Original paper

Quality of Semi-dry Fermented Sausage Containing Sugary Kefir Grains

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Semi-dry fermented sausages containing two levels of sugary kefir grains (5 %, 7.5 %) and brown sugar (4 %, 6 %) were produced and the quality attributes were evaluated. Following fermentation and heating, sausages contained 14–15 % fat. No differences ($P > 0.05$) were observed in CIE a^* for all treatments, but significantly higher CIE L^* for KB1 (4 % brown sugar/5 % sugary kefir grains) than other treatments was found. KB4 (6 % brown sugar/7.5 % kefir grains) had significantly higher ($P < 0.05$) textural fracturability and chewiness than KB1 and KB3 (6 % brown sugar/5 % kefir grains), and KB4 and KB3 were higher in shear force value than others. Total plate counts (TPC) of all treatment were not higher than 10^5 cfu/g after 12 weeks. Untrained affective evaluation showed that KB2 treatment received 49 % (total of 300 consumer ballots) of high score (11–15) ballots, followed by KB4 of 37.7 %. Results demonstrated good microbial stability and acceptable quality of semi-dry fermented sausages containing sugary kefir grains.

Keywords: semi-dry fermented sausage, sugary kefir grains, brown sugar, affective sensory evaluation, textural profile

Introduction

Kefir is a fermented beverage resulted from the fermentation process by natural lactic acid bacteria (LAB), yeasts or possibly acetic acid bacteria (Liu and Lin, 2000; Guzel-Seydim *et al.*, 2011). It is originated from the Caucasus regions (Liu and Lin, 2000; Harta *et al.*, 2004) and milk is a commonly used substrate for milky kefir fermentation. Kefir grain is the colloidal substance produced by LAB and this water-soluble polysaccharide is regarded as kefirin. Sugary kefir grains, on the other hand, utilize brown sugar as substrate and produce yellowish, colloidal polysaccharide which consist of dextran (α 1-6 linkage) (Waldherr *et al.*, 2010; Hsieh *et al.*, 2012). In contrast, milky kefir grains were composed of water-soluble heteropolysaccharide with an equal amount of glucose and galactose residue (Marshall and Cole, 1985; Micheli *et al.*, 1999).

Milky kefir grains are utilized as starter cultures to produce a varieties of food and beverages. Magalhães *et al.* (2011) indicated potential utilization of kefir grains for developing

cheese whey-based beverages. Gómez *et al.* (2014) applied kefir grains-fermented, acidified milk as an ingredient to breadmaking and found the best texture qualities comparing with the control. Harta *et al.* (2004) utilized a mixture of glucose and sucrose for effective production of kefir biomass and suggested potential application of kefir grains as a baking starter culture. Good quality of bread, such as better flavor and firmer texture could be manufactured by using isolated kefir grains from kefir drink (Plessas *et al.*, 2005). Filipčev *et al.* (2007) added native or lyophilized kefir grains to bread dough and found lower bread volume, longer shelf life and milder taste of bread dough. Mantzourani *et al.* (2014) found increased antimicrobial activity and sensorial traits of sourdoughs with the addition of kefir grains as starter culture that were primarily resulted from higher contents of lactic acid and acetic acid.

Lin *et al.* (1999) reported predominant microbes in kefir grains in Taiwan were lactic acid-producing *Lactobacillus helveticus* and *Leuconostoc mesenteroides* as well as the yeast genus *Kluyveromyces marxianus* and *Pichia fermentans*. Hsieh

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et al. (2012) reported that three lactic acid bacteria species (*Leuconostoc mesenteroides*, *Lactobacillus mali* and *Lactobacillus hordei*) were isolated from the kefir grains fermented with brown sugar. Addition of LAB starter cultures or “probiotic” cultures can also have beneficial effects (Incze, 1998; Lücke, 2000; Zhang *et al.*, 2010). Regardless of bacterial species and substrates, researches have demonstrated possible antitumor activity, antioxidant capacity, antimutagenic, immunological, antiallergic and dermatological functions (Shiomi *et al.*, 1982; Liu *et al.*, 2005; Guzel-Seydim *et al.*, 2011; Medrano *et al.*, 2011; Ahmed *et al.* 2013). The heat-inactivated *Lactobacillus kefirianofaciens* M1 isolated from milky kefir grains was shown to display antiallergic activity (Hong *et al.*, 2010).

Unique flavor, aroma and texture of fermented meat products were attributed to the biochemical and physical reactions occurred during fermentation process (Zhang *et al.*, 2010). Varying metabolites including lactic acid, acetic acid, diacetyl, free fatty acids, amines, and aldehydes resulted from different starter cultures contributed to the texture and flavor of fermented meat products (Paramithiotis *et al.*, 2010). Lactic and acetic acid and subsequent pH decline in addition to bacteriocins metabolites are responsible for the preservation effect of fermented meat products (Lücke, 2000).

Farmers in Taichung city, Taiwan utilize brown sugar instead of milk or soymilk as a substrate for producing sugary kefir grains and sugary kefir. The sugary kefir grains contained high levels of LAB (Wu, 2014) and could be applied as starter culture. 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 various levels of sugary kefir grains and brown sugar with endogenous microbiota as a substitute for traditional starter culture into an innovative and quality-accepted, semi-dry fermented sausage.

Materials and Methods

Sugary kefir grains cultivation Sugary kefir grains were cultivated in 8 % (W/W) brown sugar water at a ratio of 1:20 (W/V) at room temperature (25–27 °C) for 24 hr. Sugary kefir grains were cultured in a screw-capped glass container and the cap was occasionally loosened to release gas. After decanting sugar solution, sugary kefir grains were rinsed with distilled water and cultivated again in 8 % brown sugar solution for another 24 hr, while the number of lactic acid bacteria reached approximately 10^7 cfu/g at this point (Wu, 2014). Following complete rinsing with distilled water, sugary kefir grains were homogenized (Blixer RS1 BX3, Robot Coupe Inc., Ridgeland, Miss, USA) for analyzing its composition and pH. Fresh sugary kefir grains were used for fermenting sausage.

Semi-dry sausage manufacture According to the GMP for fermented sausage products published by the American Meat Institute Foundation (1997) 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 fermentation time at 30 °C, respectively. However, addition of 10 % kefir grains resulted in porous and crumbly texture that was deemed unacceptable. Therefore, treatments of different combinations (5 % or 7.5 % sugary kefir grains and 4 % or 6 % brown sugar) were randomly manufactured. During fermentation, a 3 M Petrifilm™ Staph Express Disk was utilized to monitor possible existence of *Staphylococcus aureus*. Preliminary results indicated no existence of pathogenic *Staphylococcus aureus* (Wu, 2014) and the safety of processing procedure was assured.

Fresh, chilled (~ 1 °C), vacuum-packaged boneless ham and pork belly were purchased from a local meat purveyor. Lean 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. Samples of lean and fat sources were taken for crude fat analysis (AOAC, 2005) for sausage formulation. Fat level of fermented sausage was formulated to be 14–15 % for all treatments after fermentation and cooking processes.

Table 1 lists the formulations for semi-dry fermented sausage containing 5 % and 7.5 % sugary kefir grains. Fresh sugary kefir grains contain approximately 87.6 % water, and the amount of water added to each treatment was adjusted so that every treatment contained equal level of added water in the sausage. Meat sources, non-meat ingredients and kefir grains were evenly blended prior to stuffing into fibrous casing (VISKASE EP Shirred, 1 1/2, VISKASE Companies, Inc. Lombard, ILL. U.S.A.). Sausages were clipped every 15 cm weighing approximately 600 g. Sausages with 5 % and 7.5 % sugary kefir grains were fermented in a smokehouse (Model ASR1297 EL/WA, MAURER AG Co., Ltd, Kindlebildstr, Reichenau, Germany) at 30 °C for 21 hr and 20 hr, respectively. Following fermentation, sausages were smoked and cooked to an internal temperature of 72 °C with a build-in thermocouple inserted in the geometrical center of one sausage. After refrigeration at 2 °C for 48 hr, sausage casings were removed and then 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 °C and packages of sausages were randomly selected at 0, 4, 8, and 12 weeks for various analyses. The entire procedure was repeated three times at different time periods resulting in a total of three replications.

Physicochemical analyses and water activity Homogenized raw or cooked sausages at each sampling period were taken in triplicate for determination of proximate compositions using the AOAC (2005) methods. Water activity of cooked samples was measured (AquaLab CX-2, Decagon Devices, Inc. Pullman, WA, U.S.A.) after equilibrium at 25 °C. The pH of sausage was

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

Ingredients	Treatment ^a (%Total meat 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

^aKB1= 4 % brown sugar/5 % sugary kefir grains; KB2= 4 % brown sugar/7.5 % sugary kefir grains; KB3= 6 % brown sugar/5 % sugary kefir grains; KB4= 6 % brown sugar/7.5 % sugary kefir grains.

^bWater from kefir grains was deducted from the actual amount of water added to each treatment so that all treatments had equal amount of total water.

^cNon-meat ingredients were added on raw meat weight basis.

measured using the modified procedure of Koniecko (1985).

Water-holding capacity determination The amounts of expressed or “free” water were determined in triplicate by the press method (Wierbicki and Deatherage, 1958; Tsai and Ockerman, 1981). The area was determined with a planimeter (Placom KP-90N, Sokkia CO., Ltd., Kanagawa, Japan). The water-retaining index (WRI) was designated as the ratio between the total wetted area and the meat film area (Wisner-Pedersen, 1987; Lin and Chao, 2001). Lower WRI means less free water released and thus higher water-holding capacity of the sample.

CIE L*a*b* Color Refrigerated sausages were tempered at room temperature (25-27 °C) for 30-40 min, and two packages of sausages were randomly chosen. After removing both ends, 4 samples (1.5 cm thickness) were cross-sectioned from each sausage with a scalpel and the CIE L*, a*, b* color values were measured using a Spectro colorimeter (CM-5, Konica Minolta Co., Ltd., Osaka, Japan) at following settings: illuminant D65, 10 degree observer, and 30 mm aperture. Three different readings from various regions of the same surface were recorded from each slice. Hue angle was calculated from $\arctan(b^*/a^*)$ with lower values indicating more red color.

Lipid oxidation Thiobarbituric acid (TBA) test was used to determine the degree of lipid oxidation of fermented sausage during refrigerated storage followed by the procedures of Ockerman (1984) and Lin and Chao (2001). TBARS (thiobarbituric acid-reactive substances) value was reported as

mg malonaldehyde/kg meat. TBARS value (mg malonaldehyde/kg) = Absorbance $\times$ 7.8

Volatile basic nitrogen The volatile basic nitrogen (VBN) determination was performed according to the procedures of Cobb *et al.* (1973) and Lin and Lin (2002). The VBN value was expressed as mg/100 g following calculation below.

$$\text{VBN (mg/100 g)} = 0.01 \times 14 \times (A-B) \times F \times (100/S) \times 100$$

Where A= sample titrated volume (mL); B= blank titrated volume (mL); F= 0.01 N HCl titer; S= sample weight (g)

Texture profile analysis and shear value Two links of fermented sausages from each treatment were used for the compression method according to the procedures of Bourne (1982) and SMS (1993). Five 1.5-cm thickness sections were dissected with a scalpel from each sausage and compressed twice using Texture Analyser (Model TA-XT Plus, Stable Micro Systems, Surrey, England). A 25 kg load cell and P/45 adaptor with 1 mm/s test speed and 60 % compression strain were used to determine parameters such as fracturability, springiness, cohesiveness, and chewiness. For shear force determination, a Texture Analyser with HDP/WBS adaptor was used. Three sausages from each treatment were randomly taken and three consecutive slices (3 cm thickness) were dissected perpendicularly to the cross section

Total plate count Total plate count (TPC) of fermented sausages during refrigerated storage was determined as described by Lin and Huang (2003) with slight modification. Ten grams of homogenized sample were mixed with 90 mL

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 ±1.78	63.21 ^A ±2.11	61.00 ^A ±2.11	62.74 ^A ±1.26
Fat (%)	14.21 ^{AB} ±1.87	13.04 ^B ±1.77	16.21 ^A ±2.15	13.52 ^B ±1.30
Protein (%)	16.17 ^{AB} ±1.14	17.07 ^A ±1.47	15.52 ^B ±0.92	16.29 ^{AB} ±1.51
Ash (%)	2.21 ^A ±0.14	2.21 ^A ±0.14	2.07 ^A ±0.15	2.06 ^A ±0.26
CHO (%) ^b	4.24	4.47	5.20	5.39
WRI	1.14 ^A ±0.05	1.14 ^A ±0.03	1.14 ^A ±0.02	1.12 ^A ±0.02
Cooked				
Moisture (%)	58.03 ^{BC} ±1.77	59.61 ^A ±0.97	57.12 ^C ±1.80	58.84 ^{AB} ±0.70
Fat (%)	13.91 ^A ±1.28	14.36 ^A ±0.60	15.08 ^A ±1.38	14.30 ^A ±1.21
Protein (%)	19.41 ^A ±1.31	18.72 ^A ±0.86	18.23 ^A ±1.47	18.99 ^A ±1.24
Ash (%)	1.85 ^B ±0.32	2.26 ^A ±0.22	2.04 ^{AB} ±0.17	2.25 ^A ±0.23
CHO (%) ^b	6.80	5.05	7.53	5.62
WRI	1.60 ^A ±0.03	1.61 ^A ±0.03	1.62 ^A ±0.02	1.63 ^A ±0.15
Yield (%)	83.18 ^A ±3.58	84.74 ^A ±0.57	81.97 ^A ±2.99	84.41 ^A ±2.69
a _w	0.946 ^B ±0.006	0.951 ^A ±0.004	0.943 ^B ±0.003	0.952 ^A ±0.004

^aKB1= 4 % brown sugar/5 % sugary kefir grains; KB2= 4 % brown sugar/7.5 % sugary kefir grains; KB3= 6 % brown sugar/5 % sugary kefir grains; KB4= 6 % brown sugar/7.5 % sugary kefir grains.

^bCHO (%)= 100- Moisture (%) - Fat (%) - Protein (%) - Ash (%)

^cMeans (+SD) within the same row bearing unlike upper-case letters (A-C) are significantly different ($P < 0.05$).

sterilized 0.1 % peptone solution in a stomacher bag and blended for 30 s. A one milliliter aliquot of sample homogenate at a selected dilution was pipetted to a 3 M Petrifilm™ (Aerobic Count Plate) and incubated at 37 °C for 48 hr. TPC was reported as a logarithmic value of colony forming units (cfu)/g meat sample.

Affective sensory evaluation Due to the unfamiliarity of consumer in Taiwan with fermented sausage, an affective sensory evaluation was conducted to the students at Providence University. Samples from all treatments were randomly selected and served on campus at three different time periods. Refrigerated sausages were tempered at room temperature (25–27 °C) for 30 min and then unpacked, sliced, cut in half, and served without re-heating to students. Students were asked to mark on a 15-cm non-ticked rule identified with 0 and 15 at both ends and gave an overall comment on the evaluation sheet as well. Each treatment was given a 3-digit random code. The number 0 and 15 refer to dislike extremely and like extremely, respectively. The marked length was then transferred to actual score and tabulated. One hundred valid evaluation sheets were collected for each replication and a total of 300 score sheets for all treatments were obtained.

Statistical analysis Data collected from all experiments, except for consumer tests, were statistically analyzed as a completely randomized design of SPSS (SPSS, 2011) 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 $P < 0.05$.

Results and Discussion

Sugary kefir grains consist of 87.6 % moisture, 0.98 % lipid, 1.0 % protein, 0.7 % crude fiber, 0.1 % ash, and approximately 9.63 % carbohydrates. From Wu (2014), sugary kefir grains contain approximately 10⁷ CFU/g lactic acid bacteria. Following cooking, refrigeration and prior to entering storage period (0 week), all treatments contained similar fat content ($P > 0.05$) in the range of 14–15 % (Table 2). No differences ($P > 0.05$) were noted for cooking yield and water-holding capacity (WRI) among treatments.

Treatment KB4 containing 6 % brown sugar/7.5 % kefir grains was the highest ($P < 0.05$) in chewiness and fracturability values (Table 3), followed by KB2 (4 % brown sugar/7.5 % kefir grains). Both treatments contained higher levels of sugary kefir grains. No significant differences ($P > 0.05$) were noted for springiness among treatments. KB4 and KB3 (6 % brown sugar/5 % kefir grains) were found to have significantly ($P < 0.05$) higher shear force values than other treatments. Higher level (7.5 %) of sugary kefir grain

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

Parameter ^b	Treatment ^a			
	KB1	KB2	KB3	KB4
Fracturability (N)	223.51 ^C ±21.86	276.46 ^B ±26.94	230.92 ^C ±27.70	302.88 ^A ±11.58
Springiness	89.89 ^A ±3.24	89.25 ^A ±3.02	91.91 ^A ±3.11	90.00 ^A ±3.37
Cohesiveness	0.41 ^B ±0.03	0.47 ^A ±0.03	0.41 ^B ±0.03	0.47 ^A ±0.04
Chewiness (N)	82.80 ^C ±15.60	112.74 ^B ±17.09	90.95 ^C ±15.30	135.29 ^A ±7.11
Shear force (N)	71.08 ^B ±7.25	72.69 ^B ±7.92	79.66 ^A ±4.87	84.78 ^A ±7.43

^aKB1= 4 % brown sugar/5 % sugary kefir grains; KB2= 4 % brown sugar/7.5 % sugary kefir grains; KB3= 6 % brown sugar/5 % sugary kefir grains; KB4= 6 % brown sugar/7.5 % sugary kefir grains.

^bMeans (±SD) within the same row bearing unlike upper-case letters (A-C) are significantly different ($P < 0.05$).

Table 4. CIE color values^b of semi-dry sausage containing sugary kefir grains

	Treatment ^a			
	KB1	KB2	KB3	KB4
L^*	48.86 ^A ±1.84	47.14 ^B ±1.30	46.86 ^B ±2.47	45.10 ^C ±1.13
a^*	10.63 ^A ±0.90	10.74 ^A ±0.46	10.53 ^A ±0.90	10.59 ^A ±0.84
b^*	13.42 ^{AB} ±0.84	12.94 ^B ±0.33	13.70 ^A ±0.63	13.25 ^{AB} ±0.71
Hue Angle ^c	51.62	50.31	52.45	51.37

^aKB1= 4 % brown sugar/5 % sugary kefir grains; KB2= 4 % brown sugar/7.5 % sugary kefir grains; KB3= 6 % brown sugar/5 % sugary kefir grains; KB4= 6 % brown sugar/7.5 % sugary kefir grains.

^bMeans (±SD) within the same row bearing unlike upper-case letters (A-C) are significantly different ($P < 0.05$).

^cCalculated hue angle (θ)= $\tan^{-1}(b^*/a^*)$

resulted in semi-dry fermented sausage harder and chewy.

The CIE color values of semi-dry sausages with the addition of sugary kefir grains was shown in Table 4. KB4, being higher in sugary kefir grains, had the lowest ($P < 0.05$) CIE L^* value among treatments; in contrast, KB1 containing the least amounts of kefir grains and sugar was the highest in CIE L^* . KB3, on the other hand, was lower in CIE L^* than KB1 partly resulted from sugar browning during cooking. No differences were found in CIE a^* among treatments. The instrumental differences in L^* a^* b^* were too marginal, although statistical differences existed, to be detected by human eyes.

The pH values ascended from 5.07-5.10 for all treatments at the end of cooking cycle to 5.62-5.74 after 24 hr of cooling in a cooler (Table 5). Hughes *et al.* (2002) reported a similar pattern that the pH values of control and *Staphylococcus carnosus*-treated semi-dry fermented sausages gradually increased after three days of ripening to the end of 35 days ripening period. Authors concluded that enzymatic activity resulting in the production of ammonia and biogenic amines was responsible. At the beginning of cold storage (0 week), KB2 has the lowest ($P < 0.05$) pH value, followed by KB4, both contained higher level of sugary kefir grains and resulted

in higher fermentation activity. Afterwards, the pH values of individual treatment stayed relatively the same as they entered cold storage, although slight fluctuation existed. After 12 weeks of refrigerated storage, KB2 was the lowest ($P < 0.05$) in pH among treatments.

All treatments showed low volatile basic nitrogen (VBN) values during 12 weeks of storage (Table 6) and treatments with higher levels of sugary kefir grains (KB2 and KB4) were higher than others during storage period. Both treatments were lower in pH values following fermentation, heating, and storage (Table 5). These low molecular non-protein nitrogen compounds were possibly resulted from protein degradation during fermentation (Ruiz-Capillas and Jiménez-Colmenero, 2005). In addition, total plate counts (TPC) of individual treatment merely changed during storage and all treatments showed TPC number less than 10^5 cfu/g (Table 7). KB2 and KB4, both containing 7.5 % sugary kefir grains, showed lower TPC than KB1 and KB3. Strains of lactic acid bacteria could provide protective action against certain pathogens through the production of bacteriocins in addition to metabolites such as lactic acid and acetic acid. (Hugas and Monfort, 1997; Lücke, 2000; Ammor and Mayo, 2007). KB2 and KB4 being higher in fermentative activities could produce more antibacterial

Table 5. Changes in pH value of semi-dry sausage containing sugary kefir grains after cooking and during refrigerated storage at 2 °C

Time	Treatment ^a			
	KB1	KB2	KB3	KB4
After Cooking				
0 hr	5.10 ^A ±0.02	5.06 ^B ±0.04	5.07 ^{AB} ±0.01	5.07 ^{AB} ±0.01
24 hr	5.74 ^A ±0.03	5.66 ^{AB} ±0.07	5.67 ^{AB} ±0.02	5.62 ^B ±0.06
Storage				
0 wk	5.70 ^A ±0.04	5.46 ^C ±0.03	5.69 ^A ±0.05	5.59 ^B ±0.14
4 wk	5.53 ^{AB} ±0.07	5.48 ^B ±0.05	5.53 ^{AB} ±0.11	5.60 ^A ±0.11
8 wk	5.64 ^A ±0.04	5.43 ^B ±0.05	5.64 ^A ±0.10	5.56 ^A ±0.14
12 wk	5.56 ^A ±0.09	5.42 ^B ±0.08	5.57 ^A ±0.07	5.55 ^A ±0.14

^aKB1= 4 % brown sugar/5 % sugary kefir grains; KB2= 4 % brown sugar/7.5 % sugary kefir grains; KB3= 6 % brown sugar/5 % sugary kefir grains; KB4= 6 % brown sugar/7.5 % sugary kefir grains.

^bMeans (±SD) within the same row bearing unlike upper-case letters (A-C) are significantly different ($P < 0.05$).

Table 6. Changes in volatile basic nitrogen (VBN)^b value of semi-dry sausage containing kefir grains during refrigerated storage at 2 °C

Treatment ^a	Storage time (week)			
	0	4	8	12
KB1	6.83 ^B ±0.14	6.93 ^B ±0.70	7.80 ^B ±0.14	8.78 ^B ±0.53
KB2	8.63 ^A ±0.55	8.81 ^A ±0.72	10.20 ^A ±0.26	10.31 ^A ±0.62
KB3	6.42 ^B ±0.70	6.52 ^B ±0.20	7.81 ^B ±0.23	8.65 ^B ±0.60
KB4	8.21 ^A ±0.59	8.21 ^A ±0.63	10.21 ^A ±0.24	10.07 ^A ±0.67

^aKB1= 4 % brown sugar/5 % sugary kefir grains; KB2= 4 % brown sugar/7.5 % sugary kefir grains; KB3= 6 % brown sugar/5 % sugary kefir grains; KB4= 6 % brown sugar/7.5 % sugary kefir grains.

^bMeans (± SD) within the same column period bearing unlike upper-case letters (A-B) are significantly different ($P < 0.05$).

Table 7. Changes in total plate counts (TPC)^{bc} of semi-dry sausage containing kefir grains during refrigerated storage at 2 °C

Treatment ^a	Storage time (week)			
	0	4	8	12
KB1	4.25 ^A ±0.12	4.27 ^A ±0.10	4.12 ^A ±0.05	4.03 ^A ±0.05
KB2	3.12 ^B ±0.44	3.02 ^B ±0.34	2.95 ^B ±0.54	2.97 ^B ±0.68
KB3	4.21 ^A ±0.10	4.21 ^A ±0.04	4.09 ^A ±0.05	4.04 ^A ±0.04
KB4	3.12 ^B ±0.30	2.60 ^C ±0.29	2.49 ^C ±0.30	2.75 ^B ±0.30

^aKB1= 4 % brown sugar/5 % sugary kefir grains; KB2= 4 % brown sugar/7.5 % sugary kefir grains; KB3= 6 % brown sugar/5 % sugary kefir grains; KB4= 6 % brown sugar/7.5 % sugary kefir grains.

^bMeans (± SD) within the same column bearing unlike upper-case letters (A-C) are significantly different ($P < 0.05$).

^cTPC was expressed as cfu/g.

products resulting in lower TPC values. There is no current regulations on the numbers of VBN and total aerobic counts for fermented meat products (Council of Agriculture, 2013).

However, total bacterial counts for fully cooked, refrigerated, ready-to-eat meat products are not allowed to exceed 1×10^6 cfu/g, in addition to free of salmonella and listeria and

Table 8. Changes in TBARS value^{bc} of semi-dry sausage containing kefir grains during refrigerated storage at 2 °C

Treatment ^a	Storage time (week)			
	0	4	8	12
KB1	0.34 ^C ±0.04*	0.48 ^{AB} ±0.07	0.42 ^B ±0.05	0.45 ^B ±0.05
KB2	0.53 ^B ±0.09	0.45 ^B ±0.02	0.51 ^B ±0.02	0.50 ^B ±0.02
KB3	0.39 ^C ±0.05	0.52 ^{AB} ±0.07	0.51 ^B ±0.05	0.53 ^{AB} ±0.07
KB4	0.64 ^A ±0.16	0.59 ^A ±0.20	0.62 ^A ±0.17	0.61 ^A ±0.20

^aKB1= 4 % brown sugar/5 % sugary kefir grains; KB2= 4 % brown sugar/7.5 % sugary kefir grains; KB3= 6 % brown sugar/5 % sugary kefir grains; KB4= 6 % brown sugar/7.5 % sugary kefir grains.

^bTBARS (mg malonaldehyde/kg meat) = Absorbance x 7.8.

^cMeans (±SD) within the same column bearing unlike upper-case letters (A-C) are significantly different ($P < 0.05$).

Fig. 1. Schematic processes for the manufacturing and analyses of fermented sausage containing sugary kefir grains.

limited number of *E. coli* and *Staphylococcus aureus*. From present results, semi-dry fermented sausage containing sugary kefir grains appears to be sound in hygienic properties.

TBARS (thiobarbituric acid-reactive substances) values for all treatments were no higher than 1 (mg malonaldehyde/kg meat) (Table 8) with slightly higher for KB4 at each storage period, although statistical significances existed at different storage period. Results suggested that addition of sugary kefir grains did not speed up the rate of lipid peroxidation. Ockerman (1984) reported TBARS value of 1 mg malonaldehyde/kg meat or above to be detected rancid flavor.

Lipid oxidation and possibly unacceptable levels of 0.5 and 1 mg malonaldehyde/kg meat, respectively, have been reported (Warriss, 2000). Results from VBN, TPC and TBARS suggested good microbial stability of fermented sausages with the inclusion of sugary kefir grains.

Scores from consumer evaluation were tabulated and organized in three categories in a 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 and 45 % of medium score (6-10), followed by KB4 of 38 % high score (11-

Fig. 2. Consumer preference evaluation of semi-dry sausage containing sugary kefir grains.

15) ballots and 43 % of medium score (6-10). Both KB2 and KB4 contained higher amount of sugary kefir grains (7.5 %). KB2 has the lowest pH value because of high fermentation activity. However, untrained panels evaluated KB1, KB3 and KB4 to be “slight sourness”. As shown in Tables 5 and 6, KB2 was promoted to protein degradation by fermentation more than other treatments. Since KB2 produced possibly not only VBN but also free amino acids which exhibited umami and sweetness. Therefore, the sourness of KB2 would be considered to be masked. Furthermore, “good to eat” was the most frequent comment consumers gave on both treatments that was an overall perception based on moisture, tractability, and/or chewiness, while “slight sourness” was given to KB1, KB3, and KB4. In light of texture, microbial stability and consumer preference, manufacture of semi-dry fermented sausage by incorporating up to 7.5 % sugary kefir grains appears to be feasible.

Conclusions

Fermented sausages have been on the market for a long time attributed to its unique aroma and texture; however, higher fat content could also have an adverse impact on consumer acceptance. The present study demonstrated feasible application of sugary kefir grains with endogenous microbiota as a substitute for commercial starter cultures. An innovative fermented sausage which has slightly acidic taste, unique flavor, acceptable texture, and high microbial safety, but lower in fat as compared with typical fermented sausages could be manufactured.

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