STARCH AND FIBRE IN WHOLE-MUSCLE COOKED HAM: YIELD, MICROSTRUCTURE AND SENSORY DISCRIMINATION

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Abstract – The properties of rice starch (RS) and fructo-oligosacharides (FOS) as binding agents and phosphate substitutes in whole muscle cooked hams were investigated by response surface methodology (d-optimal). These 'clean label' ingredients might help to obtain a healthier product, but their inclusion needs to be optimised to prevent compromising the technological and eating quality. Four numerical factors: RS, phosphates (STPP), dextrose (DEX) and FOS and one categorical factor: muscle type (*Biceps femoris* and *Semimembranosus*) were combined in 25 brine formulations (runs). The muscles were injected to 120 % of their weight, tumbled, netted, and steam cooked. Cook loss and total yield were well predicted by linear models and new brine formulations were proposed to optimize them. The yields were obtained with a combination of STPP and RS. Visualization of the microstructure provided insights into the mechanisms governing the altered processing properties of substitute ingredients. Sensory analysis by triangle test showed that the inclusion of RS and FOS in cooked hams is organoleptically perceived.

Key Words – Cooked ham, Fructo-oligosacharides, phosphates, Microstructure, Rice starch

I. INTRODUCTION

Nowadays, there is an increasing demand for healthier foods. Regarding meat products, major concerns are related to the use of additives such as salt, nitrite and phosphates. Inorganic phosphate, a common ingredient in cooked brined ham, can negatively affect human health, especially in patients with advanced chronic kidney disease [1]. However, phosphates play important roles, mainly improving the water holding capacity and juiciness and allowing the reduction of salt addition in meat products [2]. FOS has been previously used as a sugar replacer and may have additional beneficial effects to human health [3].

The aim of this study was to investigate the effect of rice starch (RS) and FOS as substitutes for phosphate and dextrose in the processing of whole muscle cooked hams. Furthermore, microscopy analyses were conducted to visualise possible changes in the microstructure. Finally, a triangular test was used to evaluate potential sensory differences between hams made with the substitute ingredients *versus* the traditional ones.

II. MATERIALS AND METHODS

Experimental design and processing of hams

A response surface methodology (RSM) based on d-optimal experiment was designed using Design Expert (v. 7.6.1, Stat-Ease Inc.). Four numerical factors (ingredients) were included: RS (Remyline XS, Beneo remy), STPP, dextrose (DEX) and FOS (Beneo Synergy 1, Beneo orafti) (Table 1), with the following constraints: RS + STPP $\geq$ 0.3; DES + FOS $\geq$ 0.2; RS + STPP + DES + FOS $\leq$ 3.3. All 25 brine formulations (runs) generated were applied to two major pork muscles: *Biceps femoris and Semimembranosus* from the left legs of female carcasses and with a categorical factor: muscle type, included in the design.

Table 1. Factors and levels for the d-optimal response surface experimental design

Level	RS	STPP	DEX	FOS
- 1	0	0	0	0
+ 1	1.2	0.3	0.2	3

Levels expressed in % by weight of the injected muscle (picking salt at 2.5 % and sodium ascorbate at 0.05 % were also included in all the hams).

The muscles were pumped to 120 %, using a 20-needle brine injector and then tumbled for 12 h (6 rpm: 30 min on/off). Tumbled muscles were netted, vacuum packed, heat shrink-wrapped and steam cooked at 85 °C, 85 % RH, to a core temperature of 72 °C. pH readings from the green muscle (72 h *post-mortem*), brine and tumbled muscle were recorded. Muscle weights were recorded in the green state and after injection, tumbling netting, cooking and chilling. Brine uptake, cook loss and total yield were subsequently calculated. Data analysis was preformed with Design Expert software. Automatic reduction algorithms were applied to reduce the number of insignificant terms in the models. Formulation optimizations were also assessed. In addition, Pearson correlations between the brine pH and independent variables were calculated, using SPSS software (v. 18.0).

Microstructure analysis

Light and confocal microscopy analyses were performed on cooked samples of whole muscle ham (*Biceps femoris*). Samples (1 cm^3) were frozen in liquid nitrogen, cryo-sectioned in sections of 20 µm for both light and confocal microscopy and stained with a mix of Fast Green and Iodine (ratio 10:1) and Nile Blue and FITC (ratio 40:1), respectively.

Triangular test

A triangular test for difference was performed according to the British Standard ISO 4120 [4]. A 16 member panel evaluate two triads (one for each muscle) per pair comparison in individual booths under red light. Muscles from the left and the right side from the same animal were compared.

III. RESULTS AND DISCUSSION

Brine pH and green and tumbled muscle pH

As expected, there was no effect ($P > 0.05$) of the different ingredients on green muscle pH. Brine pH was significantly and positively correlated with the addition of STPP ($r = 0.838$, $P < 0.001$) and negatively correlated with FOS ($r = -0.420$, $P < 0.05$), but not affected by DEX and RS ($P > 0.05$). However, there was no significant rise in tumbled muscle pH caused by phosphate addition (Table 2).

Table 2. Characteristics of the response surface models for processing attributes in cooked hams.

	Tumb. m. pH	Brine uptake	Cook loss	Total yield
Sig. model	0.023	< 0.001	< 0.001	< 0.001
Lack of Fit	ns	ns	ns	ns
Model	2Flr	2FIr	LMr	LMr
R^2	0.249	0.431	0.692	0.569
R^2 Adj	0.164	0.366	0.679	0.560
R^2 Pred	0.085	0.273	0.656	0.535
Sig. RS	0.390	0.240	0.030	
Sig. STPP			< 0.001	< 0.001
Sig. DEX	0.066	0.047		
Sig. FOS	0.978	0.164		
Sig. RS x DEX	0.054			
Sig. RS x FOS		0.004		
Sig. DEX x FOS	0.003	< 0.001		

Although the model for brine uptake was significant, the low regression coefficients indicate that brine uptake was not well predicted (Table 2). Cook loss and total yield had models with higher coefficients and were both affected significantly by the level of added-STPP (Table 2). Cook loss was also reduced by the inclusion of RS, although to a lesser extent than STPP (Figure 1).

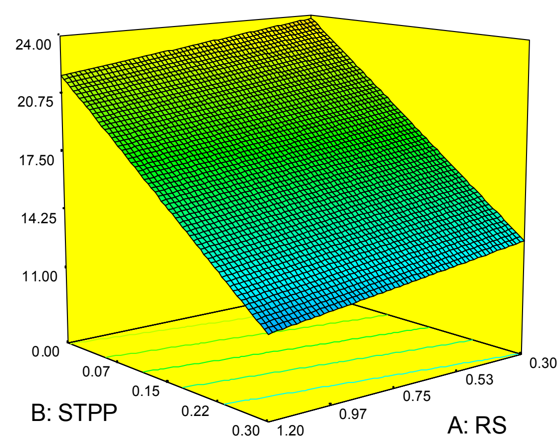

Figure 1. 3D plot of the cook loss (A: RS, B: STPP, C: DEX 0.2 %, D: FOS 0 %, E: muscle average)

Formulation optimization

Through the use of the optimization tool of the RSM, using the criteria 'minimizing cook loss, maximizing yield', 98 formulations were predicted and these are summarized in Table 3. The best predicted results are when 0.3 % of STPP and 1.2 % of RS are injected into the muscle (Table 3, *C1a*).

Table 3. Formulation to optimize processing quality of the cooked hams, according to different criteria (*C1* and *C2*).

	C1a	C1b	C2a	C2b	C2c	C2d
N	52	46	80	9	2	4
RS	1.2	0-1.2	1.1-1.2	0.6-0.9	1.20	0.2-0.3
STPP	0.3	0.3	0.1-0.2	0.2	0	0.1-0.2
DEX	0-0.2	0-0.2	0-0.2	0-0.2	0.08	0
FOS	0-1.8	0-2.9	1.5-2.0	1.5-2.6	1.47	1.6-2.9
PCL	11.07	12.51	16.11	16.83	21.66	18.28
PTY	101.51	101.50	95.35	95.83	88.53	94.07

C1: minimize cook loss and maximize yield. *C2*: minimize cook loss and maximize total yield, minimizing STPP and maximizing RS and FOS. N: number of solutions summarized. PCL: predicted cook loss. PTY: predicted total yield.

The optimization based on the second criteria, aiming for total or partial replacement of phosphates, showed that no added phosphates in combination with the inclusion of RS and FOS would result in a ham with 22 % cook loss and 89 % of total yield (Table 3, *C3c*). In the present study, the focus has been on weight loss/yield. This is an important consideration for processors but other quality aspects might be examined for the inclusion of these clean label ingredients. For example, starch can increase the perception of juiciness [5], whereas the inclusion of dietary fibre would help to obtain a healthier meat product.

Microstructure

Figure 2 shows the light micrographs of cooked hams without (A) and with (B) added RS, stained with fast green and iodine in a 10:1 ratio. It is possible to see the agglomeration of RS particles seen as a gelanitinized structure. In general, the 'RS gel' is located in free spaces, i.e. where the connective tissue was present or between myofibres. These gaps may have originated during tumbling and/or cooking [6]. The size of the 'RS gel' could reach around 40 mm^2, and therefore, they may be visible macroscopically, affecting the appearance of the product.

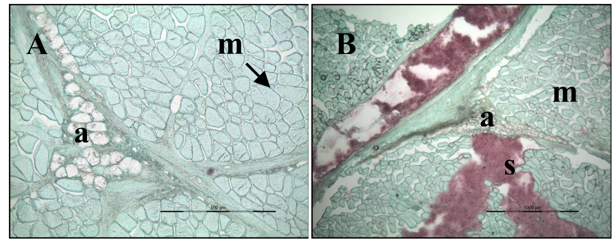

Figure 2. Light micrographs of cooked ham, A: 0 RS, 0.3 STPP, 0 DEX, 3 FOS (x10 obj.); and B: 1.2 RS, 0 STPP, 0.2 DEX, 0 FOS (x4 obj.). Showing starch (s), myofibres (m), adipose tissue (a)

RS was also positively identified in the confocal images (stained dark green), and the presence of FOS is suggested in Figure 3 (stained yellow). No interaction between RS and proteins was observed but they appear embedded in the protein phase, as previously reported [7].

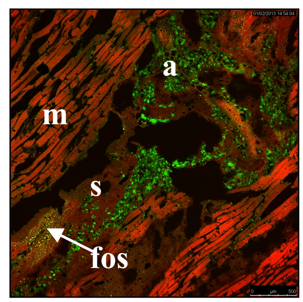

Figure 3. Confocal images of cooked ham (1.2 RS, 0 STPP, 0 DEX, 0.2 FOS). Showing RS (s), myofibres (m), fos and adipose tissue (a).

Table 4. Triangular test for 'no difference hypothesis' in cooked hams prepared with different formulations

	TRA *vs.* RS 0.30	TRA *vs.* RS 1.17	TRA *vs.* FOS
Biceps femoris			
Correct responses	31.25%	75.00%	75.00%
P value	> 0.200	0.001	0.001
Semimembranosus			
Correct responses	81.25%	68.75%	31.25%
P value	< 0.001	0.010	> 0.200
Both muscles			
Correct responses	56.25%	71.88%	53.13%
P value	0.010	< 0.001	< 0.050

TRA (traditional): phosphate 0.3%. RS: 0.30% and 1.17% of rice starch, respectively. FOS: phosphate 0.3 and fructo-oligosacharides 3.00%. All expressed by weight in the injected muscle (picking salt at 2.5 %, dextrose at 0.2 % and sodium ascorbate at 0.05 % were also included in each ham).

Triangular test

Differences in the eating characteristics were present in the *Semimembranosus* but not in the *Biceps femoris* between control hams containing phosphate and hams containing RS (0.3 %) (Table 4). Greater differences between the two hams in eating characteristics appeared at higher levels of RS inclusion. However, panellists perceived both hams to be acceptable. The addition of FOS at 3 % also modified the sensory characteristics of the *Biceps femoris* hams compared to controls, with most comments citing changes in texture, juiciness and salty taste intensity.

IV. CONCLUSION

Phosphates and rice starch are complementary in their effect on water binding capacity of whole muscle cooked hams and the best results were obtained when both were included. Partial or total substitution of phosphates with the inclusion of rice starch and/or fructo-oligosacharides is feasible; while it leads to a certain reduction in yield, it provides a healthier product.

Sensory analysis showed that discriminatory testing could identify organoleptic differences between samples produced with clean label ingredients and further studies are necessary to evaluate the acceptability of these products.

ACKNOWLEDGEMENTS

Research funding was provided under the National Development Plan, through the Food Institutional Research Measure (08/RD/TAFRC/671), administered by the Department of Agriculture, Food and the Marine, Ireland. We thank the technical support of Eugene Vesey and visiting student Hamza Chakhman.

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