



An Exploratory Study into the Use of Black Soldier Fly (*Hermetia illucens*) Larvae in the Production of a Vienna-Style Sausage

Leah W. Bessa^{1,2}, Elsje Pieterse², Gunnar Sigge¹, and L. C. Hoffman^{2,3*}

¹Department of Food Science, Stellenbosch Univ., Private Bag X1, Matieland 7602, South Africa

²Department of Animal Sciences, University of Stellenbosch, Stellenbosch 7600, South Africa

³Centre for Nutrition and Food Sciences, Queensland Alliance for Agriculture and Food Innovation, University of Queensland, Coopers Plains, QLD 4108, Australia

*Corresponding author. Email: Louwrens.hoffman@uq.edu.au (L. C. Hoffman)

Abstract: Western consumers are becoming more aware of the environmental impact of consuming meat, which has led to an increase in research into protein alternatives with specific interest in insects as a viable option. To determine whether insects could be used in an emulsified sausage application, 4 different treatments were investigated to obtain a Vienna-style sausage made from black soldier fly larvae (BSFL). Treatments consisted of a commercially formulated pork Vienna (control) and 3 BSFL treatments with varying concentrations of BSFL (28, 31, and 34%). Proximate composition, and texture profile analysis on d 1 and d 14 (refrigerated storage) were determined. The pork treatment was higher ($P < 0.001$) in protein and moisture than all of the BSFL treatments, however, there was no difference between treatments with regard to fat or ash content. Texture profile analysis indicated that the treatments had no effect on perceived hardness and cohesion on d 1. With regard to gumminess and springiness, the pork Vienna sausage had the highest ($P < 0.001$) gumminess values. After 14 d of refrigerated storage the pork treatment retained its hardness, however, there was a decrease ($P < 0.001$) in the hardness of all of the BSFL treatments. Cohesion values differed between treatments on d 14 ($P < 0.001$), but both the 34% and 31% treatments were comparable to the pork treatment. On d 1 there was a difference in springiness between the 34% treatment and 28% treatment ($P < 0.05$), and only the control and 28% treatment retained its springiness at d 14. This study establishes that although BSFL Vienna-style sausages differ from pork Vienna sausages in terms of nutritional composition and perceived texture, out of the 3 BSFL treatments, the 28% BSFL treatment was most comparable to the pork treatment in terms of protein content, ash content, perceived hardness, cohesion, and gumminess, thereby showing the potential of BSFL for partial use in emulsified meat products.

Keywords: black soldier fly larvae, emulsion sausage, entomophagy, insects as meat alternatives, meat alternative

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Introduction

With the world's population predicted to be 9.6 billion in 2050, there will be an increase in demand for food sources to feed everyone (Mitsuhashi, 2010; Premalatha et al., 2011). It has been suggested that the demand for future food requirements will largely be in favor of protein-based products, and meat production alone will need to increase to 200 million tons per yr by 2050 (Barnes, 2012). Western consumers

are becoming increasingly aware of the environmental impact of consuming meat and are beginning to seek alternatives to animal protein in efforts to shift to a more sustainable diet (Cole et al., 2018; Schösler et al., 2012; Springmann et al., 2018). All of these factors have led to an increasing interest in insects as a protein source, and according to independent market researchers, the global insect market is expected to be worth between (USD) \$722.9 million and \$1.2 billion by 2023 (Meticulous Research, 2018; Persistence

Market Research, 2018). While these values vary substantially, it has generated interest in the market and marks a potentially valuable industry on the rise, which warrants in-depth research into the products that will make this industry sustainable.

Consumer acceptance is one of the main barriers preventing the commercialization of insects in the Western culture (van Huis et al., 2013). In Western culture insects are not part of the cultural diet, resulting in a strong aversion toward incorporating insects into their diet (Hartmann et al., 2015; Tan et al., 2015). Schösler et al. (2012) reported that when Western consumers were required to describe their willingness to try mealworms and locusts in various dishes, they were willing to try insects in a dish, but were less likely to buy, consume, or prepare meals where the insects were easily visible as such. Other studies yielded similar results, and it has been established that products with visible insects are met with more disgust and apprehension by Western consumers than those where insects are processed and disguised in the product (Caparros Megido et al., 2016; Hartmann et al., 2015; Tan et al., 2015). The degree of processing also has an effect on consumer acceptance, and products where insects are completely disguised, such as insect flour, are more readily accepted than products where insects are still visible (Gmuer et al., 2016). It has therefore been suggested that to introduce insects as a commercial food source into Western culture, insects should be incorporated into familiar food products that are currently consumed (Hartmann et al., 2015; Schösler et al., 2012; Tan et al., 2015, 2016). Further investigations have also established that the majority of the consumers associate insects with meat products and meat dishes, as opposed to other savory or sweet options. Therefore, insects should be incorporated into a meat product to introduce insects into the Western food market (Caparros Megido et al., 2016; Schouteten et al., 2016).

Insects have been processed into burger patties at varied percentage inclusions (Caparros Megido et al., 2016; Schouteten et al., 2016), yet currently there is no research on the production of black soldier fly larvae into emulsified meat products. Kim et al. (2017) found that house cricket flour, when used at low inclusion levels, has potential to be used in emulsion products as a partial meat replacer. Vienna (hot dog) sausages are defined by their characteristic cylindrical shape with hemispherical ends and by the stable meat emulsion that is formed (Ranken, 2000). Emulsions are a two-phase colloidal system, consisting of 2 immiscible liquids, where 1 liquid is dispersed as small particles in another liquid of a different composition, usually

it comprises of an aqueous phase and a hydrocarbon phase (Allais, 2010). Vienna sausages are small caliber sausages made from meat and fat which is finely chopped into a homogenous batter and filled into a casing. Thereafter it is smoked and cooked (Allais, 2010; Ranken, 2000). Vienna sausages, like most cooked sausages, are fully cooked to an internal temperature of 70°C and then chilled rapidly to prevent microbial spoilage (Ranken, 2000). Typically, in an emulsified meat sausage sodium chloride is added to extract the salt soluble myofibrillar proteins, namely actin and myosin, which allows the proteins to surround the fat globules and bind water to form an emulsified batter. These salt soluble proteins in meat contribute to the characteristic structure found in emulsified sausages, which contributes to the texture and mouthfeel of the sausage (Allais, 2010). The concern when using black soldier fly larvae (BSFL), as with any alternative protein source in the Vienna sausage, is the potential negative effect it would have on texture due to the absence of meat proteins. Due to the fact that Western consumers would be more willing to consume insects as a meat alternative in a processed form, specifically as a meat alternative, the aim of this exploratory study was therefore to determine whether BSFL could have the potential to replace meat as a functional ingredient in an emulsion based product, namely a Vienna sausage. Furthermore, the intention of this study was to indicate whether these BSFL sausages would be comparable to a traditional pork Vienna sausage both in texture, structural integrity, and nutrition. Larvae of the black soldier fly were selected, as they are farmed on a mass scale in South Africa, they are soft bodied, and they do not have a hard exoskeleton, resulting in less of a mouthfeel in a product.

Materials and Methods

Experimental design

A randomized block design was used to compare the difference between a control Vienna sausage (pork standard) and BSFL Vienna sausages with three varying concentrations of BSFL (34% BSFL, 31% BSFL, and 28% BSFL). There were 4 different Vienna sausage treatments produced and 5 batches of each treatment.

Vienna sausage production

Ingredient preparation. All of the pork and pork fat was obtained from Winelands Pork (Cape Town, South

Africa), vacuum sealed, and frozen at -20°C until used. The pork consisted of defatted longissimus trimmings while the fat was made up of subcutaneous fat trimmed from pork carcasses; both lean meat and pork fat were derived from numerous pig carcasses. The BSFL was obtained from Agriprotein at 18 d old (Cape Town, South Africa) where they had been fed a commercial chicken layer mash, blanched for 3 min at 100°C , vacuum packed, and frozen at -20°C until needed. The salt packs, Vienna spice packs, soya concentrate, carrageenan, and collagen casings were obtained from Deli Spices (Cape Town, South Africa) and the lecithin was obtained from CJP Chemicals (Cape Town, South Africa).

Production. At the time of this investigation, there was no available information regarding BSFL as a meat alternative in an emulsified sausage, therefore pilot trials were conducted using a traditional Vienna sausage recipe used in South Africa (obtained from Deli Spices) as a basis to start from. The BSFL Vienna sausages did not retain shape after cooking, therefore additional ingredients were investigated in the pilot trial to assist in maintaining a structure that would allow it to be analyzed. The successful BSFL Vienna formulation developed in the pilot trials was used as a basis for the BSFL Vienna sausage treatments and contained carrageenan and lecithin which aided in stabilizing the Vienna structure. While lecithin has been found to destabilize meat emulsions, the pilot trials indicated that lecithin seemed to have a positive effect in stabilizing the BSFL emulsion, however further investigations would need to be conducted on its use in different processed products (Whiting, 1987).

Four different treatments were investigated to obtain a BSFL Vienna sausage that was comparable to that of a commercial pork Vienna sausage. Each batch consisted of 5 kg of ingredients, as this allowed for optimum functioning of the bowl chopper. The BSFL and soya concentration fluctuated inversely throughout the treatments. Lecithin and kappa carrageenan were added only to the BSFL sausages to aid in binding, emulsification, and water retention (determined in pilot trials). The formulations of each treatment are shown in Table 1. All 4 treatments were produced in the same manner, with the exception of the additional ingredients used in the production of the BSFL Vienna sausages, namely lecithin and kappa carrageenan. Prior to production, the pork, BSFL, and fat were thawed at 14°C for 6 h. A 6-blade bowl chopper (Mainca CM-21, Equipamientos Cárnicos, Barcelona, Spain) was used to process the sausages according to the flow diagram in Fig. 1 until a sticky, shiny batter was formed. The temperature was carefully controlled, and the various ingredients were added into the

Table 1. Formulation of treatments

Ingredient, %	Control	28% BSFL	31% BSFL	34% BSFL
Soya concentrate	3	9	6	3
Minced fat	19.56	19.56	19.56	19.56
Cold water	28.5	28.5	28.5	28.5
Potato starch	5	5	5	5
Spices	6.5	6.5	6.5	6.5
Salt pack	2.84	2.89	2.89	2.89
Pork	34.6	0	0	0
BSFL	0	28	31	34
Lecithin	0	0.5	0.5	0.5
Kappa carrageenan	0	0.06	0.06	0.06
Total	100	100	100	100

bowl chopper at specific temperatures (Fig. 1). The final temperature of the batter in the bowl chopper was below 14°C . A Talsa hydraulic sausage filler (DMD Foodtech, Epping, South Africa) was used to fill the sausage batter into the 25 mm collagen casings (Deli Spices), which were then twisted every 20 cm to produce individual sausages. To reduce potential variations in individual treatments, only 1 skilled person filled the casings with the sausage batter. The sausage treatments were labeled, weighed, and hung in a processing chamber (Reich Airmaster UKF 2000 BE, Urbach, Germany). The sausages were smoked and cooked (Table 2) to an internal temperature of 72°C (Ranken, 2000), measured using a thermocouple probe that was inserted into the center of a randomly selected sausage.

Proximate analysis

Two sausages from each treatment and batch were tested in duplicate for moisture content according to Official Method 934.01 (AOAC, 2002), ash content according to Official AOAC Method 942.05 (AOAC, 2002), crude fat using the 2:1 chloroform/methanol extraction method (Lee et al., 1996), and protein content according to the Official AOAC Method 992.15 (AOAC, 1992) using the defatted samples from the chloroform/methanol extraction. A sample of the blanched BSFL used in production were tested according to the above methodologies to give an indication of its nutritional profile, with the crude fiber determined using the Fibertec analyzer FIWE6 (Velp Scientifica; Trident Instrumentation, Cape Town, South Africa) according to Official AOAC Method 962.09 (AOAC, 2002).

Physical analysis

The samples were cooled by submerging in ice water for 10 min, removed and dried.

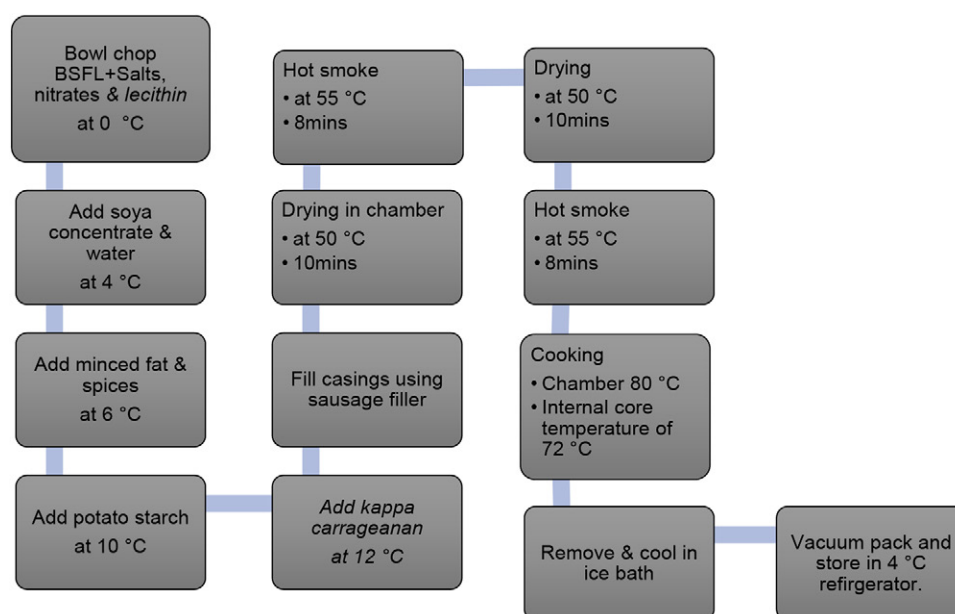


Figure 1. Flow diagram showing the sequence of steps in the production of the Vienna sausages, where the words in italics are the ingredients only used in the black soldier fly Vienna sausages and were not included in the pork sausages.

Texture profile analysis (TPA) was conducted on d 1 on the sausages after they were cooled in ice for 10 min, dried by gently dabbing with paper towel, vacuum sealed and analyzed at room temperature (20°C). The sausages were analyzed once again after 14 d of storage in a vacuum sealed bag at 4°C, using an Instron Universal Testing Machine (Instron 3345, Instron Corp., Norwood, MA) and Bluehill 2 software (Instron Corp.; Desmond and Troy, 2004; Schutte, 2008). A 30-mm diameter circular plate attached to a 5 kN load cell was used to perform a cyclic compression test with a cross head speed of 100 mm/min. Two sausages from each batch and treatment were selected, and 5 cores of 20-mm thick and 20-mm diameter were cut from each sausage. The cores were placed on the platform, directly underneath and parallel to the anvil. The cyclic compression test was conducted with a 40% compression to determine the hardness (N), gumminess (N/cm²), cohesion energy (ratio), and springiness (mm) of the sausages (Choe et al., 2013; Henning et al., 2016; Schutte, 2008; Yang et al., 2010). A 40% compression was performed to obtain descriptive results, while still maintaining the integrity of the Vienna structure.

Statistical analysis

Statistical analysis was conducted using Statistica version 12, Dell Inc. (2015; Dell, Inc., Santa Clara, CA). A complete randomized plot design with 4 treat-

ments and 5 batches of each treatment were tested using a mixed model repeated measures ANOVA.

Results and Discussion

Proximate analysis

Protein and moisture were significantly different between the treatments (Table 3), whereas there was only a slight difference between the ash and fat contents of the control sausage and the BSFL sausages. The control sausage had the highest moisture content ($P < 0.001$), and the 28% BSFL sausage had the lowest moisture content. There were no differences in moisture content between the 34 and 31% BSFL sausages. The low moisture content of the 28% BSFL Vienna was unexpected, as it contained the highest soya concentration, which is known for its high water binding capacity, therefore

Table 2. Smoking/cooking schedule for black soldier fly larvae Vienna sausages

Schedule	Chamber temperature, °C	Time, min
Drying	50	10
Hot smoke	55	8
Drying	50	10
Hot smoke	55	8
Cooking	80	19
End of schedule	80	55

Table 3. Means and standard deviations of proximate composition of the four Vienna treatments ('as is')

Item	Treatments				
	Control	34% BSFL	31% BSFL	28% BSFL	LSD
Moisture, %	58.2 ^a ± 1.99	55.0 ^b ± 1.18	55.3 ^b ± 1.04	51.6 ^c ± 0.91	$P < 0.001$
Protein, %	17.9 ^a ± 0.93	15.4 ^d ± 1.15	16.2 ^c ± 1.03	16.4 ^b ± 0.91	$P < 0.001$
Fat, %	7.7 ^c ± 2.15	9.5 ^a ± 1.66	8.8 ^{a,b} ± 0.88	8.4 ^b ± 1.13	$P = 0.001$
Ash, %	1.8 ^b ± 0.36	2.1 ^a ± 0.10	2.1 ^a ± 0.17	2.2 ^a ± 0.51	$P = 0.005$

^{a-d}Means within a row with different superscripts are significantly different ($P < 0.05$).

it was expected to retain more water during the cooking process (Joly and Anderstein, 2009). The moisture content of the 34% BSFL and 31% BSFL were similar ($P = 0.74$), but they differed from both the control and 28% BSFL ($P < 0.001$). The control had the highest protein content ($P < 0.001$), which was expected as BSFL typically has a lower protein content (11 g/100 g wet basis; Table 4) than pork (20 to 25 g/100 g wet basis; USDA, 2016). Within the BSFL treatments there was an increase ($P < 0.001$) in protein content as the BSFL concentration decreased. This is attributed to the increase in soya concentrate, which has a higher protein content (63 g/100 g) in its powder form, than the BSFL in its wet form. Although 28% BSFL had the highest protein content out of the black soldier fly treatments, it was still substantially less ($P < 0.001$) than the protein content of the control. Up to date there is no research on insects in sausage products, therefore there are no findings to compare to the results found in this study.

However, the total recoveries (fat + moisture + protein) are very low, at 78.6 to 85.6% (Table 3). The remainder (14.4 to 21.4%) could be accounted for by carbohydrate from part of the soya concentrate, starch, part of the spices, carrageenan, and presumably part of BSFL as well as from fiber. The BSFL had 4.5% fiber (Table 4). Also, the fat contents reported are low at 7.7 to 9.5% (Table 3) for a product that had 19.56% minced fat added (Table 1). This could either be due to the method of determination of lipid content (Lee et al., 1996) in that the volume of solvent was inadequate for removal of all the lipid and/or that the pork fat used had low levels of lipid. Although backfat used in this study was not tested for lipid content, it has been observed in a study comprising of 2107 pigs that South African pigs produced on average 75% lipids in their backfat (Hugo and Roodt, 2015). It is suggested that

future research also measure the carbohydrates, fiber, and actual lipid content of the fat used.

Physical analysis

Texture is primarily a sensory attribute determined best by the human senses, however, TPA using the compression test gives a good indication of the perceived texture of products. The texture profile of the sausages were defined by the following parameters: the maximum force (N), required to compress the sample (hardness), the cohesion energy was measured as a ratio of the total energy of the first compression, and the total energy of the second compression indicating the extent to which the samples deformed during the compression, gumminess indicated the force (N) that would be required to disintegrate the sample for swallowing (hardness × cohesiveness), and springiness measured the samples ability to recover to its original form after compression (Herrero et al., 2008; Mendoza et al., 2001). The texture of food is derived largely from the structure of the food and due to the fact that there was no meat in the BSFL, it was expected that there would be differences in the texture profile between the control and the BSFL treatments. The Vienna sausages were tested after 14 d of storage at 4°C refrigeration to give an indication of the shelf-life quality of the product in terms of perceived texture and hardness.

With regard to the hardness (max force) of the samples, there were no notable differences between the treatments with regard to perceived hardness on d 1 (Fig. 2). This result was unexpected, as the moisture content differed significantly between treatments (Table 3), and it was expected that the hardness would increase as the moisture content decreased (Li et al., 1998; Pereira et al., 2011). After 14 d of refrigerated storage the control retained its hardness; however, there was a significant decrease ($P < 0.001$) in the hardness of the BSFL treatments (Fig. 2). Within the BSFL treatments on d 14 there was a notable increase in hardness as the percentage of BSFL decreased (Fig. 2) which corresponds to the decrease in moisture content and increase in soya content (Table 3). It is known

Table 4. Means and standard deviations (measured in triplicate) of proximate composition of blanched black soldier fly larvae ('as is')

	Moisture	Crude protein	Crude fat	Crude fiber	Ash
<i>H. illucens</i>	75.8 ± 0.44	11.0 ± 0.42	5.5 ± 0.88	4.5 ± 0.66	0.7 ± 0.01

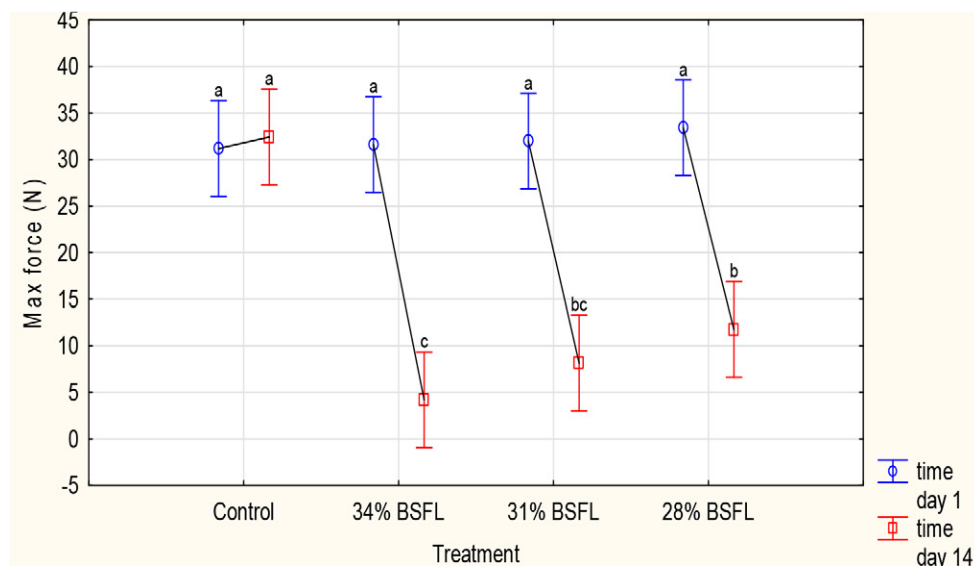


Figure 2. Hardness (mean max force values) of the four Vienna sausage treatments for d 1 and d 14, where the means with different superscripts are considered significantly different ($P < 0.05$).

that soya isolate typically increases the hardness of emulsified sausages, and could have contributed to the retention in hardness within the BSFL treatments on d 14 (Ensor et al., 1987; McCord et al., 1998; Youssef and Barbut, 2011). These results indicate that there will be a noticeable change in perceived hardness in the BSFL sausages for consumers after 14 d of storage; therefore they are not expected to have a good shelf-life in terms of quality. At this stage it is unknown as to why the hardness of the BSFL decreased over 14 d; further investigations are required to explain this phenomenon and its correlation with the other parameters (such as water binding capacity).

There were significant differences in gumminess values between the treatments ($P < 0.001$). Overall, the control had the highest ($P < 0.001$) gumminess value, which was expected, as soya (found in the BSFL treatments) has been found in previous studies to decrease the gumminess of meat products due to its dense structure (Savadkoohi et al., 2014; Ulu, 2004; Youssef and Barbut, 2011). There were no differences in gumminess between the BSFL treatments on both d 1 and d 14 (Fig. 3), indicating that the decrease in BSFL concentration did not affect the gumminess of the Vienna sausage.

On d 1, there were no differences in cohesion values between the treatments, however on d 14 there were differences ($P < 0.001$) between treatments (Fig. 4). On d 14, the 34% BSFL treatment had the lowest cohesion value and was the only treatment that differed ($P < 0.001$) from the control treatment. The 34% BSFL treatment was the only treatment that retained its cohesion

values, whereas the cohesion values of the other treatments all increased ($P < 0.001$) between d 1 and d 14.

On d 1, the BSFL treatments compared well to the control treatment, with little variation in the springiness between the treatments, except for the 38% BSFL (Fig. 4). The only difference between treatments on d 1 was between the 34% BSFL and 28% BSFL treatment ($P < 0.05$). The control retained its springiness at d 14, as did 28% BSFL, but the springiness of the 34% BSFL and 31% BSFL treatments decreased substantially ($P < 0.001$) over 14 d (Fig. 5). There was also a slight increasing trend within the BSFL treatments on d 14 where the springiness increased as the BSFL concentration decreased. This increase in springiness could be attributed to the increase in soya concentration and correlating decrease in moisture content, both of which have been found to increase springiness of sausages (Li et al., 1998). Overall, the BSFL treatments did not compare favorably to the control with regard to springiness on d 14 and the results from this study are consistent with previous findings that non-meat ingredients have been found to decrease the springiness of meat sausages (Li et al., 1998; Ulu, 2004; Youssef and Barbut, 2011). Overall, it is evident from the results that there are changes occurring in the BSFL Vienna sausages over time, causing significant changes in the textural properties. These changes could be as a result of a weak protein structure within the BSFL Vienna sausages or as a result of some unknown structural degradation, however the exact cause is unknown at this stage. Furthermore, it was

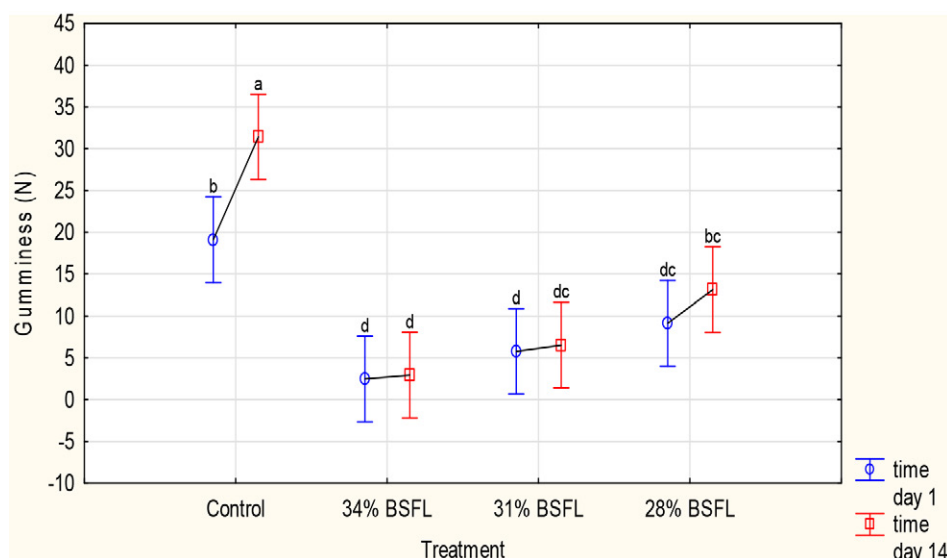


Figure 3. Mean gumminess values the four Vienna sausage treatments for d 1 and d 14, where the means with different superscripts are considered significantly different ($P < 0.05$).

noted that many of the BSFL sausages burst and fell on the floor while cooking in the chamber, so it was therefore not possible to calculate percentage mass loss during this process. It was observed that there was a slightly higher number of the 34% BSFL sausages that burst, however, it was inconsistent between batches and therefore no trend could be established. It is speculated that there was poor water binding in the BSFL sausages, allowing the free water to expand during heating, resulting in an increased vapor pressure

that would cause the casings to burst. These 2 factors will need to be investigated together with the use of different emulsifiers, and concentrations thereof prior to commercialization of BSFL Vienna sausages.

Conclusions

The focus of this study was to determine whether BSFL could successfully be processed into a Vienna-style sausage that was comparable to a traditional pork

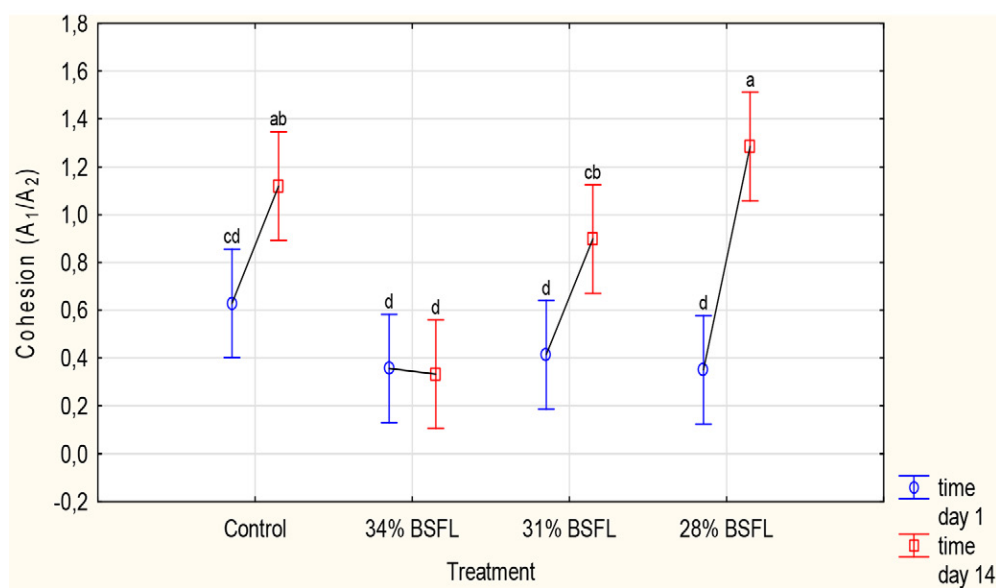


Figure 4. Mean cohesion values the four Vienna sausage treatments for d 1 and d 14, where the means with different superscripts are considered significantly different ($P < 0.05$).

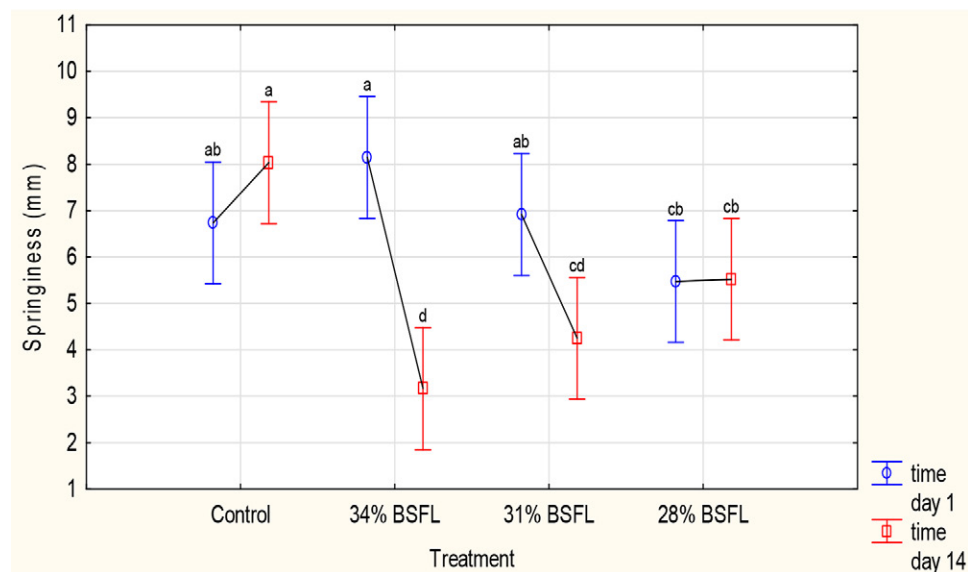


Figure 5. Mean springiness values the four Vienna sausage treatments for d 1 and d 14, where the means with different superscripts are considered significantly different ($P < 0.05$).

Vienna. Overall, it was established that the BSFL sausages do not behave like pork sausages, however of the 3 BSFL treatments, the 28% BSFL sausage compared best to the control in terms of protein content, ash content, perceived hardness, cohesion, and gumminess. The TPA indicated no notable differences between the treatments on d 1 with regard to perceived hardness and cohesion, however, on d 14 there was a drastic decrease in the hardness of the BSFL treatments and the springiness of 34% BSFL and 31% BSFL. Inversely, on d 14 there was an increase in cohesion in the control, 31% BSFL, and 28% BSFL Vienna sausages. These results indicate that there will be a noticeable change in perceived texture for consumers during 14 d of storage, and therefore BSFL Vienna sausages may not have a good shelf-life in terms of quality, indicating that the change during storage warrants further investigation. Of the treatments in this study, it would be recommended that the 28% BSFL formulation be used for further investigations such as sensory evaluation to validate the TPA results. The 28% BSFL formulation could also be used as a basis for further processing investigations to further improve texture and water binding capacity, which will improve the chances of acceptability by consumers. Furthermore, a more in-depth look into the structural degradation of the BSFL sausages over time, while looking at ingredients that could aid in maintaining structural integrity, would contribute to using BSFL as a meat alternative. As this was an exploratory study, a more in-depth investiga-

tion is recommended to address some pertinent follow up questions raised in this study.

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