The effect of Locust bean gum (LBG) on meat batter

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Abstract – Locust bean gum (LBG), as with many other non-meat ingredients, is used as a filler in meat products. Filling ingredients can influence the formation of the continuous gel matrix, modifying the physiochemical properties of the aqueous phase and influencing the final product. The main objective of this work was to evaluate the effects of dry, solubilised and gelled LBG on meat batter. The addition of 0.5,1 and 2% dry(w/w), solubilised and gelled locust bean gum (LBG) to a 65 % meat gel, improved the desirable characteristics of beef meat sausages. 1% dry LBG increased the hardness, gumminess and chewiness of samples. Addition of gelled LBG at a level of 1% incresed the cohesiveness and resilience of the product. However, adhesiveness only occurred with the addition of 2 % solubilised LBG. The present work reveals a significant change in the texture of beef sausage when adding LBG to meat batter. This study reports, Differential Scanning Calorimetry (DSC) and rheology results which show the effect of LBG on the thermal stability and the gelling proprties of myofibrillar proteins in meat batter. Differential scanning calorimetry studies of myofibrillar protein and dry LBG showed a small effect meat protein with upon addition of 1% dry LBG to meat. The effect was slight, but consistent with Pro-Poly interactions. The rheology results also confirm a weak interaction, with the G´ observed by small strain oscillatory rheology being similar to meat alone .This study confirms that the structure-forming events observed using rheology correspond in temperature to the wide endothermic transition observed in the DSC traces for identical samples run under identical conditions. Confocal Laser Scaning Microscopy (CLSM) showed that a phase separation between protein and LBG aggregates also takes place, and that the protein matrix is presumed to be weak because the protein network is not uniformly distributed through the sample. The size of the cavities increased when adding 1% dry LBG (650–700 μm) compared to 65 % meat (500–550 μm).The mean diameters of the fat droplets also decreased in a 1 % dry LBG/65%. In general, the addition of locust bean gum did not affect the textural properties and the overall acceptability of the product.

Key Words – LBG-Meat-Interaction-TPA-DSC-Rheology-CLSM

I. INTRODUCTION

The functionality of comminuted meat products depends on numerous factors, such as the water-holding capacity of the meat, non-meat ingredients, ionic strength, pH and temperature and rate of heating treatment (Acton et al., 1982; Asghar et al., 1985; Gordon et al., 1992; Smith, 1988). Non-meat ingredients are useful in comminuted meat products because of their functional properties, including emulsification, water- and fat-binding capacity and improving of texture and appearance (Hoogenkamp, 1992; Shand et al., 1990). Non-muscle proteins and polysaccharides are often used to enhance the texture and binding properties of comminuted meat products (Whiting, 1988). The protein–polysaccharide (Pro-Poly) interaction plays a significant role in the structure and stability of many processed foods. Functional properties of food protein, such as gel-forming ability, conformation stability, solubility and emulsifying and foaming properties are effected by their interactions with polysaccharides. Pro–Poly interactions are well documented for non-meat proteins. However, more studies are required regarding the interactions of meat proteins with hydrocolloids. Locust bean gum is obtained from the carob bean (Ceratonia silique), a Mediterranean tree. Carob gum or locust bean gum (LBG) is a galactomannan plant containing (1→4)-B-D-mannopyraonsyl backbone with attachment of (1→6)-α-D galactose single units (Dey, 1978). For the locust bean gum, the ratio of mannose to galactose varies between three and five, depending on the source and preparation method. Furthermore, LBG reduces syneresis and helps to produce gels of

equivalent strength with lower total polysaccharide concentration (Therkelsen, 1993). Synergistic effects appear when it is mixed with other hydrocolloids (Dea, 1979). Widely used as a thickener, LBG is well known to form a gel after a freeze/thaw cycle (Dea et al., 1977). It dissolves in hot water and forms a viscous solution (Doublier and Launay, 1981). For gel formed through a freeze/thaw cycle, cross-link formation results from the association of galactose uninhabited mannan regions, where the weight-average mannan block length is greater than six monomer units (Dea et al., 1986). The gelation rate initially increased with decreased temperature until the maximum gelation rate was found close to -5˚C (Richardson and Norton, 1998). Gel properties were dependent on galactose content, gelation temperature and concentration (Richardson et al., 1999). There are few papers to be found in the literature that deal with the effects of LBG addition on the properties of formulation meat products. Among these, Ramırez et al. (2002) reported that locust bean gums are not appropriate to be employed as a surimi additive; however, these gums presented a profitable effect when used with a xanthan ratio of 0.25/0.75. Also, Damásio et al. (1994) and García-García and Totosaus (2008) reported that if locust bean gum and k-carrageenan are added to the meat batter it causes an improvement in texture and water retention, with only a minor effect on sausage colour. A strong synergetic effect between locus bean gum and kappa carrageenan has been reported (Chen et al., 2001; Lundin and Hermansson, 1997; Stading and Hermansson, 1993). And finally, Luruena-Martınez et al. (2004) reported that the addition of locust bean/xanthan did not affect the textural properties and the overall acceptability of the product. In spite of these few published studies, the meat processing sector, particularly beef meat processors, need more scientific data to deliver formulated products that are able to meet the special requirements of consumers. Examples of such requirements may be an improvement in the cohesiveness, gumminess and chewiness of the final product, linking such properties to product microstructure. The present work consequently seeks to evaluate the influence of dry, solubilisation and gelation of locust bean gum addition on meat emulsion stability, textural properties such as hardness, springiness, cohesiveness, gumminess, chewiness and resilience and, finally, fracturability in sausage texture formation. In addition, the study investigates , looks into microstructure such as location of protein network, LBG and fat in cooked sausages, since no research has been conducted using dry, solubilisation and gelation of locust bean gum in meat products. Furthermore, the study aims to elucidate the role of heat heat-induced gelation in texture formation of processed meat products and it also involves the investigation of the interaction between meat protein in LBG because an understanding of the formation of locus bean gum/meat composite would be helpful in the development of new products.

II. MATERIALS AND METHODS

The fat trimmed from top side meat was obtained from a local butcher (Meat4u, Beeston, Nottingham, United Kingdom) 24 h after slaughtering. The top side was minced on a 5mm plate, using a Manica mincer PT-82/22. The meats were pre-weighed following the formulation, vacuum-packed separately and frozen at -20°C until use. The ingredients used in the homogenous formulation included locus bean gum (CP Kelco, Atlanta, Georgia, United States), sodium chloride(Saxa, Spalding, United Kingdom), and sodium triphosphate (Kilo, West Midlands, United Kingdom). Before the meat was processed, it was stored at 4°C for 24 hours (pH 5.67). Sausage formulations were prepared. The mincemeat was chopped with sodium chloride (NaCl) and sodium triphosphate (Na5P3O10) and half of the ice was added using the high speed setting for 1 minute; mixtures were kept in the bowl chopper for 5 minutes to allow enough time for protein extraction under high salt concentration(pH 5.87). After 6 minutes, hydrocolloids (dry, solubilisation, and gelation) were added to the batter. The bowl chopper was set to operate at high speed (knife speed: 2800 rpm, bowl speed: 24 rpm) for 60 seconds and the remaining ice was added to the mix (for dry hydrocolloids). This was then chopped for an additional 3 minutes (knife speed: 2800 rpm, bowl speed 24 rpm); the temperature of the mix did not exceed 13°C (pH 5.99).Generally, the higher quality the meat product, the less the particle size must be reduced. Immediately after chopping, the prepared sausage mixture was stuffed into 26 mm-diameter cellulose casing (Tehran Navid, Tehran, Iran). After the sausages were mechanically linked to standard size (10 cm in length and 70-80g in weight each), they were cooked in hot water (Ban Mari, UK) at 100°C for 1 hour, which was sufficient for the interior of the products to reach 72°C. A thermocouple probe (Omega Engineering, Inc., Stamford, Conn, USA) positioned in the geometric centre of the sausage was used to monitor product temperature (pH 6.1).

The sausage were then rinsed in cold water for 15 minutes, after which the water was wiped off the products with a clean towel and they were stored in a refrigerator (0-4°C) for periodic measurements. All samples were produced in triplicate. Solubilised LBG was prepared from aqueous suspensions according to the relevant formulas. Hydrocolloids were mixed by reverse-osmosis in water for 1 hour in a mixer at moderate speed and by heating to 95°C for 1 hour with constant stirring. After cooling at ≈50°C, the solutions were added to the meat batter. For the gelation of LBG, the hot solution was placed in the freezer for 12 h; after defrosting, the solid LBG was mixed into the meat batter.

III. RESULTS AND DISCUSSION

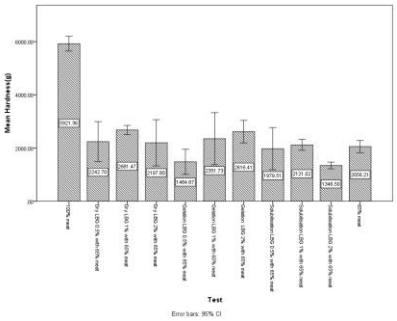


Figure 1

In this study we have chosen the highest level of each treatment for analysis of Anova and compare the highest one with the others. Analysis of variance (ANOVA) showed that hardness differed significantly by ID-Test types, so, Scheffe's test was applied in order to compare the means of the hardness ID-Test. In hardness, figure 1 shows that we have the highest mean in standard samples (100% meat), so that the p-value of the 100% meat vs. all of the other ID-Tests (dry of LBG 0.5%, 1% and 2%; gelation of LBG 0.5%, 1% and 2%; solubilisation of LBG 0.5%, 1% and 2%;65% meat) is less than .01, which means that the hardness results of these ID-Tests differ statistically with the level of 100% being at meat at $\alpha = 0.01$. After the maximum hardness (100% meat), dry of LBG 1% with 65% meat has the highest hardness. This is related to the protein matrix because LBG in meat batter is aggregated during heating and consequent stabilised the meat matrix and as a result of this improving hardness achieved . The softest sausage samples were those containing the solubilisation of LBG with a concentration of 2% and this is probably due to the water-holding capacity of beef meat sausages because LBG binds with large amounts of water and when added to meat batter it improves the water holding capacity and as a consequence the softness of the product improves. This result agrees with the results of Luruena-Martınez et al. (2004), who reported there was no significant change in hardness when adding LBG into meat batter.

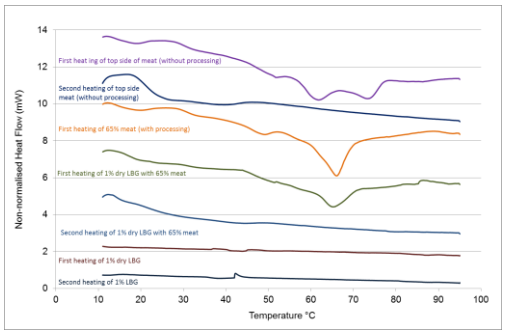


Figure 2

In this study the function of 1% dry LBG with 65% meat on the formulation of sausage batter composite during heating was investigated. The curve of endothermic topside meat (without processing) shows meat fat to be completely melted at 35 °C, which is in agreement with a previously reported study by Quinn et al. (1980).From the curve given for top side meat, three transitions can be observed: one at 51˚C, which is myosin, a second at around 61˚C, which is sarcoplasmic, and a third transition apparently at 73 ˚C, which is actin. Upon completion of the initial scanning, samples were immediately cooled to 5 ˚C and re-scanned to determine the reversibility of some of the changes in the system. There was no evidence of a peak in top side meat, indicating a complete irreversible denaturation of meat protein. The addition of 2.7% salt and 0.6% sodium triphosphate to the batter (65% meat) resulted in the disappearance of the three peaks. With the addition of salt and sodium triphosphate to the meat batter the first peak transition is less affected and the temperature shifted to the lower temperature (45 ˚C). However, the second and third transitions disappeared and shifted to the higher temperature. The small peak represents myosin and the large peak represents the denaturation of sarcoplasmic protein, connective tissue and actin (66.1 ˚C).

It is surprising that the initial scanning of 1% dry LBG with 65% meat indicates a wide transition with an endothermic peak of 65.4 ˚C. It seems that two peaks – myosin denaturation and a combination effect of the melting of LBG (coil→ aggregate transition) and sarcoplasmic and actin denaturation –

merge together and it is not possible to distinguish them from each other. and this is may be due to the LBG melting point, because Dušek and Prins (1969) reported that between the temperatures of 45 and 85 °C the LBG treatment began to melt;, furthermore, natural salt such as sodium chloride has only a slight effect on LBG (Maier et al., 1993). This result is accepted by previous findings (Igoe, 1982), which indicated that locust bean gum solubilised before fish gel formation can accrue. The curve of the second scan of 1% locust bean gum with 65% meat shows no response and indicates an irreversible transition from the LBG and proteins. The presence of 1% locust bean gum has very little influence on the transition enthalpy in that the small peak melting temperature occurs at around 44.44˚C and this supports our previous findings (Goncalves et al., 1997). We did not get a response with the second reheating of 1% LBG and this indicates an irreversible transition. Table 1 shows the onset and offset and peak temperatures for the endothermic peaks observed on heating the individual components or meat/LBG mixtures. The raw heat change data has been corrected for the weight of the specified component in the mixture. The table 1 shows the maximum onset related to 65% meat, which is 59.2˚C, and maximum offset related to the first heating of the 1% LBG with 65% meat, which is 85.6 ˚C. Within the error of measurement, baseline fitting and enthalpies it seems that any interaction between LBG and meat is unlikely to occur.

samples	T_0	T_e	T_m	ΔH (J/g)
First heating of top side meat (without processing)	55.4	77.7	61.7	2.0
First heating of 65% meat (with processing)	59.2	67	66.1	2.1
First heating of 1% dry LBG with 65% meat	58.6	85.6	65.1	2.0

Table 1 Endothermic heating transition parameters for top side meat ((first heating); 65% meat (first heating); 1% dry LBG with 65% meat (first heating); 1% dry LBG with NaCl and sodium triphosphate (first heating).T_0 = onset temperature; T_e = end set temperature; T_m = peak temperature and ΔH = non-normalised rnthalpy of transition.

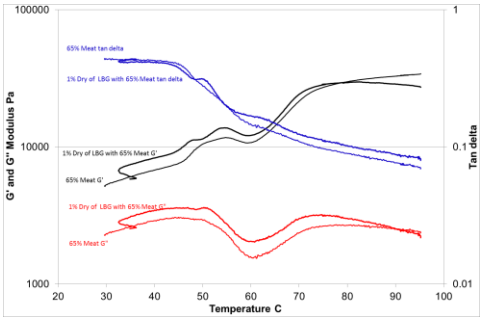


Figure 3

The 1% dry LBG with 65% meat samples were analysed for storage (G ´) and loss (G˝) moduli and tangent (tan δ) by dynamic oscillatory test, as well as 65% meat for control measurement as a function of temperature (30–90 °C)(figure 3)

Along the gelation for both curves (1% dry LBG with 65% meat and standard samples, which are 65% meat), four characteristic phases were defined, as shown in figure 3:

Phase 1 – beginning of structure build-up during the heating stage (30–44 ˚C);

Phase 2 – peak maximum (44–54˚C);

Phase 3 – minimum after the peak (54–60˚C);

Phase 4 – end of the heating stage (60–90˚C).

Gel point, defined as the increase in G´(pa) conceding in decreased Tangent δ value measured.(Hermansson, 1989) was recorded at 38 °C. Also, in this phase 1, denaturation of the head proteins of myosin and subsequent aggregation are responsible for the initial increase in G´ that occurs (Verbeken et al., 2005; Wang and Smith, 1994; Wang et al., 1990; Xiong, 1997).In phase 2, the result of G' in standard samples (65% meat) showed that there are two peaks, which represent the unfolding of myosin molecules;: the irreversible interfilamental interaction between the myosin heads (45–50 ˚C) (Egelandsdal et al., 1986; Sano et al., 1988); and another shoulder that may be further explained by the denaturation and precipitation/coagulation of myosin (Ikeuchi et al., 1994; Samejima et al., 1981)). It is interesting to note that the two peaks in 65% meat are merging together and this is because of the fast interaction of the myosin heads or the interaction of myosin with other components, such as tropomyosin (Sano et al., 1988). However, in LBG treatment at temperatures greater than 45˚C, showing two peaks. The storage modules increase gradually as the temperature increases, and this is due to the melting point of the LBG treatments (Richardson and Norton, 1998). Also, between the range of 45 and 50˚C, the unfolding of myosin molecules takes place. Dušek and Prins (1969) reported that between the temperatures of 45 and 85 °C the LBG treatment began to melt, and this is confirmed in this study because the melting point (coil→ aggregated transition) started at 45 ˚C and this transition conform by DSC transition . Also, the increase in G´ was reasoned to be a result of an increase in chain entropy with temperature. Storage modules at above 48 ˚C, there was an initial decrease followed by a gradual increase in temperature, indicating the loss of junction zones through melting, and this is reflected by an initial shoulder for tan delta (tan δ =G˝ / G´) which then decays with increasing temperatures. After 50 ˚C, the loss tangent of all samples decreased sharply; this indicates a sol-to gel transition. After 60 °C tangent δ decreased at a slower rate and reached equilibrium, suggesting gel development was complete. The second peak in the LBG treatments is explained by the denaturation and precipitation/coagulation of myosin. Also, at a temperature around 52˚C, denaturation of the myosin tails takes place, which leads to an increase in the protein network. Another hypothesis regarding the second development stage of G' in 1% dry LBG with 65% meat was referred to as 'gel setting' , in which the loose gel structure was formed and this is the conformation of the myosin molecules having changed.

In phase 3 the dissociation of the actin–myosin complex has also been suggested to contribute to the decrease in G´ between 54 and 60 ˚C. This is maybe due to the breaking hydrogen bonds of myosin as the temperature increases. Also, in this phase denaturation and precipitation/coagulation of myosin heads takes place (Ikeuchi et al., 1994; Samejima et al., 1981).

And finally, in phase 4 which is protein gel starting point. It is also during this final thermal phase that significant gelation of myosin is achieved (Ishioroshi et al., 1979; Ziegler and Acton, 1984). Future increases in G´ (60–70 ˚C) in temperature, after the point where gelation starts, have been attributed to a network formation by the aggregation and entanglement of unfolded protein molecules. The contribution from the connective tissue proteins in increasing G´ during heating (figure 3) must also be acknowledged, as collagen fibre contraction can occur up to the one-quarter of their original length under heating at 60–70 °C and if they are unrestricted (Tornberg, 2005), as would be expected in a high moisture content environment, such as the meat batter considered here. As the temperature reaches between 60 and 70 °C, the connective tissue and the muscle fibres are longitudinally contracted, and such a contraction increases with the temperature (Siripurapu et al., 1987). The slight increase in G´ in both figures during heating between the ranges of 70 to 80˚C is related to titin denaturation. Towards the end of the heating stage (at around 90 °C), a slight decrease in G' can be seen in both figures, which may be attributed to the connected tissue and collagen softening at this stage, as observed by Siripurapu et al. (1987) in meat emulsion products. In all samples the storage modulus (G´) was greater than the loss modulus (G˝) during the test (G´> G˝), which indicates the predominantly elastic behaviour of the sample for the whole range of deformation tested.

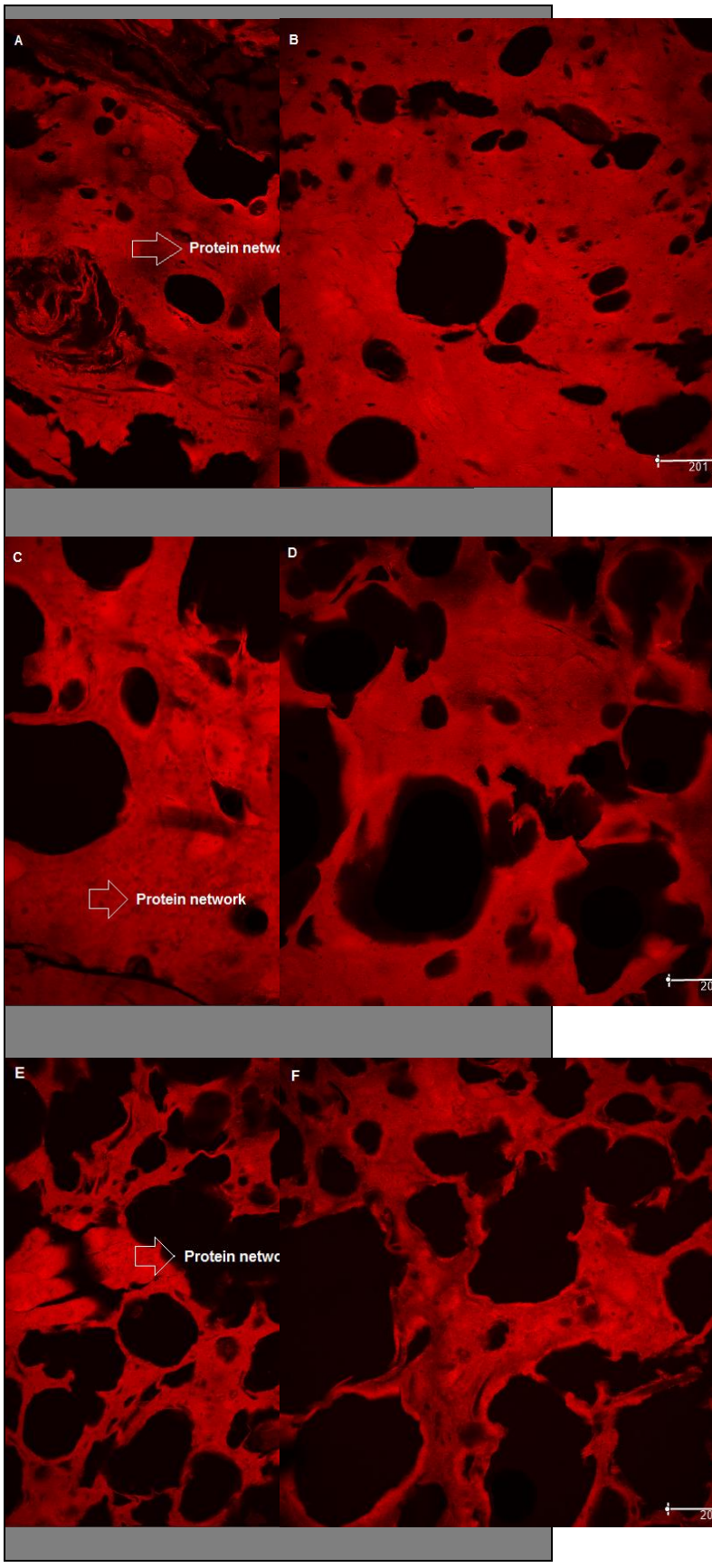
Food scientists are faced with many challenges in product development, quality control, and basic research. Confocal laser scanning microscopy (CLSM) offers the opportunity to study many important phenomena, such as physical aggregation, phase separation, and the effect of additives and processing conditions on microstructure. Interaction and phase separation of proteins and polysaccharides are important in determining the properties of many foods. Various instrument techniques can be used to give some insight into structure, such as nuclear magnetic resonance, rheometry etc., but microscopy, particularly CLSM, offers a more visual and direct approach (Vodovotz et al., 1996). Applying CLSM, the microstructure of *beef sausage standard samples*

(100% and 65%) and 1% dry LBG with 65% meat was visualised.

Sausage standard images (A and B) of 100% meat and images (C and D) of 65% meat (figure4) show the protein matrix or muscle fibre (red colour) completely distributed in system. Because of the formulation of standard samples, the size of the cavities in 65% meat (500–550 µm) is more than 100% meat (200–300 µm).

Adding dry LBG to batter caused a near complete breakdown of the continuous network and this is clear in the CLSM of the sausage images (E and F). Also, the size of the cavity is increased when adding 1% dry LBG (650–700 µm) when compared to standard samples, which are 65% meat (500–550 µm), because according to the thermogram results, LBG has already aggregated; after that, myosin, sarcoplasmic and actin create a gel and as a consequence of that, LBG is aggregated throughout the meat matrix and this aggregation of LBG is not trapped within the protein gel structure and for this reason the size of the cavity of this treatment is increased. Furthermore, it seems that the LBG rather than the protein continues in this system and the network of LBG treatment is weak and this supports our previous findings (Chattong et al., 2007). The confocal results are inconclusive because in this study we did not succeed in staining LBG with fluorescein isothiocyanate (FITC, 0.02% w/v in water/ethanol) in the protein matrix and it was not possible to definitively locate LBG.

Confocal scanning laser microscopy has been used to see the location of fat in the system. Labelling the fat with BODIPY493/503 allowed the fat material, which is green (round area), to be identified as being fairly evenly distributed in the protein network (continuous area) throughout the structure. Standard samples (65% meat) and 1% dry LBG with 65% meat show that fat coated by protein continues (round area) phase forming the typical sausage emulsion-like structure. Furthermore, the fat droplets decrease in mean size when adding dry LBG to the system. The size of the fat droplets is 60 µm or less; however, the fat droplets of standard samples are between 60 and 100 µm because the LBG is essentially 1–4 linked liner polymers and may be regarded as a natural polymer. Hence, LBG – being both non-ionic and with only ‘single sugar’ side groups – was observed to produce only a limited reduction in fat droplet size (Glicksman, 1969).



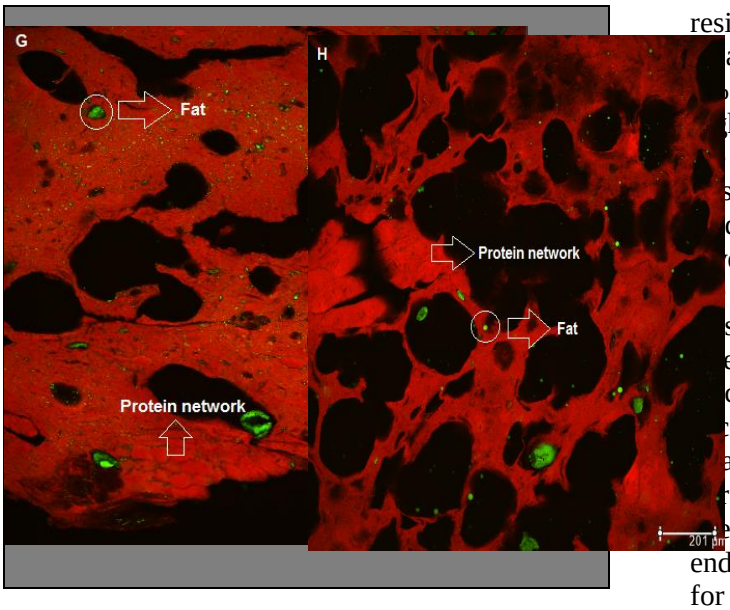


Figure 4 CLSM images of sausage containing different concentrations.
Images A and B are standard samples (100% meat) stained for protein matrix; image C and D are the standard samples (65% meat) stained for protein matrix; E and F images related to 1% dry LBG with 65% meat stained for protein matrix; image G is a standard sample (65% meat) stained for fat and protein matrix, and finally image H is 1% dry LBG with 65% meat stained for fat and protein matrix.

IV. CONCLUSION

The locust bean gum, like other non-meat ingredients, is a filler ingredient. Filling ingredients can act to influence the formulation of the continuous gel matrix, modifying the physiochemical properties of the aqueous phase and influencing the final product. The results obtained from the texture analyser show the mean value of 100% meat (standard sample) is the highest (less than .01) in hardness, springiness, chewiness and gumminess; however, after the standard samples, which are 100% meat, adding 1% dry LBG to meat batter with 65% meat improves hardness, gumminess and chewiness. Furthermore, an increase in cohesiveness and resilience of sausage samples related to the 1% ation of LBG with 65% meat, and finally the solubilisation of LBG with 65% meat has the hest mean value of adhesiveness. In addition, softest and firmness of the sausage is related to s range. Differential scanning calorimetry dies of myofibrillar protein and dry LBG ealed a slight effect on the meat protein with addition of 1% dry LBG to meat. The effect s slight, indicating notable Pro-Poly interactions. e rheology results confirm similar weak coelastic behaviour observed by small strain illatory rheology because the G´ of LBG atment is very similar to standard samples. Also, study confirms that the structure-forming ents correspond in temperature to the wide endothermic transition observed in the DSC traces for identical samples run under identical conditions. The CLSM images showed that the phase separation between protein aggregation and LBG aggregation also takes place, and that the protein matrix is weak because the protein network in not evenly distributed in this system. In general, the addition of locust bean gum did not affect the textural properties and the overall acceptability of the product. For this reason, in most meat applications, mixing the locust bean with kappa carrageenan can be used, because the kappa carrageenan/locust bean gum interaction improves texture and water retention.

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