QUALITY OF SEMI-DRY FERMENTED SAUSAGE CONTAINING SUGARY KEFIR GRAINS

C. H. Wu, P. M. Wang and K. W. Lin*

Department of Food and Nutrition, Providence University, Taichung, 43302 Taiwan

*Corresponding author email: kwlin@pu.edu.tw

Abstract – Semi-dry fermented sausages containing two levels of kefir grains (5%, 7.5%) and brown sugar (4%, 6%) were produced. Following fermentation and heating processes, semi-dry fermented sausages contained approximately 14-15% fat, and cooking yields for all treatments ranged from 82% to 85%. No differences in a_w among treatments were observed (P>0.05). WHC (water holding capacity) for all treatments was not different suggesting no additional benefit with the addition of kefir grains. KB4 (6% brown sugar/7.5% kefir grains) had significantly higher textural fracturability and chewiness than KB1 (4% brown sugar/6% kefir grains) and KB3 (6% brown sugar/5% kefir grains) (P<0.05). The pH values of cooked, fermented sausages ranged from 5.4 to 5.7 and thiobarbituric acid-reactive substances values did not exceed 1.0 after 12 weeks of refrigerated storage. Total plate counts (TPC) of all treatment were not higher than 10^4 cfu/g after 12 weeks of refrigerated storage. After 12 weeks of storage, volatile basic nitrogen values among treatments were in the range of 8.65-10.31 mg%. Results demonstrated high and acceptable quality of fermented sausages. Untrained affective evaluation showed that KB2 (4% brown sugar/7.5% kefir grains) treatment received 49% (total of 300 consumer ballots) of high score (11-15) ballots, followed by KB4 of 37.7%.

Key Words – fermented sausage, semi-dry, kefir grains, brown sugar, affective sensory evaluation.

I. INTRODUCTION

Kefir is a fermented beverage resulted from the fermentation process by natural lactic acid bacteria (LAB) and yeast [1,2], and milk is a commonly used substrate for milky kefir fermentation. Kefir grains are the colloidal substance produced by LAB. This water-soluble colloidal substance is termed as kefiran, which is a water-soluble polysaccharide consisting of dextran (α 1-6 linkage) [3,4]. Recently, many researchers have demonstrated possible antitumor, immunological and antioxidant capacity, and dermatological functions of kefir and kefir grains. Three lactic acid bacteria species (*Leuconostoc mesenteroides*, *Lactobacillus mali* and *Lactobacillus hordei*) were isolated from the kefir grains fermented with brown sugar [4]. Sugary kefir and kefir grains produced by utilizing brown sugar as substrate contain high LAB counts. Until today, no information concerning the utilization of sugary kefir grains in the production of fermented sausages was available. Thus, the aim of this research was to apply the sugary kefir grains with endogenous microbiota as a substitute for starter culture into an innovative and quality-accepted, semi-dry fermented sausage.

II. MATERIALS AND METHODS

Kefir grains were obtained from a farmer in Taichung City, Taiwan and cultivated in 8% brown sugar water (W/W) at a ratio of 1:20 (W/V) at room temperature for 24 hr. Kefir grains were rinsed with distilled water and cultivated again in 8% brown sugar solution for another 24 hr. Following complete rinsing with distilled water, kefir grains were homogenized for analyzing its composition and pH. Fresh kefir grains were used for sausage manufacture.

According to the GMP for fermented sausage products published by the American Meat Institute Foundation [5] and preliminary experiments, the time to decrease the pH of sausages containing 5% and 7.5% kefir grains (with 6% brown sugar added) to 5.3 or lower required 21 hr and 20 hr of fermentation at 30 ºC, respectively. However, addition of 10% kefir grains resulted in porous and crumbly texture. Therefore, treatments of different combinations (5% or 7.5% kefir grains and 4% or

6% brown sugar) were randomly manufactured. During fermentation, a 3M Petrifilm™ Staph Express Disk was utilized to monitor the possibility of existence of *Staphylococcus aureus*.

Lean ham tissue was trimmed of heavy connective tissue and external fat and then ground through a 9.5 mm plate (Manica PM-82A, Equipamientos Carnicos S.L., Barcelona, Spain). Pork belly as a fat source was ground through a 9.5 mm plate. Random samples of lean and fat sources were taken for percent fat analysis [6] for sausage formulation. Fat level of fermented sausage was estimated to be 15% for all treatments after fermentation and cooking.

Table 1 lists the formulations for semi-dry fermented sausage containing 5% and 7.5% sugary kefir grains. Fresh sugary kefir grains contain 87.6% water, and the amount of water added to each treatment was adjusted so that every treatment contained approximately equal level of water in the sausage.

Table 1 Formulations for semi-dry sausage contain sugary kefir grains

Ingredients	Treatment[a] (Total weight[c])			
	KB1	KB2	KB3	KB4
Pork Ham	63.8	71.5	63.8	71.5
Pork belly	36.2	28.5	36.2	28.5
Sucrose	2	2	2	2
Brown sugar	4	4	6	6
Kefir grains	5	7.5	5	7.5
Water[b]	10	10	10	10
Salt	1.6	1.6	1.6	1.6
STPP	0.2	0.2	0.2	0.2
$NaNO_2$	0.015	0.015	0.015	0.015
Na-erythorbate	0.05	0.05	0.05	0.05
Garlic powder	0.06	0.06	0.06	0.06
Nutmeg	0.06	0.06	0.06	0.06
Chili powder	0.4	0.4	0.4	0.4
Allspice	0.03	0.03	0.03	0.03

[a] KB1= 4% brown sugar/5% kefir grains; KB2= 4% brown sugar/7.5% kefir grains; KB3= 6% brown sugar/5% kefir grains; KB4= 6% brown sugar/7.5% kefir grains.

[b] Water from kefir grains was deducted from the actual amount of water added to each treatment so that all treatments had equal total amount of water.

[c] Non-meat ingredients were added on raw meat weight basis.

Meat sources and non-meat ingredients were mixed, and kefir grains were added and evenly blended prior to stuffing into fibrous casing (VISKASE EP Shirred, 1 1/2, U.S.A.). Sausages were clipped every 15 cm weighing approximately 600 g. Sausages with 5% and 7.5% kefir grains were fermented in a smokehouse (Model ASR1297 EL/WA, MAURER AG Co., Ltd, Kindlebildstr, Reichenau, Germany) at 30 ℃ for 21 hr and 20 hr, respectively. Following fermentation, sausages were smoked and cooked to internal temperature of 72 ℃. After refrigerated at 2 ℃ for 48 hr, sausage casings were removed and vacuum packaged (Multivac C350, Wolfertschwenden, Germany) in heat-shrinkable Cryovac barrier bags (CN 530, 205×270 mm). Each bag held one sausage. Sausages were stored at 2 ℃ and packages of sausages were randomly selected at 0, 4, 8, and 12 weeks for various analyses. Analyses included proximate composition, pH, water-holding capacity (expressed by water retention index, WRI), CIE color, water activity, lipid oxidation, volatile basic nitrogen, total plate counts, and consumer evaluation. Due to the unfamiliarity of consumer in Taiwan with the unique quality of fermented sausage, an affective consumer evaluation was conducted to the students on campus at Providence University. Students regardless of gender were asked to mark on a 15-cm unmarked rule identified with 0 and 15 at both ends and gave an overall comment on the evaluation sheet. The number 0 and 15 refer to dislike extremely and like extremely, respectively. One hundred valid evaluation sheets were collected for each replication and a total of 300 sheets for all replicated treatments were obtained.

The entire procedure was repeated twice at different time periods resulting in a total of three replicates. Data collected from all experiments, except for consumer tests, were statistically analyzed as a completely randomized design of SPSS [7] and analysis of variance using GLM (general linear model). Mean comparisons for treatment effects at fixed storage time or storage effects for individual treatment were performed using the Duncan's Multiple Range Test method with significant levels determined at $P< 0.05$.

III. RESULTS AND DISCUSSION

Following cooking, all treatments contained similar fat content in the range of 14-15% ($P>0.05$). No differences were noted for cooking yield and water holding capacity (WHC) among treatments ($P>0.05$).

Table 2. Proximate composition, cooking yield and water activity[c] of semi-dry sausage containing sugary kefir grains

	Treatment[a]			
	KB1	KB2	KB3	KB4
Raw				
Moisture (%)	63.17[a]	63.21[a]	61.00[a]	62.74[a]
Fat (%)	14.21[ab]	13.04[b]	16.21[a]	13.52[b]
Protein (%)	16.17[ab]	17.07[a]	15.52[b]	16.29[ab]
Ash (%)	2.21[a]	2.21[a]	2.07[a]	2.06[a]
CHO (%)[b]	4.24	4.47	5.20	5.39
WRI	1.14[a]	1.14[a]	1.14[a]	1.12[a]
Cooked				
Moisture (%)	58.03[bc]	59.61[a]	57.12[c]	58.84[ab]
Fat (%)	13.91[a]	14.36[a]	15.08[a]	14.30[a]
Protein (%)	19.41[a]	18.72[a]	18.23[a]	18.99[a]
Ash (%)	1.85[b]	2.26[a]	2.04[ab]	2.25[a]
CHO (%)[b]	6.80	5.05	7.53	5.62
WRI	1.60[a]	1.61[a]	1.62[a]	1.63[a]
Yield (%)	83.18[a]	84.74[a]	81.97[a]	84.41[a]
a_w	0.946[b]	0.951[a]	0.943[b]	0.952[a]

[a] KB1= 4% brown sugar/5% kefir grains; KB2= 4% brown sugar/7.5% kefir grains; KB3= 6% brown sugar/5% kefir grains; KB4= 6% brown sugar/7.5% kefir grains.
[b] CHO (%)= 100- Moisture (%) - Fat (%) – Protein (%) – Ash (%).
[c] Means within the same row bearing unlike lower-case letters (a-c) are significantly different (P<0.05).

Treatment KB4, containing 6% brown sugar/7.5% kefir grains, was the highest in chewiness value (Table 3), followed by KB2 (4% brown sugar/7.5% kefir grains) (P<0.05). Both treatments contained higher levels of sugary kefir grains. KB4 and KB3 (6% brown sugar/5% kefir grains) were found to have higher shear force values than other treatments.

Table 3. Textural profile analysis (TPA) and shear force value of semi-dry sausages containing kefir grains

	Treatment[a]			
Parameter[b]	KB1	KB2	KB3	KB4
Fracturability (N)	223.51[a]	276.46[b]	230.92[c]	302.88[a]
Springiness	89.89[a]	89.25[a]	91.91[a]	90.00[a]
Cohesiveness	0.41[b]	0.47[a]	0.41[b]	0.47[a]
Chewiness (N)	82.80[c]	112.74[b]	90.95[c]	135.29[a]
Shear force (N)	71.08[b]	72.69[b]	79.66[a]	84.78[a]

[a] KB1= 4% brown sugar/5% kefir grains; KB2= 4% brown sugar/7.5% kefir grains; KB3= 6% brown sugar/5% kefir grains; KB4= 6% brown sugar/7.5% kefir grains.
[b] Means within the same row bearing unlike lower-case letters (a-c) are significantly different (P < 0.05).

Treatment KB1 (4% brown sugar/5% kefir grains) was shown to have slightly higher pH values (Table 4) after fermentation, cooking and cooling processes than KB4. During storage, pH values of all treatments fluctuated slightly. After 12 weeks of refrigerated storage, KB2 was the lowest (P<0.05) in pH among treatments.

Table 4. Changes in pH value of semi-dry sausage containing sugary kefir grains after cooking and during refrigerated storage at 2 ºC

	Treatment[a]			
	KB1	KB2	KB3	KB4
After Cooking				
0 hr	5.10[a]	5.06[b]	5.07[ab]	5.07[ab]
24 hr	5.74[a]	5.66[ab]	5.67[ab]	5.62[b]
Storage				
0 wk	5.70a	5.46[c]	5.69[a]	5.59[b]
4 wk	5.53[ab]	5.48[b]	5.53[ab]	5.60[a]
8 wk	5.64[a]	5.43[b]	5.64[a]	5.56[a]
12 wk	5.56[a]	5.42[b]	5.57[a]	5.55[a]

[a] KB1= 4% brown sugar/5% kefir grains; KB2= 4% brown sugar/7.5% kefir grains; KB3= 6% brown sugar/5% kefir grains; KB4= 6% brown sugar/7.5% kefir grains.
[b] Means within the same row bearing unlike lower-case letters (a-c) are significantly different (P < 0.05).

All treatments showed low volatile basic nitrogen (VBN) values during 12 weeks of storage (Table 5). In addition, total plate counts (TPC) of all treatments did not exceed 10^4 cfu/g (data not shown). Moreover, TBARS (thiobarbituric acid-reactive substances) values (data not shown) for all treatments were lower than 1 (mg malonaldehyde/kg meat). Results suggested good microbial stability and sensorial quality of fermented sausages with the inclusion of sugary kefir grains.

Table 5. Changes in volatile basic nitrogen (VBN)[b] value of semi-dry sausage containing sugary kefir grains during refrigerated storage at 2 ºC

Treatment[a]	Storage time (Week)			
	0	4	8	12
KB1	6.83[b]	6.93[b]	7.80[b]	8.78[b]
KB2	8.63[a]	8.81[a]	10.20[a]	10.31[a]
KB3	6.42[b]	6.52[b]	7.81[b]	8.65[b]
KB4	8.21[a]	8.21[a]	10.21[a]	10.07[a]

[a] KB1= 4% brown sugar/5% kefir grains; KB2= 4% brown sugar/7.5% kefir grains; KB3= 6% brown sugar/5% kefir grains; KB4= 6% brown sugar/7.5% kefir grains.
[b] Means within the same column bearing unlike lower-case letters (a-b) are significantly different (P < 0.05).

Scores from consumer evaluation were tabulated and organized in three categories in descending

order of 11-15, 6-10, and 1-5 (Figure 1). Untrained affective evaluation showed that KB2 treatment received 49% (total of 300 consumer ballots) of high score (11-15) ballots, followed by KB4 of 38%. Both KB2 and KB4 contained higher amount of sugary kefir grains (7.5%). Furthermore, "good to eat" was the most frequent comment consumers gave on both treatments, while "sourness" was given to KB1, KB3, and KB4.

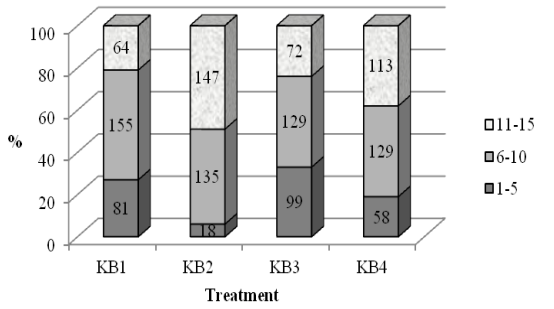

Figure 1. Consumer preference evaluation of semi-dry sausage containing sugary kefir grains. KB1= 4% brown sugar/5% kefir grains; KB2= 4% brown sugar/7.5% kefir grains; KB3= 6% brown sugar/5% kefir grains; KB4= 6% brown sugar/7.5% kefir grains.

IV. CONCLUSION

Fermented sausages have been produced for many years attributed to its unique aroma and texture; however, higher fat content in the finished fermented sausages could also have an adverse effect on consumer acceptance. Consumers in Taiwan, on the other hand, have an opportunity to try out an innovative fermented sausage which has slightly acidic taste, unique flavor, and acceptable texture and quality, but is lower in fat as compared to typical fermented sausages.

ACKNOWLEDGEMENTS

We wish to express our appreciation for funding of this study by the National Science Council (project number NSC 101-2313-B-126-005), Executive Yuan, Taiwan.

REFERENCES

1. Liu, J. R. & Lin, C. W. (2000). Production of kefir from soymilk with or without added glucose, lactose, or sucrose. Journal of Food Science 65(4): 716-719.
2. Guzel-Seydim, Z. B., Kok-Tas, T., Greene, A. K. & Seydim, A. C. (2011). Review: functional properties of kefir. Critical Reviews in Food Science and Nutrition 51(3): 261–268.
3. Waldherr, F. W., Doll, V. M., Meissner, D. & Vogel, R. F. (2010). Identification and characterization of a glucan-producing enzyme from *Lactobacillus hilgardii* TMW 1.828 involved in granule formation of water kefir. Food Microbiology 27(5): 672–678.
4. Hsieh, H. H., Wang, S. Y., Chen, T. L., Huang, Y. L. & Chen, M. J. (2012). Effects of cow's and goat's milk as fermentation media on the microbial ecology of sugary kefir grains. International Journal of Food Microbiology 157(1): 73-81.
5. American Meat Institute Foundation (1997). Good manufacturing practices for fermented dry & semi-dry sausage products. The American Meat Institute Foundation, Washington, DC.
6. AOAC. (2005). Meat and meat products. In W. Horwitz, & G. W. Latimer, Official methods of analysis of AOAC international. 18th ed. Washington, D.C.: AOAC International.
7. SPSS (2011). IBM SPSS Statistics for Windows, Version 20.0. Armonk, New York: IBM Corp.