# EFFECTS OF LACTIC ACID SOURCE ON PROPERTIES OF BEEF SAUSAGES RESTRUCTURED WITH ALGINATE GEL

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**Abstract – The aim was to compare three different acidification methods in beef sausages that use alginate as a cold-set binder. Samples were prepared and stored at either 37°C or 4°C to allow fermentation or normal cold-setting. For the high temperature group, results showed that the fermented (F) treatment developed the lowest pH (5.5), highest level of lactic acid concentration, highest binding and second highest redness ("a" value) (P<0.05). The treatment with glucono-delta lactone (GDL) had the second highest binding strength (P<0.05), while powdered lactic acid (P) and the control (C) treatments had the lowest binding strength (P<0.05). For the cold storage group, P treatment had the lowest pH values (P<0.05), lowest redness and lightness ("L" value) (P<0.05), and highest binding strength (P<0.05) along with the GDL treatment. No significant difference was found between the high temperature group and cold storage group for P treatment in binding strength (P>0.05). This study indicated that acidification by fermentation resulted in greater binding at high temperature but GDL or powdered lactic acid is better in cold storage for binding of restructured beef sausages.**

**Key Words – Binding strength, color, texture profile analysis.**

## I. INTRODUCTION

Restructuring of meat products enables the use of low-price cuts from mature animals to produce high quality meat products at reduced cost [1]. Traditional restructured meat products are based on sausage-making technology which depends upon extraction of myofibrillar proteins by salt, phosphate and mechanical action [2]. The cold-set alginate system uses an organic acid to modulate calcium solubility for greater internal binding strength [1]. Evidence that fermentation can achieve similar results has not been reported. The approach of this project is to compare different sources of acid (lactic acid bacteria, glucono-delta-lactone and powdered lactic acid) on binding strength and other selected properties of restructured beef sausages. To measure this effect, the three treatments and a control were compared after storage at 37°C to favor the fermentation or 4°C to favor the cold-set process.

## II. MATERIALS AND METHODS

Ground beef was bought locally and was well mixed before being divided into four Ziploc bags, with every bag weighing approximately 1150g and stored in a walk-in freezer (-18°C). When needed, bags of ground beef were transferred to a home-style refrigerator for 24 hours of thawing. The entire experiment was repeated four times and within each replicate, all four treatments (Control group (C), Fermentation group (F), Powdered lactic acid group (P) and Glucono-delta-lactone group (GDL)), were prepared at the same time. The culture was *Lactobacillus curvatus* (#690607, Chr. Hansen, Inc, Milwaulkee, WI) and it was enriched in deMan Rogusa Sharpe MRS broth at 37°C for 24h before cell pellets were harvested by centrifugation and suspension in peptone water to deliver 5 log CFU/g of beef. All formulations are shown in Table 1.

After blending the ground beef with the other ingredients for two minutes, the 1150g sample was divided into four parts. One part of the sample consisting of 100g was divided into five sterile petri dishes and this part of the sample was put into an incubator at 37°C for 48h and measured for pH and lactic acid concentration at 0, 10, 24, 36 and 48h. The second part consisting of 600g was put into natural casing with approximately 200g in each link of sausage and incubated. After 48h of storage, samples were taken out, cooked in a water-bath until the internal temperature reached 71°C and after cooling down to room temperature, the samples were ready for the Minolta

colorimeter, puncture test and texture profile analysis test. The third part consisting of 300g was put into natural casing with approximately 150g in each link of sausage. This part was put into a refrigerator and at 48h and 96h, one of samples was taken out to be cooked and tested as described above. The final part consisting of approximately 30g was divided into two sterile petri dishes and covered with clear plastic wrap. These were placed in a refrigerator and samples were taken out at 48h and 96h for pH determination.

Table 1. Formulations for treatments[a]

| Ingredients[b] | C | F | GDL | P |
|---|---|---|---|---|
| Ground beef | 900 | 900 | 900 | 900 |
| Sodium alginate | 32.4 | 32.4 | 32.4 | 32.4 |
| Calcium carbonate | 10.8 | 10.8 | 10.8 | 10.8 |
| Dextrose | 27 | 27 | 27 | 27 |
| Sodium nitrite | 2.24 | 2.24 | 2.24 | 2.24 |
| Lactic acid bacteria | - | 5 log/g | - | - |
| Powdered lactic acid | - | - | - | 9 |
| GDL | - | - | 9 | - |
| Peptone water[c] | 90mL | 90mL | 90mL | 90mL |

[a] C: Control, F: Fermentation, GDL: Glucono-delta-lactone, P: Powdered lactic acid.
[b] All dry ingredients were weighed in grams except culture.
[c] Peptone water: 1g peptone powder/L distilled water.

For pH measurement, 5 g ground beef was homogenized with 50 mL distilled water, and the pH was measured by a Fisher Account (Model 230A) pH meter. Lactic acid concentrations for each treatment were measured at 0h, 10h, 24h, 36h, and 48h using high performance liquid chromatography (HPLC). The Agilent system consisted of a pump, an organic acid column, an auto sampler for auto injection, UV detector and a Galaxie Chromatography data system which was used to calculate the quantity in mg/g. The mobile phase consisted of acetonitrile (6%) and 0.045N $H_2SO_4$, with a flow rate of 0.5 mL/min, the UV detector wavelength was set at 220nm and temperature was 55°C. Five grams of ground beef was homogenized with distilled water and centrifuged at 3900 rpm for 20 min at 4°C. Suspensions were obtained, which were diluted 10 times and filtered to use for the auto injections.

A puncture test was conducted as an indicator of binding strength using a Stevens-LFRA Texture Analyzer to penetrate five to six 2-cm thick sausage slices. The diameter of the spherical probe was 0.635cm and the penetrating speed was 2.00mm/sec. The highest values (in grams) were recorded. Texture profile analysis was conducted using an Instron texture analyzer. Five or six samples that were 1 cm thick and 2 cm in diameter were compressed by the probe (diameter 50mm, thickness 20mm) twice to 50% of their height. Other test conditions were: test speed 1 mm/g, pre-test speed 5 mm/s, 50 kg load cell, 5 s between the two compression cycles at 25°C.

One-way ANOVA was used to analyze the data with 0.05 as the significance level and a Tukey multiple range test was used to separate means.

## III. RESULTS AND DISCUSSION

The pH values of the F treatment and C treatment dropped as expected after 10h fermentation time; however, the F treatment declined furthest and by approximately one and half units compared to the other treatments at the end of the 48h fermentation time. The pH of the Powdered lactic acid treatment and GDL treatment at the starting point was lower ($P<0.05$) but this was attributed to the direct acidification imparted by the acids. There were no significant differences of pH value among all treatments after incubation for 48h at 37° C ($P<0.05$) as shown in Figure 1.

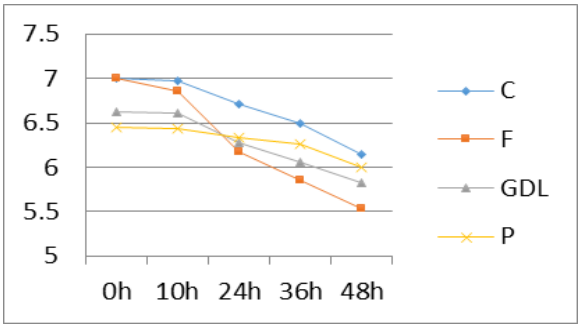


Figure 1. Changes in pH of beef sausages during 48h of incubation at 37°C

The pH can be explained by the lactic acid concentration presented in Figure 2. The

decrease in pH in the F group was due to the increase of the lactic acid concentration that is produced by the lactic acid bacteria. The lactic acid concentration of the P treatment was significantly higher than that of the other three treatments at the beginning of the storage time (P<0.05). After 48h, the lactic acid concentration of the F treatment was significantly higher than that of the other treatments (P<0.05) and there were no significant differences among the other three treatments (P<0.05).

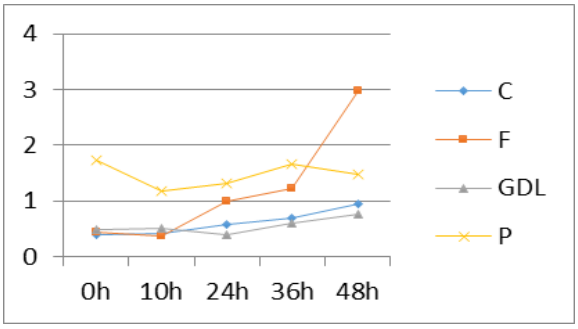


Figure 2. Lactic acid concentration (mg/g) of beef sausages during 48h of incubation at 37°C

For the cold storage group, the pH at 48h was 6.37 and 6.58 for the P and GDL treatments, respectively and were statistically lower (P<0.05) than the 6.94 and 7.00 for the C and F treatments, respectively. The lack of acidification in the F treatment at 4°C affects solubility of the calcium carbonate and thus calcium is unavailable to complete the gelation.

Table 2 has the color observations of the four treatments in the high temperature storage group. The C treatment has the lowest (P<0.05) redness ("a" value) and the second highest lightness ("L" value), which are not desirable in beef products. The F treatment had the highest redness values and lower lightness values (P<0.05), indicating the F treatment had a better color than the other treatments. The higher redness and lower lightness indicated that using fermentation has a positive effect on appearance and may influence myoglobin. Results for the group stored at 4°C were similar but are not presented here.

Table 2. Color observations of beef sausages after 8h of incubation at 37°C

| Treatment[c] | L | a | b |
|---|---|---|---|
| C | 42.24[ab] | 7.30[b] | 7.96[a] |
| F | 41.67[b] | 9.68[a] | 6.69[b] |
| GDL | 43.21[a] | 9.71[a] | 7.92[a] |
| P | 41.31[b] | 8.78[a] | 7.83[a] |

[ab] Different letters in each column indicate significant difference (P<0.05).
[c] C: Control, F: Fermentation, GDL: Glucono-delta-lactone, P: Powdered lactic acid.

The puncture test determined the force to push a probe into food samples [3] and is often used to compare binding strength for treatments. For the high temperature group, the F treatment had the highest average puncture value (P<0.05) in grams (Fig 3.), indicating that the F treatment had the highest binding strength. The GDL treatment had the second highest value (P<0.05) but there were no significant differences between the C and P treatments. Although C had the second lowest lactic acid concentration after

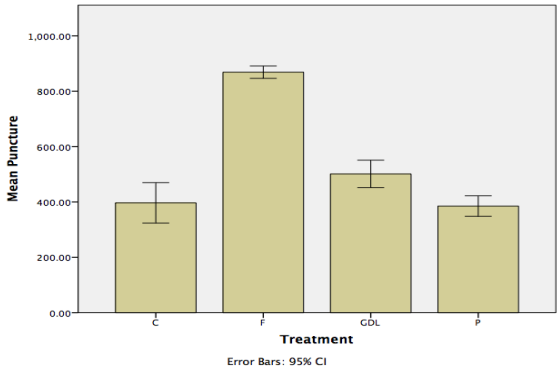


Figure 3. Puncture test (grams) of beef sausages after 48h of incubation at 37°C

48h at 37°C compared with P and F treatments (P<0.05), the binding strength was similar to that of P treatment and significant lower than GDL or F treatment. The greater acidification if F appeared to aid in the alginate gelation at 37°C [4], whereas the other three treatments did not develop as high binding strength.

For the cold temperature group, the puncture test results were quite different (Fig. 4). In that

group, the C and F treatments had the lowest values, indicating that the least binding took place. The GDL and P treatments, however, were significantly (P<0.05) higher and the result was consistent with previous cold set results [1, 5, 6] using alginate binders. The outcome indicated that acidification by fermentation at 37°C or release of acid (GDL) at 4°C were both effective in increasing binding.

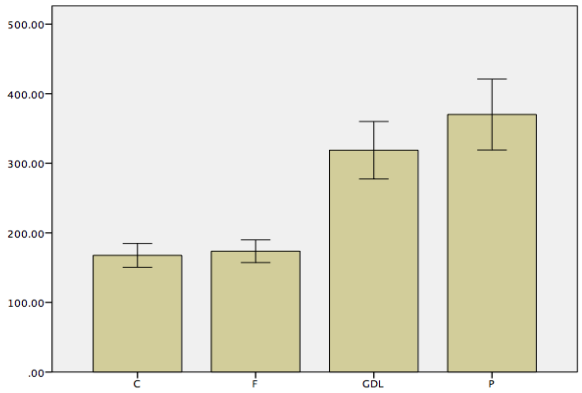

Figure 4. Puncture test (grams) of beef sausages after 48h of incubation at 4°C.

As shown in Table 3, F treatment for the high temperature storage group had the highest hardness, gumminess and chewiness (P<0.05), indicating that the internal binding and elasticity were stronger than for the other treatments. The P treatment had the lowest springiness, cohesiveness and gumminess indicating it had weaker binding properties (P<0.05).

Table 3. Texture profile analysis of beef sausages after 48h of incubation at 37°C

| Treat-ment[d] | Hard-ness | Spring-iness | Cohes-iveness | Gumm-iness | Chew-iness |
|---|---|---|---|---|---|
| C | 25.6[a] | 0.93[b] | 0.81[c] | 20.4[b] | 18.8[b] |
| F | 48.2[b] | 0.91[b] | 0.79[c] | 39.1[c] | 35.6[c] |
| GDL | 22.7[a] | 0.89[b] | 0.74[b] | 17.1[ab] | 15.5[b] |
| P | 17.9[a] | 0.76[a] | 0.64[a] | 11.6[a] | 8.9[a] |

[abc] The different letters in each column indicate significant difference (P<0.05).
[d] C: Control, F: Fermentation, P: Powdered lactic acid, GDL: Glucono-delta-lactone

There was no significant difference between C and GDL treatment in terms of hardness, springiness and chewiness (P<0.05). Cohesiveness of the C treatment was significantly higher than GDL treatment (P<0.05). Results for the cold storage group are not presented here, but the C treatment was significantly (P<0.05) lower in hardness, gumminess and chewiness compared to the other treatments.

## IV. CONCLUSION

At a high storage temperature which allows fermentation, the generation of lactic acid had a favorable impact on binding strength compared to the use of powdered acidulants. In cold storage, the use of either GDL or powdered lactic acid was more effective in increasing the binding strength. Fermentation did not occur in the cold group but acidification by either fermentation or addition of powdered acidulants is necessary for solubility of the calcium source and adequate alginate gel formation in beef sausages.

## ACKNOWLEDGEMENTS

The authors thank the University of Missouri Agricultural Experiment Station for support of the project.

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