EFFECT OF FAT SUBSTITUTION ON STRUCTURAL PROPERTIES OF COMMINUTED MEAT PRODUCTS: A MIXTURE DESIGN APPROACH

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Abstract – This study involved the full substitution of pork back fat in a typical breakfast sausage with prebiotic dietary fibre (two commercial Beneo GR and HP® inulin types) by mixture (D-optimal) design. The experimental design assigned 17 runs with each point representing a vertex, edge or surface point of the simplex. Each run represented a different substitution level for back fat with inulin, e.g. 100, 50, 33 % substitution. Sausage batters were prepared, containing 44 % lean pork shoulder, 19 % pork back fat/ inulin substitute, 28 % ice water, 7 % rusk and 2.5 % seasoning by weight, and stuffed into cellulose casings (23 mm). Samples were blast frozen and stored (-20 °C) for subsequent structural analyses (dynamic rheology, differential scanning calorimetry - DSC and microscopy). Inulin inclusions significantly increased the rheological characteristics (vield stress, storage modulus). Temperature-dependant behaviour of fat-replaced sausages was assessed using dynamic rheology and DSC. Storage modulus increased circa 30 to 40 °C which corresponded to the endothermic peaks for fat and protein recorded by DSC. Light and confocal microscopy techniques permitted visualization of the aggregation of inulin microcrystals distribution of fat respectively. Improved understanding of structural properties of meat models will aid in new product development.

Key Words – dietary fibre, response surface, dynamic rheology, calorimetry, microstructure.

I. INTRODUCTION

Health is a key driver in determining consumer purchasing decisions. Therefore, the meat industry is examining the potential to develop products with reduced levels of ingredients that are generally perceived as unhealthy, such as fat, salt or sugar and assessing the potential of meat products as vehicles for bioactive ingredients. Fat reduction is desirable from a health perspective as high consumption is linked with many chronic diseases (e.g. cardiovascular disease, late maturity onset diabetes). However, fat is a key component in the structural composition of emulsion type products like sausages and has an important role in governing the binding properties of protein molecules [1]. It also contributes to essential quality attributes such as texture, flavour and appearance. Therefore, its removal represents a significant technical challenge. Inulin (a prebiotic dietary fibre) has been shown to be a successful fat mimetic in other food products e.g. cheese [2] and fermented sausages [3]. It consists of linear non-digestible polysaccharides composed of fructose units joined together by β (2-1) linkages [4] and is usually derived from chicory root.

Previous work by Hayes et al. [5] has reported on the technological and sensorial effects of up to 7.5 % w/w inulin inclusion in breakfast sausages and concluded that inulin inclusions of up to 5 % were acceptable without significant detrimental changes in overall product quality. Previous studies by Keenan et al. [6] have reported increased TPA hardness (p < 0.001), with higher levels of fat substitution by inulin. This is an important observation as consumers are unwilling to compromise on quality arising from the removal of a constituent perceived as unhealthy or the inclusion of health promoting ingredients compared to conventional products. While the technological effects of inulin substitution for fat are well documented, information on the underlying mechanisms governing these phenomena are not widely available in real food matrices. Rheological, microscopic and calorimetric methods may better elucidate the technological responses, leading to improved understanding and a more scientific-based approach to product development. The aim of this work involved the

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rheological, microscopic and calorimetric analyses of fully fat-substituted breakfast sausages using two commercially available inulin products (Beneo GR and HP®, Beneo-Orafti, Tienen, Belgium) by mixture (D-optimal) design.

II. MATERIALS AND METHODS

Sausage batters were prepared containing 44 % lean pork shoulder, 19 % pork back fat /inulin, 28 % ice water, 7 % rusk and 2.5 % seasoning by weight and were stuffed into cellulose casings (23 mm). Samples were blast frozen (air speed 3.75 m/sec) and stored (-20 °C) for subsequent analyses. Mixture design experiments were designed and analysed using Design Expert (v. 7.6.1, Stat-Ease Inc., Minneapolis, MN, USA). The effect of ingredient proportions on comminuted meat product characteristics were studied using D-optimal design for the 3component mixture systems with constraints (all were restricted at the total maximum of 20 % of total sausage-meat weight), which comprised inulin: Orafti HP® (x_1) and Orafti GR® (x_2) ; and fat (x_3) . This assigned 17 runs with run representing a different substitution level for fat, e.g. 100, 50, 33 % substitution with inulin. Effects between component interactions on product structure were analysed by DSC dynamic rheology and microscopy using previously reported methodologies [7, 4 respectively]. Scheffe's special cubic model for three components was used to model the responses. Variables were analysed and models subjected to analysis of variance (ANOVA) to determine the significance (p < 0.05), determination coefficient (\mathbf{R}^2) and lack of fit.

III. RESULTS AND DISCUSSION

Rheological characteristics

Flow behaviour of raw sausages was assessed and recorded shear stress values were fitted to a Herschel-Buckley model $(\tau = \tau_{HB} + c\dot{\gamma}^p)$ to determine the yield stress. The yield stress is the point at which a material begins to deform plastically i.e. will not return to its original shape after the strain is removed. Results indicate that the model for yield stress was significant (p < 0.05) and that it increased with increasing inulin substitution, with a more pronounced increase for the Beneo HP inclusion at 100 % substitution than Beneo GR products (Figure 1). This is most likely due to the difference in chain length between the HP (long-chain) and GR (shorter chain), which are key determinants in gel formation [8].



Fig. 1: 3D contour plot of the mixture response surface for yield stress



Fig. 2. Frequency sweeps of the effects of fat substitution on selected extremes of raw sausages

A similar result was also obtained for oscillatory analysis of the raw sausage.



Fig. 3. Temperature-dependent behaviour of fat substituted sausages

Figure 2 shows storage modulus (G') dominating the loss modulus (G"), indicating expected paste-like behaviour for selected extremes with a tendency towards increasing storage modulus (representing the elastic or solid behaviour of the material) with increasing inulin inclusion. This can be attributed to inulin's ability to form interacting groups of micro-crystals that ultimately agglomerate into a gel network [9]. Furthermore, raw sausage samples were subjected to a heating regime (5-85 °C at 1 °C min⁻¹) under a fixed frequency to observe the effects of temperature on the gel.

Figure 3 shows that storage modulus increased for all samples between 30 and 40 °C as the onset of the hardening process followed by the chemical cross-linking (protein denaturation) occurred. Samples containing 50 % fat /Beneo HP are closest to the 100 % fat control. It also supports previous rheological trends for increasing elastic behaviour. However, a combined sample, containing 33 % fat/HP/GR, showed the largest increase in G' over the heating regime. This could imply a synergistic effect between the fat and inulin but is more likely due to a large degree of heterogeneity between the sausages.

Differential scanning calorimetry (DSC)

Two endothermic peaks were identified in the present study. The first endothermic peak (ca. 30 °C) corresponds to the melting of fat and is in agreement with previous authors [1]. The second endothermic peak corresponds to the remaining components of the sausage matrix i.e. protein from the meat (myosin, actin, collagen) and carbohydrate from inulin and rusk. Results indicate that the model for reaction enthalpy (ΔH) of peak 1 (energy needed to melt the fat) was significant (data not shown). As fat was substituted, ΔH values for endothermic peak 1 decreased while there was a concomitant significant rise in ΔH values for endothermic peak 2 (Figure 4). The increase in reaction enthalpy of peak 2 is a result of the denaturation of meat proteins (myosin 45-50 °C; collagen 60-66 °C; actin 78-80 °C [10]) and the development of an inulin micro-crystal network.



Fig. 4. Reaction enthalpy (Δ H) of endothermic peak 2 of fat-substituted sausages containing inulin.

Microstructure analyses

Figure 5a shows a light micrograph (bright field; x4 magnification) of 100 % fat-substituted cooked sausage. A network of micro-crystals can be seen bottom and top left in irregular shaped circles embedded in the protein (stained purple). In contrast, Figure 5b (100 % fat) shows more globular structures that are dispersed throughout the matrix. The distribution of fat in the sausages can also be visualised using confocal scanning laser microscopy (CSLM) and

differential staining with fast green and nile red. Fat (stained green) was reduced from the 100 % control, to total substitution with inulin (Figure 5d and 5c respectively).



Fig.5a-d. Light (a, b) and CSL (c, d) micrographs of fat substitution: a) and c) 100 % fat-substituted cooked sausage; b) and d) 100 % fat control cooked sausage

IV. CONCLUSION

Structural analyses may better elucidate the interactive mechanisms governing the technological characteristics of meat products and lead to more scientifically oriented product development strategies. Further investigation of the water holding and sensory characteristics of fat-substituted products will determine whether these effects are perceptible to consumers and help optimize fat-reduced product formulation.

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