1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Effect of stabiliser classes (animal proteins, vegetable proteins, starches, hydrocolloids and dietary fibre) on the physicochemical properties of a model lean meat product

Cristina Perez-Santaescolastica¹, Olivier Goemaere², Jana Hanskens², José M. Lorenzo^{1*}, Ilse Fraeye^{2*}

¹Centro Tecnológico de la Carne de Galicia, Rúa Galicia 4 Parque Tecnológico de Galicia, San Cibrán das Viñas 32900 Ourense, Spain

² Ku Leuven Ghent Technology Campus, Leuven Food Science and Nutrition Research Centre (Lforce), Research Group for Technology and Quality of Animal Products, Gebroeders De Smetstraat 1, 9000 Ghent, Belgium

* Corresponding author: Ilse Fraeye (email: ilse.fraeye@kuleuven.be)
Jose M. Lorenzo (email: jmlorenzo@ceteca.net)

Abstract

The effects of 11 ingredients (originating from various ingredient classes: animal proteins, plant proteins, starches, hydrocolloids and fibres) applied in 2 different concentrations on drip loss, rheological characteristics and hardness were evaluated in a lean meat model system. The high concentration of potato starch, insoluble citrus fibre, rice starch or pea protein significantly ($P<0.05$) reduced drip losses (6.92%, 17.87%, 15.70%, 17.60%, respectively) compared to control (30.80%), while rheological measures and hardness remained unchanged ($P>0.05$). Moreover, the addition of low concentrations of soluble citrus fibre did not significantly affect hardness (4.11 N), whereas its inclusion at high concentrations had a significant effect on the final hardness (7.17 N). Among all the tested ingredients, xanthan presented the lowest drip loss value (0.12% and 0.40% for high and low concentration, respectively). Finally, gelatine did not show any differences in water holding capacity, storage modulus nor hardness compared with the control group.

Keywords: drip loss, rheology, hardness, carrageenan, xanthan gum

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Introduction

Traditionally meat and meat products have been highly valued due to the presence of a number of essential nutrients. In this regard, we can specifically refer to, among others, high quality proteins, vitamins like B12 as well as highly bioavailable iron (Lorenzo *et al.*, 2019). However, in the latest years, due increased prevalence of cardiovascular diseases, cancer and obesity around the world, these products are being regarded as harmful products due to their low fibre and their high fat and salt contents (Gnagnarella *et al.*, 2018; Xu, & Zhu, 2019). Therefore, the meat industry has been forced to develop new production strategies to produce healthier products. Since many processed foods have a high sodium content, one of the strategies focused on its reduction, but this action could lead to changes in textural, flavour or shelf life product characteristics (Dominguez *et al.*, 2016). Similarly, fat reduction in processed meat product formulations could compromise the final quality, considering the important contribution of fat to tenderness, flavour and juiciness (Leites *et al.*, 2015).

The use of stabilisers has been proposed to remediate all challenges mentioned above (Parniakov *et al.*, 2018; Thirumdas *et al.*, 2018; Zugcic *et al.*, 2018). At the same time, some of these ingredients could also add health benefits to the product. For instance, fibres are into the top 10 in the consumer rank of added ingredients due to their effects against intestinal diseases (López-Marcos *et al.*, 2015). In our study, the term “stabiliser” refers specifically to ingredients contributing to stability (water and fat retention) and structure of meat products. The consumer acceptance of a large range of products in which stabilisers were incorporated has been previously studied (Hashim *et al.*, 2009; Karazhian and Mahdian, 2018; Akkarachaneeyakorn and Tinrat, 2015). More specifically, there are several studies related to different types of stabilising ingredients

in meat products: animal and vegetable proteins (Marti-Quijal *et al.*, 2018, 2019), carbohydrate gums (Lorenzo *et al.*, 2016; Heck *et al.*, 2017), starches (Luallen, 2018), dietary fibre (Mehta *et al.*, 2015). According to previous studies, vegetable proteins could be used to reduce production costs (Petracci *et al.*, 2013), while animal proteins are suitable for their effect on moisture and fat binding as well as texture (Kristensen *et al.*, 2016). In addition, gums are useful to improve texture, gelling or emulsion characteristics (McClements, 2015) and fibres are fitting in meat products to enhance water holding capacity (WHC), both at high (during cooking) and low (during storage) temperature, juiciness and tenderness (Petracci *et al.*, 2013).

In spite of the wide range of research carried out until nowadays, none of these studies compared the behaviour of more than two or three ingredients in the same meat product, and in general ingredients used belonged to the same category. A large comparing study among the representative categories of stabilisers is very useful, not only for extending the current knowledge about these ingredients but also for selecting the most suitable one based on specific processing and product requirements at every moment. This research is the first study until now that compares the effects of 11 different ingredients in two different concentrations on a single meat model in order to determine and quantify the intrinsic properties of stabilisers with respect to structure formation and stability.

Materials and methods

Lean meat system

To claim that possible effects are attributable to the addition of ingredients and to reduce other influences, a large batch of pork *Longissimus dorsi* muscle from a local supplier was used for all preparations. This batch was cleaned, grinded and stored in

1
2
3
4
5 82 homogeneous portions at -18 °C. One day before processing, one bag of homogenized
6
7 83 raw meat was thawed for each preparation. A weight of 280.0 g of thawed meat was
8
9
10 84 transferred to the chopper (Grindomix GM200, Retsch) together with 420.0 g of ice water
11
12 85 in which 1.6 % nitrite brine salt (NBS) and 0.2 % tetra sodium pyrophosphate (TSPP)
13
14 86 was dissolved. A short time (10 sec) of premixing at 2000 rpm was executed before
15
16 87 adding the ingredient. Ingredients from five different categories (animal proteins,
17
18 88 vegetable proteins, starches, hydrocolloids and fibres) were added and mixed for 5
19
20 89 minutes at 3500 rpm to a temperature of 12 °C, to prevent premature protein
21
22 90 denaturation. Two different concentrations, selected based on ingredient product sheets
23
24 91 and literature, were used for each ingredient. The ingredients used, their concentrations
25
26 92 and E number (if applicable) are shown in **Table 1** sorted by product category. In
27
28 93 addition, one control lean meat model system, lacking any ingredient was also prepared.
29
30 94 Part of the dough was separated to conduct drip loss and rheological analyses. The
31
32 95 remaining lean meat dough was immediately filled in two aluminium cans and heated by
33
34 96 air saturated with water vapour for 90 minutes at 76 ± 1 °C in an oven (Rational Belgium
35
36 97 SA, Mechelen, Belgium). After that, the cans were cooled in an ice bath for 30 minutes
37
38 98 and stored at 3 ± 0.1 °C for 5 to 6 days after which hardness was determined.
39
40 99 Preparations were made in duplicate on different days. **Figure 1 shows the elaboration**
41
42 **process of samples.**
43
44
45
46
47
48

49 101 *Physicochemical analyses*

50 102 *Drip loss*

51
52 103 Drip loss upon forced destabilization was analysed as a measure of water holding
53
54 104 capacity (WHC) of the heated dough. The determination was carried out in triplicate on
55
56 105 each preparation by weighing 30 g of meat dough into a centrifuge tube which was
57
58
59
60

heated at 75 °C for 30 min in a smoke-air cooker (Kerres Anlagen systeme, Backnang, Germany). After that, the tube was centrifuged at 447.2g for 1 min (Universal 320/R table centrifuge, Hettich, Westmaas, the Netherlands), after which the separated fluid was removed. Finally, WHC was expressed as the percentage of drip loss of the heated dough after centrifugation.

Rheological analysis

Temperature sweep was performed in duplicate using a stress-controlled AR2000 ex Rheometer (TA instruments, Zellik, Belgium), equipped with a Peltier temperature control system. The geometry consisted of two 40 mm parallel (gap = 1000 µm) cross-hatched plates, to prevent slippage of the sample during analyses. The meat dough rested for at least 90 min at 14 ± 1 °C to guarantee a uniform structure and temperature of the samples. Samples were loaded between the parallel plates at a starting temperature of 14 °C. Throughout the whole analysing sequence, a strain of 0.025 was applied at a fixed frequency of 1 Hz. In the first part of the oscillatory measurement temperature increased from 14 °C to 76 °C at a ramp rate of 2 °C/min. Then, the maximum temperature was held for 3 min before initiating the cooling process from 76 °C to 14 °C, also at a rate of 2 °C/min. In each phase of the measurement the phase angle and the storage modulus (G') were recorded. The phase angle describes the viscous-elastic character of the sample, while G' represents the elastic properties. Both parameters together can be used to compare the amount of structure between samples.

Texture

Texture analysis was performed by use of a texture analyser (model TA Plus; Lloyd Instruments, Bognor Regis, UK). One week after processing, the meat was carefully removed from the cans. These meat blocks were positioned on the texture

1
2
3
4
5
6 130 analyser and were axially penetrated by a cylindrical probe (d=12 mm; Lloyd
7
8 131 Instruments) to a depth of 30% of the total can height (5 cm) at a speed of 1 mm/s. For
9
10 132 each can, three measurements were made on different points of the surface of the meat.
11
12 133 A load cell of 100 N was selected, the maximum force (N) to penetrate the sample was
13
14
15 134 directly obtained from the force-distance curves, other texture parameters are not
16
17 135 discussed in this experiment. For each meat preparation, 2 cans were measured, each
18
19 136 through 3 penetrations, resulting in 6 measurements per preparation.
20

21
22 137 *Statistical analysis*

23
24 138 Statistical analysis was performed using the IBM SPSS Statistics 23.0 (IBM
25
26 139 Corporation, Somers, NY, USA) software package. Drip loss, hardness, G' and tan δ were
27
28 140 analysed using a mixed linear model including type of ingredient and concentration as
29
30 141 fixed effects. Differences between means due to the used ingredient, at high and low
31
32 142 concentration, were compared by Tukey test and were considered significantly different at
33
34 143 $P \leq 0.05$. All results were expressed as mean values and standard deviation of the mean.
35
36

37
38 144 **Results and discussion**

39
40 145 **Table 2 and Figure 2** show the effect of the different ingredients on drip loss,
41
42 146 hardness, G' and tan δ in a lean meat model system. Statistical analysis showed that
43
44 147 different ingredients presented different behaviours; irrespective of the category they
45
46 148 belong to. It is observed that both the addition of stabilisers and studied concentration
47
48 149 displayed statistical differences among the batches. Finally, the effect of the interaction
49
50 150 between the ingredient and concentration was visible on drip losses ($P < 0.01$), G'
51
52 151 modulus ($P < 0.01$) and tan δ ($P < 0.001$).
53

54
55
56 152 **Effects on drip losses**
57
58
59
60

Among the ingredients applied in a low concentration, only xanthan gum and potato starch significantly ($P<0.05$) reduced drip losses, whereas at high concentration almost all ingredients (except for caseinate and gelatine) showed significant drip loss reductions.

The addition of xanthan gum to the lean meat samples resulted in a very strong reduction ($P<0.05$) of drip loss values. Remarkably, xanthan is the only one of the tested ingredients which reduced drip losses entirely, irrespective of the applied concentration. Similar results were found by Chattong *et al.* (2015) in ostrich-meat emulsion where among all the ingredients tested, xanthan presented the highest WHC value. Both other hydrocolloids (especially ι -carrageenan but to a lesser extent also κ -carrageenan) improved WHC when used in high concentration. Interactions between proteins and hydrocolloids have been widely studied and water retention increases when gels are formed consisting of both compounds. In this regard, Palanisamy *et al.* (2018) observed that the addition of carrageenan improved not only the moisture content of cooked meat products but also the cooking yields resulting in better sensorial properties like mouth-feel and juiciness in the final product. For this reason, actually κ - and ι -carrageenan are widely used in the meat industry in a large range of products, such as canned meat, pet food, cooked sliced and low fat meat products (Ayadi *et al.*, 2009; Yasin *et al.*, 2016).

Both fibres (especially soluble citrus fibre, but to a lesser extent also insoluble citrus fibre) also reduced drip losses when used at high concentration. There is a large list of fibres which have demonstrated to enhance WHC in meat products, such as oat bran (Alakhrash *et al.*, 2016) and bacterial cellulose (Paximada *et al.*, 2016), which have variable contents of soluble and insoluble fibre. Despite the huge number of studies focusing on the use of fibres in meat products, insoluble citrus fibre has not been studied

extensively. In general, the main purpose of using insoluble fibres in meat products is to reduce drip losses, that is to enhance water binding in order to get products with higher yield and better sensorial or structural characteristics (Schmiele *et al.*, 2015). In this regard, this study reveals the suitability of insoluble citrus fibre for meat industry, since it allows improving WHC without altering texture and rheological properties (as discussed below), which may be desirable in specific applications. Furthermore, potato starch (both at high and low concentration), and to a lesser extent rice starch (only at high concentration), also improved WHC.

On the other hand, the great reduction in drip loss upon blood plasma addition is probably due to the demonstrated capacity of blood proteins to retain water. In this regard, Cofrades *et al.* (2000), observed higher values of WHC in samples with bovine plasma. In addition, pea protein addition also improved WHC at high concentration. In contrast, caseinate and gelatine did not show a significant difference in drip loss. According to Petracci *et al.* (2013) concentrations up to 2% of caseinate are needed to get effects in meat products suggesting that the addition of higher contents would have been necessary to see significant effects in drip loss.

Effects on texture

The highest hardness value was observed in samples with high contents of soluble citrus fibre (7.17 N), whereas samples with high concentration of xanthan presented the lowest values (0.76 N). While the soluble citrus fibre increased product hardness when added at high concentration, this effect was not observed for insoluble citrus fibre addition.

Low concentrations of blood plasma showed significant increases ($P<0.05$) in hardness compared to control samples. A similar effect was observed in fat reduced

1
2
3
4
5 201 sausages with formulation contents from 0 to 5%. It was observed that increased plasma
6
7
8 202 addition resulted in a greater hardness and chewiness (Cofrades *et al.*, 2000). In
9
10 203 contrast, hardness results were not affected by neither caseinate nor pea protein addition
11
12 204 at either concentration studied. In addition, gelatine addition also did not affect the
13
14 205 hardness of the meat models. Gelatine is water soluble and it is well known that gelatine
15
16 206 gels melt at increased temperature. Also, it is necessary to keep in mind that the meat
17
18 207 model in this study consisted of a basic, critical recipe including little additives (except
19
20 208 NBS and TSPP) and exhibiting a rather high cooking loss. Therefore, it is expected that
21
22 209 gelatine melted during heating and dissolved in the cooking loss. As a result, the final
23
24 210 gelatine content in the meat product is expected to be relatively low, therefore no
25
26 211 significant increase in hardness was measured.

27
28
29
30 212 Likewise, starch was shown not to affect the texture of the meat model system at
31
32 213 either of the concentrations. The available literature data with regard to texture effects is
33
34 214 quite variable. Studies can be found which obtained increments in hardness in meat
35
36 215 batters and processed meat products (Jairath *et al.*, 2018) while at the same time studies
37
38 216 with opposite results have been reported (Resconi *et al.*, 2016). The differences among
39
40 217 authors can be related to several factors such as type of starch, content, product to which
41
42 218 it is added, technological process settings, etc. (Genccelep *et al.*, 2015).

43
44
45
46 219 Most research reports a higher hardness after the storage in samples with
47
48 220 different contents of carrageenan (Ayadi *et al.*, 2009), although λ -carrageen is not often
49
50 221 applied since these ingredients causes a reduction in meat protein gelation, resulting in
51
52 222 softer gels (DeFreitas *et al.*, 1997). In this sense, Ayadi *et al.* (2009) found hardness
53
54 223 increments of about 1% upon increasing the ι -carrageenan concentration from 0 % to
55
56 224 1.5% in turkey sausages. However, in our study the addition of ι - and k -carrageenan
57
58
59
60

(either at low or high content) did not affect hardness significantly. In case of k-carrageenan an increase in hardness was observed, especially at high concentration, but this increase was not statistically significant. The hardness increase reported in literature could be a consequence of possible microstructural changes in the matrix. Therefore, contrasting results could be explained by the fact that structural differences between different meat origins, such as muscle fibre types, can influence protein-carrageenan interactions (Petracci *et al.*, 2013). Previous studies displayed hardness reductions in a large range of food products with low contents of xanthan (Habibi & Khosravi-Darani, 2017). In our study, xanthan addition resulted in slightly decreased hardness values with respect to the control preparation, however these differences were not statistically significant ($P>0.05$).

Effects on rheological parameters

According to Chattong *et al.* (2015), xanthan lead to the weakest material properties, in contrast to the widely accepted knowledge which describes xanthan as an ingredient to provide high viscosity solutions at low concentrations (Mohsin *et al.*, 2019). In our study, the G' modulus significantly increased in samples prepared with low concentration of xanthan (from 10 to 26 kPa for control and xanthan samples, respectively). The increase in storage modulus may be related to the reduction in drip loss, since higher water binding could involve more network formation in which water is retained. Beside that and as can be suspected, significant differences in the values of $\tan \delta$ were found in our study. This fact indicates that the increase in loss modulus (G'') was slightly stronger compared to the increase in G' suggesting a slightly more viscous character. However, differences were very small, hence the practical relevance of this difference is probably limited. In addition, marked differences ($P<0.05$) were found in G'

value at high contents of either ι- or κ-carrageenan compared to control. Control samples presented values around 10 kPa, while batches with high content of carrageenan showed values into the range of 20-24 kPa.

Similar to that, a low increase in G' value was observed due to the addition of high concentrations of blood plasma (about 19 kPa). This result is expected, since plasma protein is known to enhance the elasticity through gel formation (Li *et al.*, 2017). Similarly, a high concentration of caseinate resulted in a significant ($P<0.05$) increase in G'. Both other proteins (gelatine and pea protein) did not affect G' upon their addition at either concentration. This fact could be due to the differences among commercial products, as it was observed that pea proteins often show limited gelling capacity due to the extraction procedures which imply partial protein denaturation (Ben-Harb *et al.*, 2018). Also, G' values increased in samples with high content of soluble citrus fibre, while insoluble citrus fibre did not affect G' values.

Previous studies observed that amylopectin is responsible for an increase in elasticity and viscosity. For that and due to the high amylopectin content of starches used in this study, 80% and 75% for rice and potato, respectively, changes in the rheological profiles of samples were expected. Remarkably, our study did not show any statistical differences ($P>0.05$) compared to the control group. Still, the high content of potato starch showed the tendency to enhanced the G' modulus, but this difference was not statistically significant due to the large variance in these replicates. In addition, while most rice and potato starches used in previous studies were modified, the present study was realized using native starches, allowing their use in clean label meat products.

Conclusion

1
2
3
4
5
6 272 It can be seen that the ingredient category is not determinant for the behaviour of
7
8 273 each ingredient. In this sense, among the different ingredients, potato starch (in both
9
10 274 concentrations studied) and the high concentration of insoluble citrus fibre, rice starch
11
12 275 and pea protein only reduced drip losses, whereas rheology measures and hardness
13
14 276 remained unchanged compared to control group. At high concentration, l-carrageenan,
15
16 277 k-carrageenan, blood plasma and caseinate reduced drip losses and increased gel
17
18 278 strength. On the other hand, the high concentration of soluble citrus affected all
19
20 279 measured attributes (reducing drip losses and enhancing storage modulus values and
21
22 280 hardness). In contrast, gelatine did not affect any of the attributes. Finally, xanthan
23
24 281 showed the strongest drip losses reductions, however, its addition results in rheological
25
26 282 changes leading to phase angle increments and slight (though not significant) reductions
27
28 283 in texture. The insights generated in this study will help meat producing companies to
29
30 284 selected ingredients and concentrations which respond to their specific needs in terms
31
32 285 of WHC and product structure.
33
34
35
36

37
38 286 **Acknowledgement**

39
40 287 This research was funded by Flanders Innovation and Entrepreneurship (AIO)
41
42 288 TETRA project HBC.2017.0047 entitled ‘Func-I-Meat: Screening of the intrinsic
43
44 289 properties of functional ingredients and their application in meat products’.
45
46 290 Acknowledgements to INIA for granting Cristina Pérez Santaescolástica with a
47
48 291 predoctoral scholarship (grant number CPD2015-0212). José M. Lorenzo is member of
49
50 292 the Healthy Meat network, funded by CYTED (ref. 119RT0568).
51
52
53

54 293 **Ethical Guidelines**

55
56 294 Ethics approval was not required for this research.

57
58 295 **Conflict of Interest**
59
60

There is no conflict of interest.

Data Availability Statement

Research data are not shared.

References

- Akkarachaneeyakorn, S., & Tinrat, S. (2015). Effects of types and amounts of stabilizers on physical and sensory characteristics of cloudy ready-to-drink mulberry fruit juice. *Food Science & Nutrition*, **3**, 213-220.
- Alakhrash, F., Anyanwu, U., & Tahergorabi, R. (2016). Physicochemical properties of Alaska pollock (*Theragra chalcogramma*) surimi gels with oat bran. *LWT-Food Science and Technology*, **66**, 41-47.
- Ayadi, M. A., Kechaou, A., Makni, I., & Attia, H. (2009). Influence of carrageenan addition on turkey meat sausages properties. *Journal of Food Engineering*, **93**, 278-283.
- Ben-Harb, S., Panouillé, M., Huc-Mathis, D., Moulin, G., Saint-Eve, A., Irlinger, F., ... & Souchon, I. (2018). The rheological and microstructural properties of pea, milk, mixed pea/milk gels and gelled emulsions designed by thermal, acid, and enzyme treatments. *Food Hydrocolloids*, **77**, 75-84.
- Chatpong, U., Apichartsrangkoon, A., Chaikham, P., Supavititpatana, T., & Bell, A. E. (2015). Viscoelastic properties and physicochemical characteristics of pressurized ostrich-meat emulsions containing gum additives. *Innovative Food Science & Emerging Technologies*, **32**, 64-69.
- Cofrades, S., Guerra, M. A., Carballo, J., Fernández-Martín, F., & Colmenero, F. J. (2000). Plasma protein and soy fibre content effect on bologna sausage properties as influenced by fat level. *Journal of Food Science*, **65**, 281-287.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

319 DeFreitas, Z., Sebranek, J. G., Olson, D. G., & Carr, J. M. (1997). Carrageenan
320 effects on salt-soluble meat proteins in model systems. *Journal of Food Science*, **62**,
321 539-543.

322 Domínguez, Munekata, P.E., Cittadini, A., & Lorenzo, J.M. (2016). Effect of the
323 partial NaCl substitution by other chloride salts on the volatile profile during the ripening
324 of dry-cured lacón. *Grasas y Aceites*, **67**, e128.

325 Genccelep, H., Saricaoglu, F. T., Anil, M., Agar, B., & Turhan, S. (2015). The effect
326 of starch modification and concentration on steady-state and dynamic rheology of meat
327 emulsions. *Food Hydrocolloids*, **48**, 135-148.

328 Gnagnarella, P., Caini, S., Maisonneuve, P., & Gandini, S. (2018). Carcinogenicity
329 of high consumption of meat and lung cancer risk among non-smokers: a comprehensive
330 meta-analysis. *Nutrition and Cancer*, **70**, 1-13.

331 Habibi, H., & Khosravi-Darani, K. (2017). Effective variables on production and
332 structure of xanthan gum and its food applications: A review. *Biocatalysis and Agricultural
333 Biotechnology*, **10**, 130-140.

334 Hashim, I. B., Khalil, A. H., & Afifi, H. S. (2009). Quality characteristics and
335 consumer acceptance of yogurt fortified with date fiber. *Journal of Dairy Science*, **92**,
336 5403-5407.

337 Heck, R.T., Guidetti, R., Etchepare, M.A., dos Santos, L.A.A., Cichoski, A.J.,
338 Ragagnin, C., Smanioto, J., Lorenzo, J.M., Wagner, R., & Campagnol, P.C.B. (2017). Is
339 it possible to produce a low-fat burger with a healthy n-6/n-3 PUFA ratio without
340 affecting the technological and sensory properties?. *Meat Science*, **130**, 16-25.

Jairath, G., Sharma, D. P., Dabur, R. S., Singh, P. K., & Bishnoi, S. (2018). Standardization of corn starch as a fat replacer in buffalo calf meat sausages and its effect on the quality attributes. *Indian Journal of Animal Research*, **52**, 1521-1525.

Karazhian, R., & Mahdian, E. (2013). Effects of fat replacers and stabilizers on rheological, physicochemical and sensory properties of reduced-fat ice cream. *Journal of Agricultural Science and Technology*, **15**, 1163-1174

Kristensen, M. D., Bendsen, N. T., Christensen, S. M., Astrup, A., & Raben, A. (2016). Meals based on vegetable protein sources (beans and peas) are more satiating than meals based on animal protein sources (veal and pork)—a randomized cross-over meal test study. *Food & Nutrition Research*, **60**, 32634.

Leite, A., Rodrigues, S., Pereira, E., Paulos, K., Oliveira, A.F., Lorenzo, J.M., and Teixeira, A. (2015). Physicochemical properties, fatty acid profile and sensory characteristics of sheep and goat meat sausages manufactured with different pork fat levels. *Meat Science*, **105**, 114-120.

Li, W., Huang, M., Wang, P., & Xu, X. (2017). Rheological and nuclear magnetic resonance characterization of porcine plasma protein-stabilized gel-like emulsion affected by pH and heating. *LWT Food Science & Technology*, **75**, 460-465.

López-Marcos, M. C., Bailina, C., Viuda-Martos, M., Pérez-Álvarez, J. A., & Fernández-López, J. (2015). Properties of dietary fibres from agroindustrial coproducts as source for fibre-enriched foods. *Food and Bioprocess Technology*, **8**, 2400-2408.

Lorenzo, J.M., Maggiolino, A., Gallego, L., Pateiro, M., Serrano, M.P., Domínguez, R., García, A., Landete-Castillaejos, T., & de Palo, P. (2019). Effect of age on nutritional properties of Iberian wild red deer meat. *Journal of the Science of Food and Agriculture*, **99**, 1561-1567.

- 365 Lorenzo, J.M., Munekata, P.E.S., Pateiro, M., Campagnol, P.C.B., & Dominguez,
366 D. (2016). Healthy Spanish salchichón enriched with encapsulated n-3 long chain fatty
367 acids in konjac glucomannan matrix. *Food Research International*, **86**, 289-295.
- 368 Luallen, T. (2018). Utilizing starches in product development. In *Starch in food* (pp.
369 545-579). Woodhead Publishing.
- 370 Marti-Quijal, F. J., Zamuz, S., Tomašević, I., Rocchetti, G., Lucini, L., Marszałek,
371 K., Barba, F.J., & Lorenzo, J. M. (2019). A chemometric approach to evaluate the impact
372 of pulses, Chlorella and Spirulina on proximate composition, amino acid, and
373 physicochemical properties of turkey burgers. *Journal of the Science of Food and*
374 *Agriculture*, **99**, 3672-3680.
- 375 Marti-Quijal, F.J., Zamuz, S., Galvez, F., Roohinejad, S., Tiwari, B.K., Gómez, B.,
376 Barba, F.J., & Lorenzo, J. M. (2018). Replacement of soy protein with other legumes or
377 algae in turkey breast formulation: Changes in physicochemical and technological
378 properties. *Journal of Food Processing and Preservation*, **42**, e13845.
- 379 McClements, D. J. (2015). *Food Emulsions: Principles, Practices, and Techniques*.
380 CRC press.
- 381 Mehta, N., Ahlawat, S. S., Sharma, D. P., & Dabur, R. S. (2015). Novel trends in
382 development of dietary fibre rich meat products-a critical review. *Journal of Food Science*
383 *and Technology*, **52**, 633-647.
- 384 Mohsin, A., Ni, H., Luo, Y., Wei, Y., Tian, X., Guan, W., ... & Zhuang, Y. (2019).
385 Qualitative improvement of camel milk date yoghurt by addition of biosynthesized
386 xanthan from orange waste. *LWT*, **108**, 61-68.

- Palanisamy, M., Töpfl, S., Aganovic, K., & Berger, R. G. (2018). Influence of iota carrageenan addition on the properties of soya protein meat analogues. *LWT Food Science & Technology*, **87**, 546-552.
- Parniakov, O., Toepfl, S., Barba, F.J., Granato, D., Zamuz, S., Gálvez, F., & Lorenzo, J. M. (2018). Impact of the soy protein replacement by legumes and algae based proteins on the quality of chicken rotti. *Journal of Food Science and Technology*, **55**, 2552-2559.
- Paximada, P., Dimitrakopoulou, E. A., Tsouko, E., Koutinas, A. A., Fasseas, C., & Mandala, I. G. (2016). Structural modification of bacterial cellulose fibrils under ultrasonic irradiation. *Carbohydrate Polymers*, **150**, 5-12.
- Petracci, M., Bianchi, M., Mudalal, S., & Cavani, C. (2013). Stabiliser ingredients for poultry meat products. *Trends in Food Science & Technology*, **33**, 27-39.
- Resconi, V. C., Keenan, D. F., García, E., Allen, P., Kerry, J. P., & Hamill, R. M. (2016). The effects of potato and rice starch as substitutes for phosphate in and degree of comminution on the technological, instrumental and sensory characteristics of restructured ham. *Meat Science*, **121**, 127-134.
- Schmiele, M., Mascarenhas, M. C. C. N., da Silva Barretto, A. C., & Pollonio, M. A. R. (2015). Dietary fibre as fat substitute in emulsified and cooked meat model system. *LWT-Food Science and Technology*, **61**, 105-111.
- Thirumdas, R., Brncic, M., Rimac Brncic, S., Barba, F.J., Galvez, F., Zamuz, S., Lacomba, R., & Lorenzo, J. M. (2018). Evaluating the impact of vegetal and microalgae protein sources on proximate composition, amino acid profile, and physicochemical properties of fermented Spanish “chorizo” sausages. *Journal of Food Processing and Preservation*, **42**, e13817.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

411 Xu, R., & Zhu, J. (2019). Association between red and processed meat with breast
412 cancer: Comment on “Red and processed meat intake and risk of breast cancer: a meta-
413 analysis of prospective studies” by Guo *et al.* *Breast Cancer Research and Treatment*,
414 **173**, 241-242.

415 Yasin, H., Babji, A. S., & Ismail, H. (2016). Optimization and rheological properties
416 of chicken ball as affected by κ -carrageenan, fish gelatine and chicken meat. *LWT-Food*
417 *Science and Technology*, **66**, 79-85.

418 Zugcic, T., Abdelkebir, R., Barba, F.J., Rezek-Jambrak, A., Galvez, F., Zamuz, S.,
419 Granato, D., & Lorenzo, J. M. (2018). Effects of pulses and microalgal proteins on quality
420 traits of beef patties. *Journal of Food Science and Technology*, **55**, 4544-4553.

Table 1. Ingredients and concentrations used in the lean meat preparations, sorted by product category

Category	Ingredient	Concentration (% Weight)	
		Low	High
Animal protein	Caseinate		
	Gelatine	1	2
	Blood plasma		
Vegetable protein	Pea protein	1	2
Starch	Rice starch		
	Potato starch	1.5	3
Hydrocolloid	I-carrageenan		
	K-carrageenan	0.5	1
	Xanthan gum		
Fibre	Soluble citrus fibre		
	Insoluble citrus fibre	1	3

Table 2. Effect of different ingredients and concentrations added to lean meat samples on drip loss expressed as a percentage by weight of the total dough, rheological parameters and hardness (mean ± standard deviation)

Batch	Concentration	Drip loss (%)	Hardness (N)	Rheological parameters	
				G' (Pa)	Tan δ
Control	-	30.80±4.44 ^{67cd}	3.55±0.73 ^{123ab}	9583±1075 ^{123ab}	0.16±0.00 ^{12a}
Caseinate	L	26.76±0.48 ^{bcd}	3.95±0.01 ^{bc}	12074±1423 ^{ab}	0.17±0.00 ^{abc}
	H	19.27±0.14 ⁵⁶	4.39±0.41 ²³⁴	19265±3147 ⁴⁵	0.18±0.00 ³⁴
	Sig.	**	ns	ns	**
Gelatine	L	37.34±0.62 ^d	3.91±0.90 ^{bc}	10739±949 ^{ab}	0.18±0.00 ^{cd}
	H	38.11±0.07 ⁷	5.25±1.00 ²³⁴	7457±938 ¹	0.18±0.00 ¹²³
	Sig.	ns	ns	ns	ns
Blood plasma	L	19.66±8.55 ^{bc}	5.90±0.65 ^c	14628±1336 ^b	0.16±0.00 ^{ab}
	H	6.32±0.21 ¹²³⁴	6.03±0.50 ²³⁴	19390±460 ⁴⁵	0.17±0.00 ¹²³
	Sig.	ns	ns	*	*
Pea protein	L	23.08±0.01 ^{bcd}	4.49±0.62 ^{bc}	5467±2345 ^a	0.17±0.00 ^{abc}
	H	17.60±3.75 ⁴⁵	4.96±0.70 ²³⁴	9768±1411 ¹²³	0.17±0.00 ¹²³
	Sig.	ns	ns	ns	*
Rice starch	L	20.19±1.61 ^{bc}	3.40±0.31 ^{ab}	9660±1938 ^{ab}	0.17±0.00 ^{abc}
	H	15.70±4.99 ³⁴⁵	3.43±0.11 ¹²	8213±141 ¹²	0.16±0.01 ¹
	Sig.	ns	ns	ns	ns
Potatostarch	L	13.02±1.18 ^{ab}	3.81±0.24 ^{abc}	10338±543 ^{ab}	0.17±0.00 ^{ab}
	H	6.93±1.58 ¹²³⁴	4.59±0.01 ²³⁴	17000±5020 ²³⁴⁵	0.17±0.01 ¹²³
	Sig.	*	*	ns	ns
I-Carrageenan	L	20.31±1.69 ^{bc}	4.44±0.42 ^{bc}	14600±1068 ^b	0.17±0.00 ^{abcd}
	H	4.27±0.08 ¹²³	5.10±0.04 ²³⁴	21938±202 ⁵	0.18±0.00 ²³
	Sig.	**	ns	*	ns
K-Carrageenan	L	18.94±4.70 ^{bc}	5.53±0.45 ^{bc}	15698±2549 ^b	0.17±0.00 ^{abcd}
	H	13.58±3.73 ²³⁴⁵	6.91±0.20 ³⁴	20275±1146 ⁴⁵	0.17±0.00 ¹²³
	Sig.	ns	ns	ns	ns
Xanthangum	L	0.40±0.27 ^a	1.55±0.20 ^a	26075±6180 ^c	0.22±0.01 ^e
	H	0.12±0.17 ¹	0.76±0.11 ¹	18500±1831 ³⁴⁵	0.24±0.00 ⁵
	Sig.	ns	*	ns	*
Soluble citrus fibre	L	18.08±1.68 ^{bc}	4.11±0.11 ^{bc}	10655±859 ^{ab}	0.18±0.00 ^d
	H	1.95±0.16 ¹²	7.17±2.73 ⁴	19255±5692 ⁴⁵	0.20±0.01 ⁴
	Sig.	**	ns	ns	ns
Insoluble citrus fibre	L	29.14±1.00 ^{cd}	4.47±1.29 ^{bc}	10375±1937 ^{ab}	0.17±0.00 ^{bcd}
	H	17.87±5.25 ⁴⁵	4.96±0.16 ²³⁴	12071±63 ¹²³⁴	0.17±0.01 ¹²³
	Sig.	ns	ns	ns	ns
Sig.	I	***	***	***	***
	C	***	**	***	***
	IxC	**	ns	**	***

Concentration: L: Low concentration; H: High concentration;
I: significance due to the ingredient
C: significance due to the concentration
IxC: interaction of ingredient and concentration
Sig: significance * (P<0.05); ** (P<0.01); *** (P<0.001); ns: no significant difference
^{a-g} Means within the same column not followed by the same letter differ significantly (influence of ingredient at low concentration)
¹⁻⁷ Means within the same column not followed by the same number differ significantly (influence of ingredient at high concentration)

Figure 1. Elaboration process of samples

Figure 2. Effect of different ingredients and concentrations added to lean meat samples on drip loss expressed as a percentage by weight of the total dough (a), hardness (b) and rheological parameters (c, d) (mean $\pm$ standard deviation). Concentration: L: Low concentration; H: High concentration; ^{a-g} Means values not followed by the same letter differ significantly (influence of ingredient at low concentration) ($P<0.05$); ¹⁻⁷ Means values not followed by the same number differ significantly (influence of ingredient at high concentration) ($P<0.05$)

The effects of 11 different ingredients (animal proteins, vegetable proteins, starches, hydrocolloids and dietary fibre) in two different doses on a single meat model in order to determine and quantify the intrinsic properties of functional ingredients in respect to structure formation and stability.