ± standard deviation. Different letters in the same row (a-c) denote significant differences ($p < 0.05$) among the different processing steps, while different letters in the same column (x-y) denote significant differences ($p < 0.05$) between control and polyphosphates treatments for each amino acid. Hydroxyproline contents were bellow detection limit. Abbreviations: PP - polyphosphates; <DL - below detection limit

Amino acids	Treatment	Raw material	After brining	After dry-salting	After drying	After domestic desalting	After industrial desalting
Essential amino acids (EAA)							
HIS	control	1.71±0.26^{a}	1.95±0.14$^{a\ x}$	1.87±0.12$^{a\ x}$	2.02±0.03$^{a\ x}$	1.99±0.02$^{a\ x}$	2.06±0.05$^{a\ x}$
	6 % PP		1.92±0.09$^{a\ x}$	1.95±0.09$^{a\ x}$	1.98±0.04$^{a\ x}$	2.03±0.08$^{a\ x}$	2.06±0.14$^{a\ x}$
THR	control	4.88±0.37^{b}	5.07±0.19$^{ab\ x}$	5.29±0.20$^{ab\ x}$	5.41±0.29$^{ab\ x}$	5.04±0.45$^{ab\ x}$	5.66±0.07$^{a\ x}$
	6 % PP		5.01±0.18$^{b\ x}$	5.19±0.25$^{ab\ x}$	5.44±0.23$^{ab\ x}$	5.78±0.05$^{a\ x}$	5.76±0.14$^{a\ x}$
TYR	control	3.53±0.23^{d}	3.90±0.10$^{abc\ x}$	3.93±0.16$^{abc\ x}$	4.14±0.06$^{a\ x}$	3.63±0.12$^{cd\ x}$	4.08±0.04$^{ab\ x}$
	6 % PP		3.81±0.16$^{b\ x}$	3.98±0.21$^{ab\ x}$	4.17±0.04$^{a\ x}$	4.02±0.03$^{ab\ x}$	4.04±0.02$^{ab\ x}$
VAL	control	4.81±0.25^{b}	5.24±0.16$^{ab\ x}$	5.13±0.20$^{ab\ x}$	5.36±0.12$^{a\ x}$	4.79±0.13$^{b\ x}$	5.29±0.04$^{ab\ x}$
	6 % PP		5.08±0.19$^{a\ x}$	5.18±0.24$^{a\ x}$	5.36±0.09$^{a\ x}$	5.24±0.05$^{a\ x}$	5.29±0.01$^{a\ x}$
MET	control	2.94±0.38^{a}	3.30±0.21$^{a\ x}$	3.06±0.13$^{a\ x}$	3.27±0.37$^{a\ x}$	2.84±0.45$^{a\ x}$	2.79±0.06$^{a\ x}$
	6 % PP		3.13±0.19$^{a\ x}$	3.28±0.31$^{a\ x}$	3.25±0.27$^{a\ x}$	2.61±0.03$^{a\ x}$	2.69±0.15$^{a\ x}$
PHE	control	3.95±0.17^{b}	4.30±0.15$^{a\ x}$	4.16±0.18$^{ab\ x}$	4.37±0.10$^{a\ x}$	3.82±0.11$^{b\ x}$	4.20±0.02$^{ab\ x}$
	6 % PP		4.18±0.19$^{a\ x}$	4.18±0.19$^{a\ x}$	4.35±0.08$^{a\ x}$	4.11±0.08$^{a\ x}$	4.15±0.03$^{a\ x}$
ILE	control	4.42±0.24^{b}	4.82±0.15$^{ab\ x}$	4.74±0.19$^{ab\ x}$	4.96±0.13$^{a\ x}$	4.40±0.09$^{b\ x}$	4.87±0.03$^{ab\ x}$
	6 % PP		4.67±0.18$^{a\ x}$	4.78±0.23$^{a\ x}$	4.96±0.09$^{a\ x}$	4.83±0.05$^{a\ x}$	4.88±0.01$^{a\ x}$
LEU	control	7.92±0.42^{c}	8.63±0.22$^{ab\ x}$	8.58±0.37$^{ab\ x}$	8.99±0.11$^{a\ x}$	8.01±0.14$^{bc\ x}$	8.79±0.08$^{ab\ x}$
	6 % PP		8.37±0.36$^{b\ x}$	8.62±0.38$^{ab\ x}$	9.03±0.11$^{a\ x}$	8.68±0.09$^{ab\ x}$	8.81±0.02$^{ab\ x}$
LYS	control	7.36±1.28^{a}	7.51±0.17$^{a\ x}$	6.61±0.22$^{ab\ x}$	5.36±1.13$^{b\ x}$	7.01±1.05$^{ab\ x}$	7.69±0.56$^{a\ x}$
	6 % PP		6.96±0.65$^{a\ x}$	6.08±0.48$^{ab\ x}$	5.31±0.83$^{b\ x}$	7.81±0.31$^{a\ x}$	7.56±0.23$^{a\ x}$
Σ EAA	control	42.7±1.5^{b}	45.0±0.4$^{a\ x}$	44.0±0.6$^{ab\ x}$	43.9±0.6$^{ab\ x}$	43.5±1.0$^{ab\ x}$	45.4±0.5$^{a\ x}$
	6 % PP		44.3±1.1$^{a\ x}$	43.5±0.9$^{a\ x}$	43.8±0.5$^{a\ x}$	45.1±0.6$^{a\ x}$	45.2±0.2$^{a\ x}$
Non-essential amino acids (NEAA)							
ASP	control	10.35±0.44^{bc}	10.81±0.23$^{abc\ x}$	10.90±0.33$^{abc\ x}$	11.31±0.10$^{a\ x}$	10.25±0.20$^{c\ x}$	11.09±0.10$^{ab\ x}$
	6 % PP		10.60±0.31$^{b\ x}$	11.03±0.37$^{ab\ x}$	11.29±0.06$^{a\ x}$	10.98±0.05$^{ab\ x}$	11.00±0.07$^{ab\ x}$
GLU	control	16.61±0.75^{b}	17.17±0.37$^{ab\ x}$	17.48±0.54$^{ab\ x}$	18.04±0.23$^{a\ x}$	16.38±0.24$^{b\ x}$	17.60±0.17$^{a\ x}$
	6 % PP		16.73±0.40$^{b\ x}$	17.66±0.54$^{ab\ x}$	18.12±0.08$^{a\ x}$	17.65±0.26$^{ab\ x}$	17.60±0.09$^{ab\ x}$
SER	control	4.79±0.05^{b}	4.79±0.09$^{b\ x}$	4.87±0.10$^{ab\ x}$	4.98±0.04$^{a\ x}$	4.81±0.11$^{ab\ x}$	4.85±0.04$^{ab\ x}$
	6 % PP		4.76±0.03$^{b\ x}$	4.93±0.07$^{a\ x}$	5.01±0.05$^{a\ x}$	4.87±0.03$^{ab\ x}$	4.86±0.01$^{ab\ x}$
GLY	control	5.95±0.89^{a}	4.84±0.25$^{b\ x}$	4.87±0.41$^{b\ x}$	4.44±0.11$^{b\ x}$	5.65±0.36$^{ab\ x}$	4.44±0.24$^{b\ x}$
	6 % PP		5.06±0.61$^{a\ x}$	5.04±0.69$^{a\ x}$	4.42±0.12$^{a\ x}$	4.64±0.35$^{a\ x}$	4.55±0.18$^{a\ x}$
ARG	control	6.55±0.10^{bc}	6.37±0.25$^{c\ x}$	6.78±0.12$^{a\ x}$	6.82±0.03$^{a\ x}$	6.54±0.05$^{abc\ y}$	6.74±0.03$^{ab\ x}$
	6 % PP		6.52±0.05$^{b\ x}$	6.86±0.07$^{a\ x}$	6.86±0.04$^{a\ x}$	6.86±0.06$^{a\ x}$	6.82±0.03$^{ab\ x}$
ALA	control	6.37±0.07^{a}	6.26±0.08$^{ab\ x}$	6.20±0.11$^{b\ x}$	6.31±0.07$^{ab\ x}$	6.10±0.03$^{b\ x}$	6.14±0.05$^{b\ x}$
	6 % PP		6.17±0.09$^{a\ x}$	6.27±0.09$^{a\ x}$	6.28±0.05$^{a\ x}$	6.15±0.07$^{a\ x}$	6.17±0.07$^{a\ x}$
TAU	control	0.93±0.19^{a}	0.67±0.14$^{b\ x}$	0.31±0.06$^{c\ x}$	0.33±0.04$^{c\ x}$	<DL	<DL
	6 % PP		0.66±0.16$^{a\ x}$	0.27±0.04$^{b\ x}$	0.28±0.03$^{b\ x}$	<DL	<DL
PRO	control	4.04±0.60^{a}	3.72±0.10$^{a\ x}$	3.85±0.14$^{a\ x}$	3.89±0.27$^{a\ x}$	4.29±0.39$^{a\ x}$	3.70±0.13$^{a\ x}$
	6 % PP		3.75±0.20$^{a\ x}$	3.98±0.22$^{a\ x}$	3.90±0.26$^{a\ x}$	3.74±0.10$^{a\ x}$	3.77±0.08$^{a\ x}$
Σ NEAA	control	57.3±1.5^{a}	55.0±0.4$^{b\ x}$	56.0±0.6$^{ab\ x}$	56.1±0.6$^{ab\ x}$	56.5±1.0$^{ab\ x}$	54.6±0.5$^{b\ x}$
	6 % PP		55.7±1.1$^{a\ x}$	56.5±0.9$^{a\ x}$	56.2±0.5$^{a\ x}$	54.9±0.6$^{a\ x}$	54.8±0.3$^{a\ x}$

The sudden increase of moisture could explain some apparent loss in the amino acids contents, but the loss of salt must also be taken into consideration. Amino acids contents, expressed in dry weight, raised with the desalting procedure being the highest increase observed for glutamic acid (Table 2).

After desalting, the proportion of most amino acids did not change for cod desalted in industrial environment (Table 3). As exceptions, taurine contents decreased to levels below detection limit, while those of lysine increased. On the other hand, cod desalted in domestic environment evidenced a significant decrease in the contents of aspartic acid, glutamic acid, taurine, tyrosine, valine, phenylalanine, isoleucine, and leucine. To balance, other amino acids contents increased, although not significantly.

The amino acids loss is explained by the solubilization of amino acids released from the muscle to the desalting water. In the study of Wu & Cao [29] on Chilled round scad, during drying, proteolysis increased as well as the activity of several endogenous enzymes, such as cathepsin B and L, and leucyl and arginine aminopeptidases, and free amino acids were mainly produced during this processing stage. A similar protein degradation mechanism might have occurred in cod, which would explain the release of produced free amino acids in the following processing stage (desalting).

3.2. Effect of Polyphosphates Addition to Cod during Processing

Polyphosphates were added during brining to evaluate their effects in the nutritional quality of cod during the processing of dried salted cod. Polyphosphates interact with proteins and are well known to increase the water holding capacity of food products, which could influence the diffusion and loss of amino acids from cod muscle.

After brining, total phosphates levels were *ca*. 6 times higher in cod treated with polyphosphates than in control [17]. Despite this difference, polyphosphates did not affect the amino acids composition (ww or dw; data not shown) of cod in this processing step. In the study of Larsen *et al*. [7], brine also had polyphosphates, but it is not conclusive if the effects observed in free amino acids composition were accounted to the presence of these additives, due to the lack of a control treatment without polyphosphates.

In wet salted cod, polyphosphates affected the amino acids composition of wet salted cod, although total phosphates levels decreased to *ca*. 5 g P_2O_5/kg during this processing step [17]. Threonine and lysine contents were lower in wet salted cod processed with polyphosphates (0.9 and 1.1 g/100 g ww, respectively), while no statistical differences were found in the remaining amino acids contents, when comparing with the control. Results expressed in dry weight reveal differences among treatments for more amino acids. In particular, wet salted cod treated with polyphosphates presented lower levels of threonine, phenylalanine, leucine, lysine, and serine, being the highest significant difference among treatments observed for lysine (2.6 g/100 g dw in cod with polyphosphates).

Phosphates loosen the electrostatic forces within the actomyosin complex contributing to the solubility of muscular proteins, as a result of the binding of the negatively charged phosphate ions with the positively charged Mg^{2+} or Ca^{2+} ions, that play a vital role in muscle contraction [37]. In this sense, the presence of polyphosphates might have increased the protein solubility, in comparison with cod processed only with sodium chloride. During dry-salting, amino acids might have been lost in the water drained out, together with added phosphates.

The loss of amino acids during dry-salting, especially in the treatment with polyphosphates, also affected their contents in the following processing step. Dried cod processed with polyphosphates had lower amino acids contents than control samples, although not significant for methionine and lysine (considering data in wet weight), and for glycine, threonine, and proline (considering data in dry weight). The highest difference between treatments was observed for glutamic acid, followed by aspartic acid and leucine (7.8 g/100 g dw, 4.9 g/100 g dw, and 3.9 g/100 g dw, respectively, in dried salted cod processed with polyphosphates).

In desalted cod, no significant differences were found in the amino acids composition (data in wet weight) between control and polyphosphates treated cod, for both desalting procedures. However, results expressed in dry weight showed some differences. In particular, threonine, isoleucine and leucine contents were higher in cod with polyphosphates (4.5 g/100 g dw, 3.8 g/100 g dw, and 6.8 g/100 g dw, respectively) after domestic desalting, while threonine and tyrosine levels were higher in cod with polyphosphates (4.7 g/100 g dw and 3.3 g/100 g dw, respectively) after industrial desalting.

In general, the proportion of the different amino acids was not changed due to the presence of polyphosphates, compared with the control treatment, in all processing steps (Table 3). Only arginine levels were significantly higher in the treatment with polyphosphates in cod after domestic desalting.

Wet salted cod or green cod is a heavily salted cod product, not dried, which is also appreciated in south Europe. Being a problem for consumers of wet salted cod, yellow-brown discoloration is overcome with the use of polyphosphates, also added to avoid lipid oxidation [15]. It is conceivable to assume that changes in amino acids of wet salted cod during desalting will be similar to those reported here, as drying did not cause major changes in amino acids composition.

3.3. Nutritional Quality of Fresh Cod (Raw Material)

The amino acids composition of fresh cod muscle shows that the total amino acids content was 14.7 g/100 g ww, being glutamic acid, aspartic acid, leucine, and lysine the most abundant amino acids, which accounts for more than 40 % of total amino acids (Table 1). These amino acids were also reported as being among the most abundant in the muscle of several marine fish species [38,39,40]. In contrast, hydroxyproline contents in fresh cod were below the detection limit of the method.

The amino acids composition obtained in our study is comparable to the one described by Jensen *et al*. [4], especially for wild cod. In particular for other similar species, Pacific cod (*Gadus macrocephalus*) and Alaska

pollock (*Gadus chalcogrammus*) also presented glutamic acid, aspartic acid, leucine, and lysine as the most abundant amino acids [40], but the amino acids contents were, in general, higher than those of cod analyzed in the current study.

Regarding the non-essential amino acids, the most abundant ones were glutamic acid, aspartic acid, arginine, and alanine (Table 1). Some of these, such as glutamic and aspartic acids, dissolve well in water, and are well-known for producing the umami taste [41], and may explain the characteristic sensory properties of cod. In relation to essential amino acids, which cannot be synthesized in the body, and thus need to be supplied by the diet [21], leucine, lysine, threonine, and valine were those with the major contents (Table 1).

Protein quality indexes are presented in Figure 1 and Table 1. The highest essential amino acids scores were obtained for threonine (219±14 %) and phenylalanine+tyrosine (203±7 %), while histidine had the lowest score (99±17 %) in fresh cod. To meet the daily requirements for this amino acid, it is necessary a portion with *ca*. 270 g of fresh cod (for an adult with 70 kg). The ratio between essential and non-essential amino acids was high in fresh cod. The estimated rat-PER was comparable to those obtained by Bechtel & Johnson [42] for Alaska pollock and Pacific cod. In relation to EAAI, cod muscle accounted for 70.1 % of the essential amino acids contents in the reference protein (whole egg), being valine the amino acid with the lowest amount face to the reference protein. In terms of nitrogen retained by the organism, the estimated BV shows a 65.4 % value for fresh cod. For comparison purposes, the EAAI estimated based on the reported amino acids composition of several marine fish species showed values of 57-73 %, while BV was 51-67% [43].

3.4. Nutritional Quality of Desalted Cod

Total amino acids contents of desalted cod were *ca*. 15-16 g/100 g ww (Table 1). In general, the amino acids composition presented values close to those observed in fresh cod. Still, significant higher levels of histidine (only in cod desalted in domestic environment), threonine, tyrosine, and leucine were determined, while those of taurine were lower in desalted cod. Such differences were lower than 0.2 g/100 g ww and the apparent increase of several amino acids concentration agrees with the lower moisture levels of desalted cod (78-80% in desalted cod *vs* 82% in raw material) [17]. Results also showed that the contents of amino acids in desalted cod were not influenced by the type of desalting procedure, domestic or industrial (Table 1 and 2).

In general, the protein quality of desalted cod was higher than that of fresh cod (Figure 1 and Table 1). Desalted cod products presented amino acid scores higher than 100 % for all essential amino acids. Compared with fresh cod, only leucine, isoleucine, phenylalanine+tyrosine, and valine in desalted cod (processed industrially) had significantly higher scores. In desalted cod, the ratio between essential and non-essential amino acids was higher than in fresh cod, although not significantly for cod desalted with the domestic procedure. The index rat-PER presented similar values of those of fresh cod muscle. Another protein quality index showed that the essential amino acids contents of desalted cod muscle were more similar to those of the protein reference (whole egg) than those of fresh cod, and thus the estimated BV was also significantly higher in desalted cod.

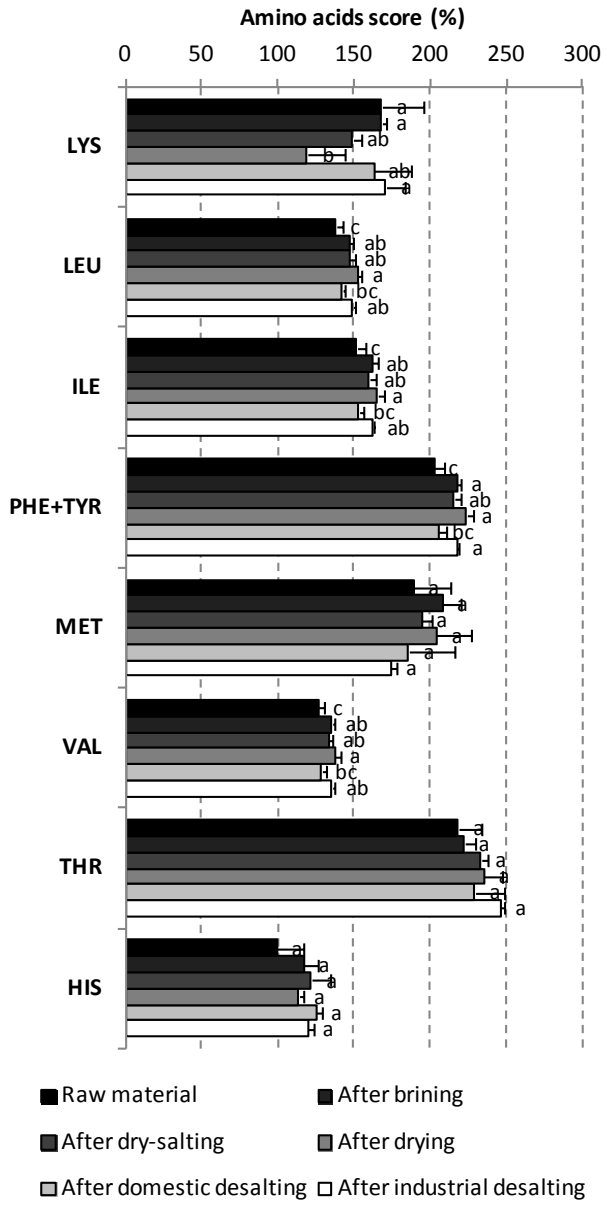

Figure 1. Amino acid scores of cod throughout the processing of dried salted cod. Bars represent the average, and error bars the standard deviation. Different letters (a-c) denote significant differences ($p < 0.05$) among the different processing steps for each amino acid

4. Conclusions

This study shows that processing affected the amino acids composition of cod muscle. Differences in the nutritional composition of cod might be due to the solubilization of amino acids in brine, in drained water during dry-salting, and also in the desalting water, which resulted in the loss of amino acids. Moreover, changes in amino acids contents were also explained based on variations in moisture and salt levels characteristics of the processing.

The addition of polyphosphates affected also the contents of some amino acids in wet salted cod (*e.g.* lower levels of threonine and lysine), dried salted cod (lower levels of all amino acids especially glutamic acid, aspartic acid, and leucine), and desalted cod (*e.g.* higher levels of threonine). However, the proportion of the different amino acids was preserved.

This study also showed that both fresh and desalted cod contained all essential amino acids and the different protein quality indexes indicate that cod muscle is a high quality protein source. Histidine had the lowest amino acid score, but still cod muscle meets 99 % of the requirements of this amino acid in the diet of an adult.

The nutritional composition presented in this study gives important information to the fishing industry, nutritionists, and consumers.

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