Protein removal from waste brines generated during ham salting through acidification and centrifugation

Journal:	*Journal of Food Science*
Manuscript ID:	Draft
Manuscript Type:	5 Food Engineering and Physical Properties
Date Submitted by the Author:	n/a
Complete List of Authors:	Gutierrez, Rosario; Politechnic University of Valencia, Food Science and Technology Muñoz-Guerrero, Hernan; Universidad de Antofagasta, Departamento de Alimentos Alcaína-Miranda, M. Isabel; Politechnic University of Valencia, Departamento de Ingeniería Química y Nuclear Barat, Jose; Politechnic University of Valencia, Food Science and Technology
Keywords:	residues, mathematical modeling, food engineering, salt, osmotic dehydration

Protein removal from waste brines generated during ham salting through acidification and centrifugation

Gutiérrez-Martínez[2], M.R.; Muñoz-Guerrero[3], H.; Alcaína-Miranda[2], M.I.; Barat[1*], J.M.

[1*]Departamento de Tecnología de Alimentos, Universitat Politècnica de València, Camino de Vera s/n, Valencia (46022), Spain. E-Mail: jmbarat@tal.upv.es

[2]Departamento de Ingeniería Química y Nuclear, Universitat Politècnica de València. Camino de Vera s/n, Valencia (46022), Spain. E-Mail: magumar@doctor.upv.es

[3]Departamento de Alimentos, Facultad de Recursos del Mar, Universidad de Antofagasta. Avda. Angamos 601, Antofagasta (1270300), Chile. E-Mail: hmunoz@uantof.cl

***Author for correspondence: Jose M Barat**

E-mail jmbarat@tal.upv.es

Phone: +34 963877365

Fax : +34 963877369

ABSTRACT

The salting step in food processes implies the production of large quantities of waste brines having high organic load, high conductivity and other pollutants with high oxygen demand. Direct disposal of the residual brine implies salinization of soil and eutrophication of water. Since most of the organic load of the waste brines comes from proteins leaked from the salted product, precipitation of dissolved proteins by acidification and removal by centrifugation is an operation to be used in waste brine cleaning.

The aim of this study is optimizing the conditions for carrying out the separation of proteins from waste brines generated in the pork ham salting operation, by studying the influence of pH, centrifugal force and centrifugation time.

Models for determining the removal of proteins depending on the pH, centrifugal force and time were obtained. The results showed a high efficacy of the proposed treatment for removing proteins, suggesting that this method could be used for waste brine protein removal.

Keywords: waste brine, protein removal, protein precipitation, ham salting, acidification.

1. INTRODUCTION

Dry-cured ham is the most important processed meat product in Spain and well known worldwide for its excellent sensory characteristics (Toldrá, 2010).

The manufacturing process of dry-cured ham may vary depending on the traditions of each area of production (Toldrá, 2010), however there are some common stages: pre-conditioning of the raw material, curing, salting, post-salting or settlement, drying, aging or maturation and refinement (Barat et al., 2004). The process generates solid and liquid waste (sewage). The dry-salting step is mainly accomplished in plastic or metallic containers that allow the drainage of the generated brine (Barat et al., 2006). The waste brine is composed primarily by water, minerals, blood, proteins and other soluble compounds (Barat et al., 2006). The waste brine exceeds most of the limits permitted by law for direct disposal, and thus it must be treated before disposal. Nevertheless, the treatment of the waste brine can't be done through conventional treatments because of its very high conductivity as a consequence of the high salt content (Barat et al., 2006). Thus it must be managed as a special waste, with an increase in the production cost in addition to the environmental impact.

Figure 1 shows the general dry-salting process of Spanish dry-cured ham, were the wastes generated during the process have been highlighted.

Figure1.

Barat et al. (2006) estimated the production of saturated residual brine during Spanish dry-cured ham production in the year 2003, which was in the

range from 29000 to 31700 m^3 / year, corresponding to nearly the 10% of the weight of the raw hams.

Due to the large variety of stages involved in the overall process, the residual brine has a high content of pollutants. The levels of chlorides, chemical oxygen demand (COD), nitrate and total suspended solids obtained exceeded the limits established by law (L.BOP, 1995) (Barat et al., 2006) for their direct discharge, as they are likely to cause harmful effects to the environmental status of waters. The main problems are related to eutrophication due to the presence of salts such as nitrates and phosphates; unfavourable oxygen balance due to the presence of organic compounds, and soil and water salinization due to the high electrical conductivity, as a consequence of the high concentration of salts (Buckley et al., 1987).

Waste brines coming from ham salting are rich in proteins, since the raw ham contains around 20% of proteins, free amino acids, dipeptides, and nucleotides (Warris, 2003). The proteins found in the brine are soluble in concentrated salt solutions and myofibrillar type (Lawrie,1998) such as actin, tropomyosin, troponin, α-actinin, β-actinin, myosin, protein C, protein M. Most proteins are denatured at relatively low temperatures (<60 ºC) and also in acidic conditions, a fact used to produce protein precipitation in the brine to treat (Cheftel, 1989).

1.1 Waste brine treatment alternatives

To minimize the environmental impact of waste brines, various techniques have been used such as natural evaporation, forced evaporation, or vacuum evaporation (Diez et al., 2000). One disadvantage of this approach is that the

residual solid obtained must be discharged as a special polluting waste, and the high consumption of resources, especially energy.

Another alternative technique is the regeneration of the brine for reuse (Barat et al., 2005). This approach consists in the removal of the existing organic nature solutes in the brine (Cuartas et al., 2007) with the consequent recovery of usable substances that currently are discarded (Bes-Pía et al., 2008). Because most of the organic pollution of the brine are proteins, the aim of this work is the study of protein removal by acidic precipitation and centrifugation, as a pre-treatment before filtration stages, allowing the increase in the yield of the filtration processes and delaying the fouling of membranes (Lee et al., 2004).

2. MATERIALS AND METHODS.

2.1 Waste brine

The waste brine used in this study was obtained from a dry-cured ham processing plant. A total of 50 kg of drained brine from salting containers containing hams with a weight ranging from 11 to 12 kg were collected for the study.

2.2 Brine characterization analysis

The main physicochemical properties of the waste brine were determined, as well as those of the main parameters related to their polluting load.

Those determinations were: chloride, pH, conductivity, total solids, chemical oxygen demand (COD), nitrates and fats.

A 1:2000 dilution of the brine was done to determine the chloride content by means of a chloride analyzer (CIBA Corning Mod 926) (Barat et, al., 2006). A dilution of 1:1000 was used for determination sample pH and conductivity, and was measured with a pH meter CRISON® model MM 40 (CRISON Instrument SA, Barcelona, Spain) with a PT100 sensor for temperature compensation.

The COD measurement was carried out by photometric determination of the chromate concentration two hours after oxidation with potassium dichromate, sulphuric acid and silver sulphate, using a photometer NANOCOLOR model 300D (Macherey-Nagel GmbH & Co. KG) and Test 0-33 (NANOCOLOR COD 300 - DIN 34409-H41-1 and in accordance with ISO 15705). Since chloride content higher than 1500 mg/L causes interference in the method, it was necessary to dilute the sample (dilution 1:50 v/v). For the determination of nitrates in the brine a photometer NANOCOLOR®300D(Macherey-Nagel GmbH& Co. KG) was used, with the kit reagents and Test 0-64 (NANOCOLOR ® Nitrate 50. DIN 38 405-D9-3). This analysis is based on the photometric determination with 2,6-dimethylphenol, in a mixture of sulfuric /phosphoric acid. A dilution of 1:2000 brine was done to ensure that the sample is within the range where there was no interference, both from chlorine and nitrite. Total solids in the brine were determining by means of the method APHA 2540 B and fat content was determined using an automatic unit Soxhlet™ 2055 (APHA 5520D, 1998) FOOs, Hillerod, Denmark.

Analytical determinations were done in triplicate and the Statgraphics®Plus 5.1 sotware was used to do the statistic of the obtained results.

2.3 Acidification and centrifugation experiments

Denaturation of proteins in the residual brine was carried out by means of acidification (Lawrie, 1998). Considering that the isoelectric point of proteins is in the range of pH from 4.5 and 5 (Flores, 1997), the waste brine pH was adjusted from the initial brine pH (6.2±0.01) to 2, 3, 4 and 5 by means of concentrated hydrochloric acid (5N) to avoid dilution and introducing elements that are not naturally present in the brine.

Protein removal from the acidified brine was studied by means of centrifugation. Four centrifugation times were used in the study (5, 10, 15 and 20 minutes) and 5 centrifugation speeds (1000, 3000, 6000, 10000 and 15000 rpm).

A factorial design was used (80 combinations) and three replications were done at every combination. At every studied point, 15 milliliters of the acidified brine solution were used.

At every point, the collected pellet was weighted, and its moisture determined by drying at 104ºC for 24 hours (according to the official standard ISO R-1442). The salt content of the dehydrated pellet was calculated considering that the salt concentration of the retained liquid of the pellet was the same than that of the supernatant.

The centrifugation of the brine was done by means of a BL MEDIFRIGER® 7001085 centrifuge. The relative acceleration at every centrifugation speed was determined by means of equation 1:

$$RCF = 0.0000118 \cdot r \cdot v^2 \qquad (1)$$

where:

RCF = Relative acceleration in g; $RCF_{maximum}$ = 19584 g

r = Radius of rotation (r = 8.2 cm)

174 v = Spin speed (rpm); Spin speed maximum = 15000 rpm

175 Table 1 shows the equivalence between spin speed and relative
176 acceleration (RCF) calculated by means of equation 1.

177

178 ***Table 1***

179

180 **2.4 Protein content in brine**

181 Total protein content in the supernatant was determined by means of the
182 bicinchoninic acid method (microplate) (Laemmlí, 1970).

183

184 **2.5 Statistical analysis**

185 The experimental design and statistical analysis of the results was carried
186 out by analysis of variance (ANOVA) using Statgraphics Plus 5.1 software
187 package (Statistical Graphics Corp.), the confidence level chosen was 95%.

188 When the factors were significant, we analyzed the differences between the
189 various levels by contrast analysis using the LSD test. In all cases a
190 significance level of 95% was used.

191

192 **3. RESULTS AND DISCUSSION.**

193 **3.1 Waste brine characterization**

194 The characteristic parameters of the waste brines used in the study can be
195 seen in table 2.

196

197 ***Table 2.***

198 The maximum values accepted by Spanish Laws (R.D.606/1993) for direct
199 disposal of effluents are shown in the third column of table 2. As it can be
200 observed, all the analyzed parameters had higher values than the accepted by
201 laws for direct disposal. As stated in the introduction section, the most
202 remarkable fact is the big contamination with organic compounds combined with
203 the very high conductivity of the waste brines.

204 Considering that all the chlorides present in the brine come from the NaCl
205 use for ham salting, it can be confirmed that the residual brine collected from
206 the salting containers is saturated as expected. In addition to the very high salt
207 content of the brine, a significant concentration of nitrates was determined,
208 which indicates the partial leakage of the used nitrates in the nitrification stage.

209

210 **3.2. Influence of pH and time for a constant speed**

211 A model of the experimental precipitate weight fraction obtained (weight of
212 collected precipitate/weight of brine) after centrifugation at every processing
213 condition was obtained by means of a nonlinear regression analysis. The
214 mentioned model was obtained at every centrifugation speed as a function of
215 pH and time.

216 Table 3, shows the obtained models and the regression coefficients at a 95%
217 confidence when using 1000, 3000, 6000, 10000 and 15000 r.p.m.,
218 respectively.

219

220 ***Table 3***

221

It can be observed a good fitting of the model for all the other experimental conditions except for 1000 r.p.m.. In all cases the obtained precipitate was dependant of pH and centrifugation time. In case of centrifugation at 1000 r.p.m. the bad fitting was due the very low precipitate collected at any pH or centrifugation time.

The response surfaces obtained from the fitted models for 3000, 6000, 10000 annd 15000 rpm can be observed in figure 2.

Figure 2.

As a general behaviour, it is observed that the collected pellet increased with centrifugation time and with the decrease in pH. As expected, the collected pellet at a pH of 5 was very small, and it would consist in other compounds than denatured proteins. When lower the pH, a higher denaturation of the proteins is expected, and the results confirm this point. Nevertheless, the increase in the collected pellet when pH decreased from 3 to 2 was very small. That difference was even smaller when increasing the centrifugation speed.

Figure 3 shows the protein concentration in the supernatant of the residual brine after centrifugation at 6000 rpm and 5 minutes depending on the used pH. A sharp decrease in the protein content is observed when the pH is reduced from 5 to 4, while no significant differences were observed between pH 2 and 3.

Figure 3

Attending to that result, it seems that a pH of 3 would be the best value to be employed in an industrial treatment, since the protein separation is quite high, as much as at pH 2,, with less acid to be added, lower dilution of the waste brine and better preservation of the equipment than with pH 2. It is known that at low pH values the corrosion rate increases exponentially with pH decrease (DOE-HDBK-1015, 1993).

When the centrifugation speed increased, the maximum weight fraction of collected pellet was achieved at shorter time. In some experimental conditions (6000 rpm and pH 2), a decrease of the collected pellet can be observed when the centrifugation time increases. It could be explained by the compaction of the pellet, thus reducing the retained brine in the pellet and as a consequence of that the water content. To confirm this hypothesis, the protein content in the supernatant at a pH 3 and the dry pellet were determined.

In the previous figure it can be observed that the maximum precipitate fraction obtained at centrifugation speeds of 3000, 6000 rpm at pH values 2 and 3, was achieved at 20 and 5 minutes, respectively.

Figure 4 shows the total quantity of dry pellet collected after centrifugation and the percentage of removed protein from the supernatant working at pH 3 for 5 and 20 minutes. As it can be seen, the maximum protein removal from the brine was close to 90% of the initial content. This value is an asymptotic one and no significant differences were observed depending on the centrifugation time. Attending to this observation, it seems that a further protein cleaning should have to be attained by using filtration techniques.

On the other hand, when the quantity of collected dry solid is analyzed, it is confirmed an initial increase in the collected pellet when the centrifugation

speed increases simultaneously with the increase in protein removal from the brine. Nevertheless, once a maximum value is achieved, a decrease in the collected dry pellet is observed, even maintaining the quantity of removed proteins, and confirming the mentioned hypothesis that points out towards a compactation of the pellet when the centrifugation speed and time increases, with no increase in the protein removal.

Figure 4

3.3 Influence of centrifugation time and speed to pH 3.

As mentioned in the previous section, it was considered the pH 3 as the optimum for protein removal. That's why the study of the influence of centrifugation time and speed was done at that pH value. The wet pellet weight fraction collected at pH 3 (with standard deviation bars) depending on the centrifugation speed and time can be observed in figure 5.

Figure 5

As a general pattern it can be observed that the collected pellet achieved the maximum values at centrifugation speeds of 6000 and 10000 rpm, with a drastic decrease at a centrifugation rate of 15000 rpm, pointing out to the compaction of the collected pellet occurred with time and increasing centrifugation speed.

Figure 6, shows the collected wet pellet at pH 3 at every centrifugation time, depending on the centrifugation speed. It can be clearly seen that no influence of centrifugation time exists at a centrifugation speed of 1000, probably because it

was a too small speed for collecting the denatured proteins. On the contrary, a clear influence of centrifugation time was observed form 3000, 6000 and 10000 rpm speeds, with a common behaviour, reaching a maximum in the collected pellet at a certain time, and decreasing the collected pellet at further time, due to the compaction action previously mentioned. In case of 15000 rpm, the maximum compaction would be achieved before the 5 minutes, indicating that no benefits would be achieved at longer centrifugation times except for a reduction of the retained brine in the precipitate.

Figure 6

It can be seen that for the same centrifugation time, the maximum collected pellet is achieved at lower centrifugation speed than the maximum one, giving support to the compaction of the brine, which was independent of time for the maximum speed.

4. Conclusions.

The use of acidification of residual brine coming from pile salting during Spanish dry-cured ham production is an effective technique for protein removal.

The best pH value to be used in an industrial process seems to be 3, because a large quantity of proteins is removed while the pH is no very low. The industrial processing should have to be followed by the neutralization of the supernatant.

The obtained results indicate that almost 90% of the proteins from the brine can be removed by acidification followed by centrifugation. A further protein

321 removal from the brine should have to be achieved by using filtrating
322 techniques, which efficiency could be highly improved as a consequence of the
323 previous treatment through acidification and centrifugation. Further studies are
324 needed to confirm this aspect.
325
326 **Acknowledgements**
327 We would like to thank the Spanish Government (MCYT) and the EU
328 (FEDER program) for the financial support of the project (AGL2004-05064-
329 C02).
330
331 **5. Bibliography.**
332 Akili D.Khawaji, Ibrahim K Kutubkhanah and Jong-Mihn Wie (2008), Advances
333 in seawater desolation technologies, *Desalination*,221, 47-49.
334 Barat J. M, Grau R., Pagán-Moreno M.J. and Fito P. (2004), Replacement of
335 pile by simultaneous brine thawing-salting in Spanish cured ham
336 manufacturing, *Meat Science*, 66, 603-608
337 Barat J.M., Grau R., Ibañez J. B. and Fito P.(2005) Post salting studies in
338 Spanish cured ham manufacturing. Time reduction by using brine thawing-
339 salting., *Meat Science,* 69, 201-208.
340 Barat J.M., Vidal-Brotóns D., López-Pascual E.,.Gras M.L (2006) Quantification
341 and kinetics of the residual brine generation during ham and shoulder pile
342 salting, *Meat Science*, 73, 576-580.
343 Bes-Piá A., Cuartas-UribeB., Mendoza-Roca J.A., Galiana-Alexandre M.V.,
344 Iborra Clar M.I., Alcaina-Miranda M.I.(2008). Pickling wastewater
345 reclamation by means of nanofiltration, *Desalination,* 221,225-2

Buckley, C.A; Simpson,A.E; Kerr,C.A.; Schutte,C.F. The treatment and disposal of waste brine solutions, *Desalination* 1987, 67,0, 431-438.

Cheftel J.C. (1989), Food Proteins.Biochemistry, functional properties nutritional value, chemical modifications. Edition Acribia 141-165

Cruz J (2003) "Analysis of the production and marketing of ham in Spain" *Eurocarne* 113,page 23-30

Cuartas-Uribe B, Alcaína-Miranda M.I. , Soriano-Costa E. and Bes-Piá A. (2007),Comparison of the behavior of two Nanofiltration Membranes for sweet whey demineralization, *Journal of Diary Science*,90,1094-1101

Diez, V., Del Pozo, R., Salazar, G., García, L.,(2000)", Wastewater Meat Industry, treatment options and selection criteria. *Eurocarne* 87,35-46.

Doe fundamentals handbook chemistry. (1993) DOE HDBK, 1015/ 1-93, Volume 1,2 January 1993

Flores J.(1997). Mediterranean vs. northern European meat products. Processing technologies and main differences, *Food Chemistry* , 59,505-510

Gallard Jornet, L;Escriche Roberto , I; Fito Mauppey, P (2005), The salting of fish, a tradition in the Mediterranean diet, Polytechnic University of Valencia. 111

Joo,S.T,.Kauffman, R.G.Kim, B.C., G.B.(1999).The relationship of sarcoplasmic and myofibrillar protein solubility to colour and water-holding capacity in porcine longissimus muscle. *Meat Science* 52, 291-197

Laemmlí UK,(1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227,680-685

Lawrie, R.A.,(1998)"*Meat Science*"3ª edition , Ed, Acribia , Zaragoza. 123

Lee Nohun, Amy Gary, Groué Jean Philippe and Buisson Herve, (2004),Identification and understand ding of fouling in low pressure membrane (MF/UF) filtration by natural organic matter (NOM)*Water Research*, 38,4511-4523

Li J.,Shi Y.M., Whan R.S.,Li X.D.,Xie G.F.,(2009) Analysis of the performance of a sintered stainless steel wire mesh filter for cryogenic liquid purification.,*Cryogenics*,49,27-37

Ministry of the environment program A.G.U.A. *Proceedings for the management and use of water*

Provincial Legislation Official Gazette (L. BOP 162) ;(10/07/1995)

Toldrá F and Aristoy C ,Dry-Cured Ham, In Toldrá .,editor, *Handbook of Meat Processing*, first ed,IOWA:WILEY-BLACKWELL;2010,354-357.

Ventanas J. (2001). "Technology Iberian ham. In traditional systems the rational explotation of the flavor and aroma". Editions Mundi-prensa

Vineet K.Gupta, Nishith Verma (2002),Removal of volatile organic compounds by cryogenic condensation followed by adsorption, *Chemical Engineering Science*, 57,2679-2926.

Warris, P,D.(2003). *Meat Science,* Ed. Acribia Zaragoza.

Figure index

Figure 1

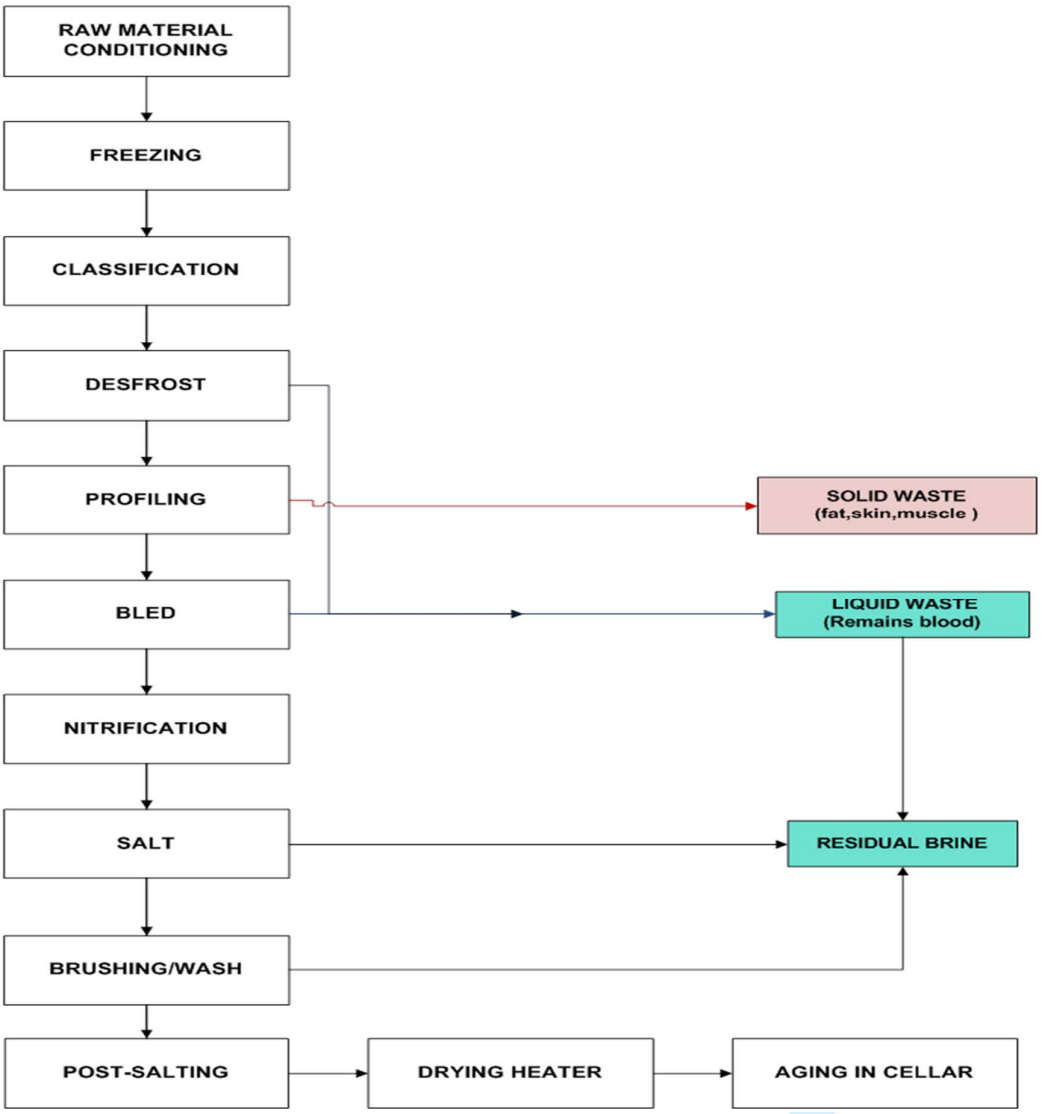
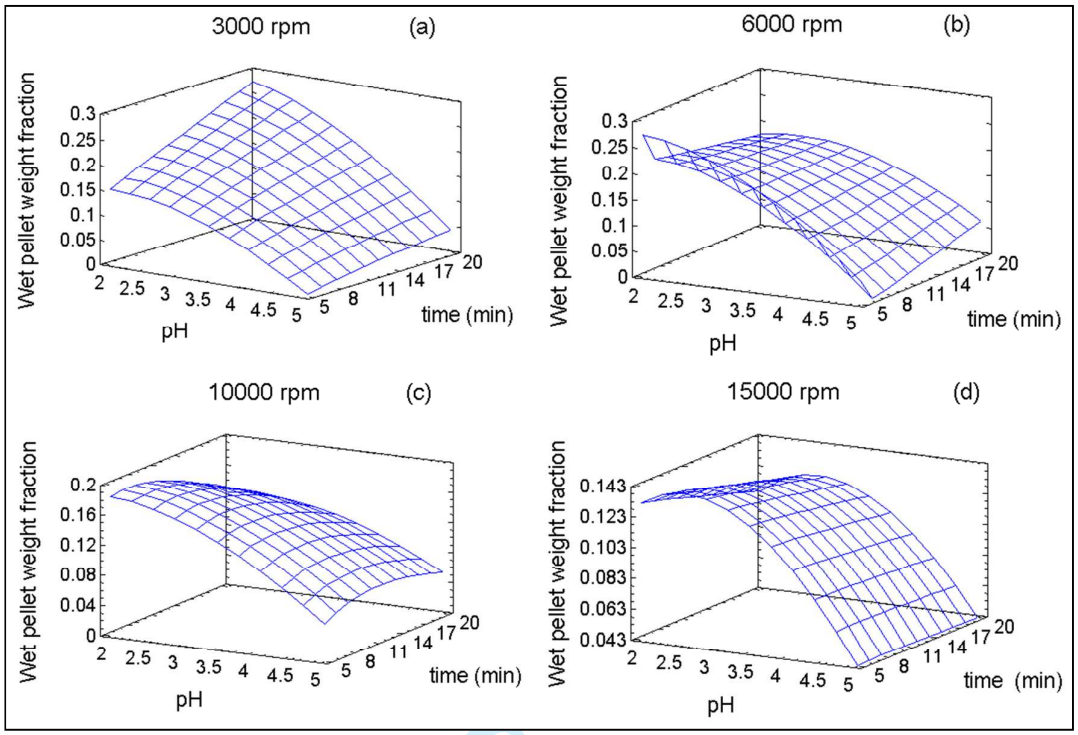
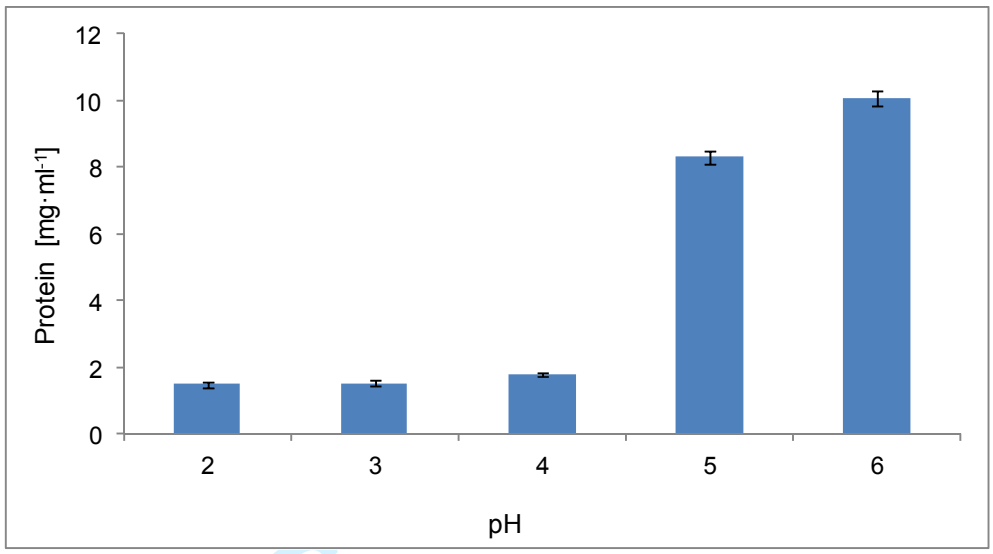

RAW MATERIAL CONDITIONING
FREEZING
CLASSIFICATION
DESFROST
PROFILING
BLED
NITRIFICATION
SALT
BRUSHING/WASH
POST-SALTING
DRYING HEATER
AGING IN CELLAR
SOLID WASTE (fat,skin,muscle)
LIQUID WASTE (Remains blood)
RESIDUAL BRINE

Figure 2



441 **Figure 3**



442

443

444

445

446

447

448

449

450

451

452

453

454

455

456

457

458

Figure 4.

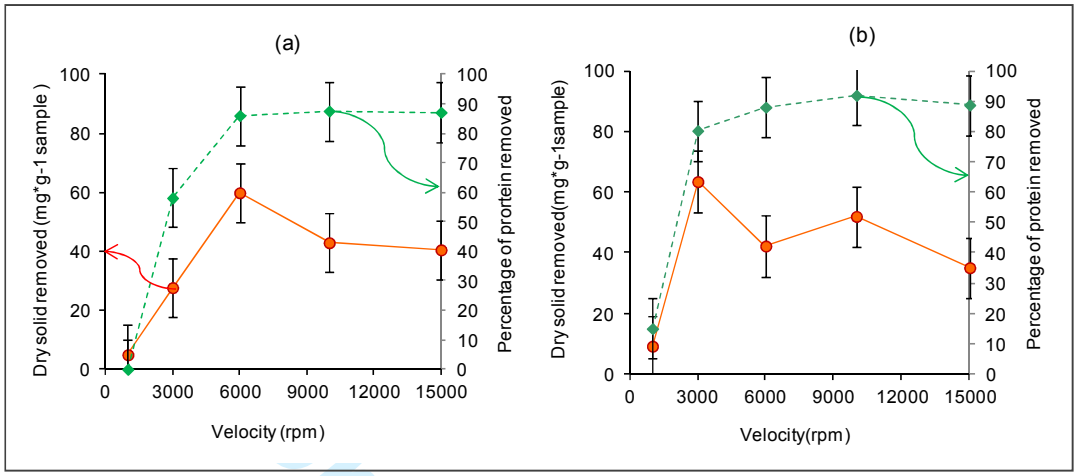


Figure 5

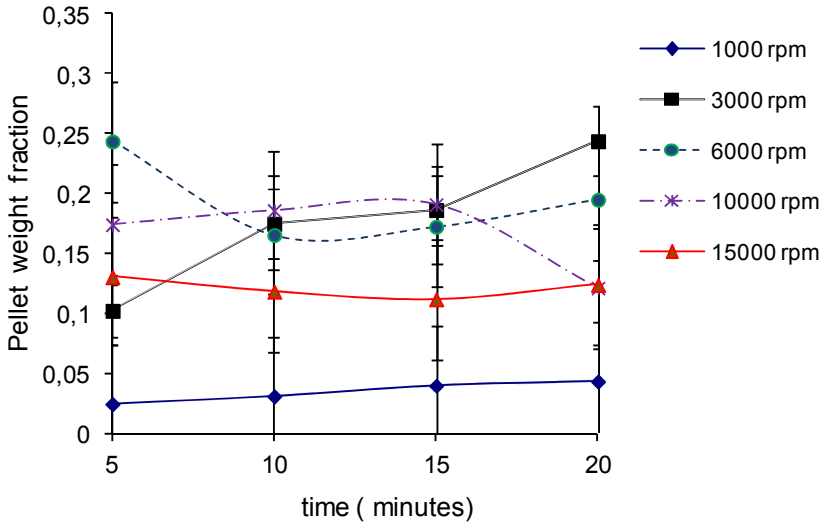
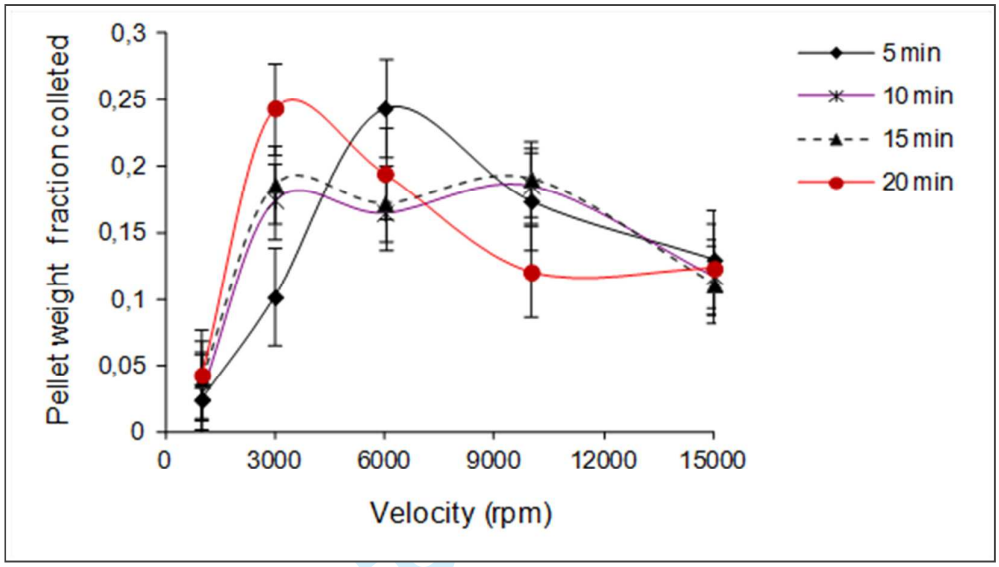


Figure 6



514

515 Index table

516 Table1. Equivalence of spin speed used in the experiments and relative
517 acceleration (g)

518 Table 2. Physicochemical properties of the waste brine used in the study.

519 Table 3. The models and the regression coefficients at different velocities

520

521

522

523

524

525

526

527

528

529

530

531

532

533

534

535

536

537

538

Table 1

Spin speed (rpm)	1000	3000	6000	10000	15000
Relative acceleration (g)	92	825	3300	9168	20627

561

562 **Table 2**

Analysis	Values of analysed waste brine	Maximum accepted values for disposal from Spanish Laws (R.D. 606/1993)	Analytical method
pH	6.2 ± 0.01	5.5 - 9.5	pH meter CRISON MM40
Conductivity [mS/cm]	582 ± 7	5000	pH meter CRISON MM40
Chloride [g/L]	175.5 ± 0.5	2	Chloride Analyzer SHERWOD 926
COD [mg/L]	25867 ± 2013	500	Photometer Nanocolor Test DQO 300
Solids [mg/L]	11650 ± 240	300	Method 2540-B
Nitrates [mg/L]	470 ± 1.2	20	Photometer Nanocolor Test NO_3-50
Fat [mg/L]	381 ± 20	40	Soxtec System 2055 Tecator

563

564

565

566

567

568

569

570

571 **Table 3**

rpm	Model*	Regression coefficient (R^2)
1000	$x^{pp} = 28.14 \cdot pH^{0,56} - 0.24 \cdot t^{-0,26} + 7.62 \cdot 10^{-4} \cdot pH \cdot t - 0.11 \cdot pH - 4.09 \cdot 10^{-3} \cdot t$	7.2
3000	$x^{pp} = \mathbf{0}.63 \cdot pH^{0,64} - 0.29 \cdot t^{-0,06} - 1.89 \cdot 10^{-3} \cdot pH \cdot t - 0.30 \cdot pH + 0.01071 \cdot t$	89.9
6000	$x^{pp} = 2.64 \cdot pH^{0.95} + 3.29 \cdot 10^{9} \cdot t^{-15.44} + 25{,}48 \cdot pH \cdot t - 2.43 \cdot pH - 7.83 \cdot 10^{-3} \cdot t$	88.8
10000	$x^{pp} = 1.45 \cdot pH^{0,94} + 0.18 \cdot t^{0.96} + 1.51 \cdot 10^{-3} \cdot pH \cdot t - 1.31 \cdot pH - 0.16 \cdot t$	78.2
15000	$x^{pp} = 1.01 \cdot pH^{0.832} - 0.22 \cdot t^{-0.004} + 5.50 \cdot 10^{-4} \cdot pH \cdot t - 0.72 \cdot pH - 0.00287 \cdot t$	91.0

572 * (x^{pp} = wet pellet weight fraction $-\ g/g\ -$, $t = time\ (min)$)

573

574