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Garlic inulin as a fat replacer in vegetable fat incorporated low-fat chicken sausages 10

Abstract 11

Inulin is a non-digestible carbohydrate and a prebiotic that can also act as a fat replacer in 12 various foods. This study examined the effect of replacing vegetable oil with garlic inulin on the 13 quality traits of chicken sausages. Water-based inulin gels were prepared using garlic inulin or 14 commercial inulin to imitate fats in chicken sausages. Chicken sausages were prepared 15 separately replacing vegetable oil with water-based inulin gels to reach final inulin percentages 16 of 1, 2, and 3 (w/w). The control was prepared using 3% (w/w) vegetable oil with no inulin. The 17 physicochemical properties and thiobarbituric acid reactive substance (TBARS) value of 18 prepared sausages were analyzed over 28-d frozen storage. Sausages with 2% garlic inulin 19 recorded higher flavour and overall acceptability scores (p < 0.05). Ash, moisture, and protein 20 contents of the sausages were increased with increasing levels of inulin while fat content was 21 reduced from 13.67% (control) to 4.47-4.85% (p<0.05) in 3% inulin-incorporated products. 22 Sausages incorporated with 2% inulin had lower lightness (L*) values than the control (p < 0.05). 23 Water holding capacity was similar (p>0.05) among the samples. During storage L* value, pH, 24 and water holding capacity decreased while redness (a*) and yellowness (b*) values increased in 25 all the samples. In addition, TBARS values were increased during the storage in all samples 26 within the acceptable limits. In conclusion, garlic inulin can be used successfully as a fat 27 substitute in sausages without altering meat quality parameters. 28

Keywords: Fat substitute, Garlic, Meat quality, Prebiotic, TBARS 29

30 Introduction

Meat as a nutritionally dense food of animal origin is vital for a healthy and well 31 balanced diet. Meat and meat products have a unique role in optimal human growth and 32 development throughout the history (Higgs, 2000; Pereira and Vicente, 2013). These are rich in 33 34 protein of high biological value, in particular essential amino acids such as isoleucine, leucine, lysine, methionine, tryptophan, valine, and phenylalanine, and micro nutrients like vitamin B_{12} , 35 vitamin A, folic acid, iron, selenium, zinc, and copper that human body needs to function 36 37 properly (Pereira and Vicente, 2013; Verbeke et al., 2010). Despite the nutritional value, meat and meat products are considered disease-promoting food primarily due to the fat content 38 (Pereira and Vicente, 2013) that is originally present and/or added during processing including 39 vegetable oil. Some epidemiological data has revealed that prolonged intake of high fat meat 40 products is directly associated with the occurrence of several chronic diseases such as cancer and 41 cardiovascular diseases (Domingo and Nadal, 2017; Han and Bertram, 2017). 42

Fat as a vital ingredient in the production of meat products contributes to the flavor, 43 texture, mouth feel, and appearance of the end products (Alaei et al., 2018; Cáceres et al., 2004; 44 Luisa García et al., 2006). Commercial sausage manufacturers in Sri Lanka use vegetable oil as a 45 46 main fat source in their products. Vegetable oils are composed mainly of triacylglycerols (around 95%) along with some free fatty acids, monoacylglycerols, and diacylglycerols and the 47 remainder comprises of minor components which include phospholipids, free and esterified 48 49 sterols, triterpene alcohols, tocopherols and tocotrienols, pigments, and hydrocarbons (Oswell et al., 2005). However, consumer demand for healthier meat products with lower fat, cholesterol, 50 nitrates, and calories, and higher health promoting components has increased over the last few 51

years (Wolk, 2017). Numerous extensive approaches have been proposed to reduce fat content of meat products by incorporating health promoting ingredients such as dietary fibers while retaining the sensory and organoleptic properties.

Addition of dietary fibers such as inulin into meat products has become an emerging 55 trend in recent years due to their potential health benefits including inhibiting colon tumor 56 growth, lowering the risk of coronary heart diseases, preventing cholesterol absorption and 57 reducing obesity risk (Hathwar et al., 2012). Inulin is being widely applied in the food industry 58 either individually or combined with other related ingredients as a prebiotic, dietary fiber, low 59 calorie sweetener, fat replacer, gelling agent, viscosity modifier, and a texture modifying 60 ingredient in various foods (Melilli et al., 2021; Mensink et al., 2015). In aqueous systems, upon 61 vigorous mixing and consequent cooling, inulin forms a smooth gelling structure which provides 62 a combination of creamier and juicier mouth feel to the end product which can potentially 63 replace fat, without altering its rheological properties but resembling the mouth feel of fat in low-64 fat meat products upon incorporation (Franck, 2002; Mudannayake et al., 2015b). Hence, it can 65 possibly be used as a fat substitute in formulation of low fat meat products (Illippangama et al., 66 2022) with a low calorie content of about 1-1.5 kcal per gram (Weiss et al., 2010). 67

Inulin naturally occurs in tubers, rhizomes, and bulbs of plants such as chicory (*Cichorium intybus*), garlic (*Allium sativum*), Jerusalem artichoke (*Helianthus tuberosus*), leeks (*Allium porrum*), dahlia (*Dahlia pinnata*), onion (*Allium cepa*), asparagus (*Asparagus falcaus*), etc (Mudannayake et al., 2015a). The commercial production of inulin is primarily based on two plant species, *C. intybus* and *H. tuberosus* at present and limited to European countries (Moongngarm et al., 2011). The use of inulin as an ingredient in food industry is very limited in Sri Lanka due to its high cost. Garlic belongs to the family of Amaryllidaceae and Genus Allium. Garlic (*A. sativum*) bulb is found as a rich source of inulin (18-19 g/100 g fresh weigh;
Mudannayake et al., 2015a) and is available in Sri Lanka. Garlic can be used to extract inulin
which potentially delivers enhanced flavors and organoleptic properties in food products.

Vegetable oil has been successfully replaced with inulin in the formulation of emulsion-78 type chicken sausages (Alaei et al., 2018; Berizi et al., 2017). Guedes-Oliveira et al. (2019) 79 produced reduced fat lamb patties incorporating vegetable fat with inulin. Furthermore, Menegas 80 et al. (2013) produced dry-fermented chicken sausage replacing corn oil with inulin for the 81 enhancement of physicochemical, microbiological, and textural characteristics. However, 82 literature reveals that scientific data on effect of garlic inulin as a fat substitute on quality of meat 83 and meat products are scant. Therefore, this study examined the extraction of inulin from garlic 84 and the effects of adding extracted garlic inulin and commercial (chicory) inulin as partial fat 85 replacers on physicochemical, sensory, and textural characteristics of vegetable fat incorporated 86 chicken sausages. 87

88

89 Materials and Methods

Commercial chicory inulin (BENEO Orafti[®]) was purchased from DPO Lanka (Pvt) Ltd, Colombo, Sri Lanka and garlic inulin was extracted from garlic bulbs using hot water extraction, vacuum evaporation and subsequent spray drying as described by Mudannayake et al. (2015b) with slight modifications. The ingredients employed to prepare the sausage formulations, such as chicken breast meat, vegetable oil, wheat flour, chili powder, salt, pepper, onion, cardamom and cinnamon were purchased from a local grocery store.

97 **Extraction of inulin from garlic**

Garlic bulbs (A. sativum) were first peeled, cleaned and washed thoroughly with water and 98 subsequently cut into small slices to have 3 mm thickness. Sliced garlic was then blanched in 99 boiling water, acidified with ascorbic acid (0.1% w/w) for 1 to 2 min, mixed with potable water 100 (1:10 g/mL) and kept at 80 ± 5 °C for 50 min with frequent stirring in a double-water-jacketed pan 101 on a stove. Then the mixture was filtered through 4 layers of muslin cloth and the residue was 102 weighed and re-extracted one more time following the same procedure. Both extracts were 103 combined together and concentrated to a brix value of approximately 8° at 60°C using a 104 rotavapor (Model: 1000282711, BUCHI Labortechnik AG, China) equipped with evaporator 105 flask, receiving flask, recirculating chiller, and vacuum pump. The concentrated extracts were 106 finally spray dried (Model: 005-1116-FD, SOLTEC[®], Malaysia) at a feed rate of 2.5 L/h, air 107 flow of 110 kg/h, and inlet and outlet temperatures of 60 and 200°C to obtain a light colour fine 108 powder, respectively. 109

110

111 Fourier transform infrared spectroscopy (FT-IR)

The chemical characterization of extracted garlic inulin and commercially available chicory inulin powders was conducted separately using Fourier-transform infrared (FT-IR) (Bruker FT-IR spectrophotometer, Bruker Optik GmbH, Germany) equipped with an OPUS 7.5 FT-IR software against a KBr background. Approximately 2 mg of each powder were milled separately with 200 mg of KBr using a clean motor and pestle and pressed into pellets manually. The prepared clear KBr-inulin pellets were analyzed in the FT-IR. A spectral range of 4000-400 cm⁻¹ at a resolution of 4 cm⁻¹ and 32 scans for each spectrum were used (Grube et al., 2002).

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Determination of inulin content

Inulin content of commercial inulin and extracted garlic inulin powders were analyzed 121 separately according to enzymatic spectrophotometric method (AOAC 999.03) as described by 122 AOAC (2005) using the Megazyme fructan assay kit (Megazyme, Ireland), which contained 123 sucrase, fructanase, fuctan control, sucrose control and D-Fructose standard (Mudannavake et al., 124 2015a). Briefly, 1 g of inulin sample was put into a conical flask filled with 80 mL of hot 125 distilled water (~70°C). The conical flasks were covered with parafilm and placed on a shaking 126 water bath at a constant temperature $(85\pm2^{\circ}C)$ to extract fructans and sugars. Sample extract was 127 allowed to cool to room temperature and the volume was adjusted to 100 mL with distilled water 128 and it was mixed well. Finally, the mixture was filtered through a filter paper (No.4 Whatman 129 International Ltd., Maidstone, England) and the inulin content of the extract was determined as 130 described by McCleary et al. (2000). The inulin content of each powder was calculated using the 131 following formula: 132

Inulin% =
$$\Delta A \times F \times \frac{V}{W} \times 2.48$$

where ΔA =sample absorbance-sample blank absorbance (both read against the reagent blank),

F=factor to convert absorbance values to μg of fructose [(54.5 μg of D-Fructose)/ (absorbance for 54.5 μg of D-Fructose)],

137 V=volume (mL) of extract used, and

138 W=weight (mg) of sample extracted.

139

Preparation of water based inulin gel

The water based inulin gel was prepared using 40% of inulin (w/w), 58% of water (w/w) and 2% of soya lecithin (w/w) (Kim and Wang, 2001). During the preparation of inulin gel, the aqueous solution of inulin was heated to 85°C with continuous mechanical stirring for 20 min, mixed with soy lecithin, allowed to cool to room temperature and refrigerated (5°C) for 24 h (Mudannayake et al., 2015a). After 24 h, the resultant water based stable inulin gel was incorporated into meat batter to reach 1, 2, and 3% (w/w) total inulin levels in the product.

146

147 **Experiment-1**

Experiment-1 was conducted to select the best level of inulin for manufacture of low-fat chicken sausages without altering the desired quality characteristics.

150

151 Formulations

Seven formulations of chicken sausages were prepared for the study with different 152 percentages of laboratory prepared garlic inulin or commercial chicory inulin according to 153 commercial guidelines to test the replacement of vegetable oil with garlic or commercial chicory 154 inulin. The treatments and amounts of the ingredients used in each treatment in production of 155 low-fat chicken sausages are given in Table 1. Chicken meat was first minced in a mincer (HR-156 2726, PHILIPS, China) and the remaining ingredients (Table 1) were added and mixed 157 thoroughly. The meat batter was then stuffed in cellulose casing and links were formed. The 158 products were then cooked until the core temperature of the product reached to 72°C. After heat 159 160 treatment, the products were cooled and casings were removed. Finally, the products were vacuum packed (DZ500-2D, China) and stored under frozen condition (-18°C) until further 161

analysis. The experimental units were 1.5 kg, in triplicate, and at three different time periods(three lots).

164

165 **Proximate composition**

Proximate composition of inulin powders and sausages samples was determined following 166 the method of AOAC (2016). Briefly, moisture content (AOAC 950.46) was measured by drying 167 each sample (3 g) in an aluminum dish at 104°C for 15 h (DHG-9145A, Zenith Lab Co. Ltd., 168 Changzhou, China). Crude protein content (AOAC 928.08) was measured using the Kjeldahl 169 method (DNP-3000, RAYPA, Barcelona, Spain). Crude protein content was calculated by 170 multiplying the amount of nitrogen by a factor of 6.25. Crude fat content (AOAC 960.39) was 171 172 measured using the Soxhlet extraction system (EME60250/CEB, Electrothermal, Staffordshire, UK). Crude ash content (AOAC 923.03) was determined by igniting each sample (2 g) in muffle 173 furnace (HD-230, Hobersal, Barcelona, Spain) at 600°C overnight. 174

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176 Sensory evaluation

The design of the sensory evaluation for cooked sausages was reviewed and approved by the 177 Research Ethics Review Committee of Uva Wellassa University (No. UWU/REC/2021/003). 178 Sensory evaluation of cooked sausages was performed with a panel of 30 untrained panelists in 179 individual booths as described by Menegas et al. (2013). Sausage samples were first heated using 180 a sausage griller (SG 9, ASAIN, Zhengzhou Ohfu Ind. Ent. Co. Ltd, China) just before the 181 evaluation and prepared to uniform size (1.5 cm \times 2 cm). Coded sausage samples were then 182 evaluated for appearance, texture, odour, flavour, fatty flavour, colour and overall acceptability 183 using a 9-point hedonic scale (9-like extremely, 8-like very much, 7-like moderately, 6-like 184

slightly, 5-neither like nor dislike, 4-dislike slightly, 3-dislike moderately, 2-dislike very much,
1-dislike extremely). Drinking water at room temperature was provided to rinse the mouth prior
to and between sample evaluations.

188

189 **Experiment-2**

The best formulations from Experiment-1 were selected based on the results of sensory evaluation for determination of meat quality parameters during a 28-d frozen storage. Prior to analysis, samples were thawed by packing them in a clean leak-proof package and submerging in a container of clean tap water at room temperature, changing the water every 30 min.

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195 **Determination of pH value**

Each meat sample (1 g) was homogenized with 9 mL of distilled water for 60 s by using a homogenizer (Ika Laboratory Equipment, Korea) and filtered through a filter paper (No.4 Whatman International Ltd., Maidstone, England). The pH value of each filtrate was determined with a pH meter (pH 700, Eutech instruments, Singapore) after calibration using buffers (pH 4.01, 7.00 and 10.01) at room temperature.

201

202 Determination of water holding capacity (WHC)

WHC was determined based on the technique of Hamm (1961), as described by Wilhelm et al. (2010). Sausage samples (2.00±0.10 g) were carefully placed between two pieces of filter papers (No. 4; Whatman International Ltd, Maidstone, England) on acrylic plates and left under a 10-kg weight for 5 min separately. After recording the final weight of each sample, WHC was calculated using the following equation, where *Wi* and *Wf* are the initial and final weights (g) of sample, respectively.

209 WHC (%) =
$$100 - [\frac{(Wi - Wf) \times 100}{Wi}]$$

210

211 Determination of colour values

The colour values of minced chicken sausages were measured using a colorimeter (CR-410, Konica Minolta, NIC., Japan) which was calibrated against a white reference tile (No. 20433045, Y=85.7, x=0.3170 and y= 0.3242, Konica Minolta, NIC., Japan). The average of three repeated measurements in each minced sample was used for the determination of the values of lightness (CIE L*), redness (CIE a*), and yellowness (CIE b*).

217

218 **Determination of lipid oxidation**

Lipid oxidation of sausage samples were evaluated by the determination of 2-thiobarbituric 219 acid relative substances (TBARS) values as described in Lee et al. (2021) with some 220 modifications. Each sample (5 g) was homogenized in 15 mL of deionized water using a 221 homogenizer (D-500, Velp Scientifica, Usmate, Italy) at 14,000 rpm for 30 s. Butylated 222 223 hydroxytoluene (BHT; 50-µL) (7.2% w/v in ethanol) and thiobarbituric acid/trichloroacetic acid solution (20 mM TBA and 15% [w/v] TCA; 2 mL) were added to the homogenate (1 mL) and 224 vortexed for 30 s. The mixture was then incubated in a water bath (YCW-010E, Gemmy 225 Industrial Corporation, Taipei, Taiwan) at 90°C for 30 min, and subsequently cooled for 10 min 226 in an ice-water bath. After centrifuging the samples at 3,000 rpm for 15 min (5°C) using a ST 227

40R centrifuge (Thermo Fisher Scientific, Osterode, Germany), the absorbance of was measured at 532 nm with a UV-2005 spectrophotometer (J.P. Selecta, Barcelona, Spain) against a blank prepared with 1 mL deionized water and 2 mL TBA/TCA solution. The malondialdehyde (MDA) concentration of each sample was determined against an external standard curve constructed using tetraethoxypropane. The results were expressed as mg MDA per kg of sausages.

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235 Statistical Analysis

The complete experiment was repeated three times in a completely randomized design and duplicate samples were drawn for each parameter. The data were subjected to one-way analysis of variance (ANOVA) and Tukey's comparison of the means test ($p \le 0.05$) using Minitab 17 software. Data obtained from sensory analysis was analyzed using the Friedman test.

240

241 **Results and Discussion**

FT-IR Analysis

Fig. 1 shows the FT-IR spectra of developed garlic inulin and commercial chicory inulin 243 powders. Very identical FT-IR spectra were observed for laboratory prepared garlic inulin 244 powder and commercial inulin powder. Previous studies reported that inulin had distinctive 245 hydroxyl (OH) groups at 3353 cm⁻¹ (Wu and Lee, 2000) and at 3384-3386 cm⁻¹ (Mudannayake et 246 al., 2015b), and C=O at 1745 cm⁻¹ (Wu andLee, 2000) and 1631-1640 cm⁻¹ (Mudannayake et al., 247 2015b) in FT-IR analysis. In the present study, the FT-IR spectrum of garlic inulin had 248 absorption bands at 3339, 1457, 1130, and 970 cm⁻¹. The peaks at 970 and 1130 cm⁻¹ indicated 249 the presence of inulin (Grube et al., 2002). The absorption bands in the range of 3417-2924 cm⁻¹ 250

showed the OH groups which are abundant in inulin structure while those in the range of 1635-1427 cm⁻¹ are responsible for the presence of esterified carboxyl groups. These confirmed similar chemical structure in inulin from both sources. Furthermore, FT-IR results of the current study are comparable to those of chicory, Jerusalem artichoke, globe artichoke and garlic inulin (Abou-Arab et al., 2011; López-Molina et al., 2005; Wu andLee, 2000).

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257

7 Inulin content in garlic and commercial inulin powders

Commercial chicory inulin powder contained a higher content of inulin (73.43% dry wt) 258 compared to laboratory prepared garlic inulin powder (63.28% dry wt) (Table 2) as measured by 259 enzymatic spectrophotometric method (AOAC 999.03). However, the reported amount of inulin 260 (90% dry wt) in commercial chicory inulin powder as per the product sheet of Orafti GR (2007) 261 is comparatively higher than the actual quantity of inulin measured enzymatically (73.43% dry 262 wt) in this study. Mudannayake et al. (2015b) attributed such variations between the 263 commercially reported values and the actual measured values to differences in sample 264 preparation and methods of analysis. In addition, a higher level of purity in garlic inulin may be 265 achieved through application of further purification steps such as activated carbon treatment for 266 extracted garlic juice. 267

268 **Experiment-1**

Proximate composition of inulin powders

The moisture, protein, fat and ash contents of the developed garlic inulin and commercial chicory inulin powders are presented in Table 2. Laboratory prepared garlic inulin powder had lower levels of crude fat and crude protein and higher levels of moisture and ash opposed to

commercial inulin powder (p < 0.05). The values reported for commercial inulin in this study are 273 not in agreement with those observed by Mudannayake et al. (2015b) in commercial chicory 274 inulin powder. However, Bouaziz et al. (2014) detected almost similar protein content (1.58%) 275 and a higher of ash content (1.19%) in commercial chicory inulin compared to the present study. 276 The differences in the chemical composition might be due to the variations in the species plant 277 extraction process, purification process and method of analysis. In addition, these results 278 suggested that the quality of garlic inulin powder needs to be improved through purification of 279 garlic inulin extract using activated carbon and ion exchange resin (IER) treatments 280 (Mudannayake et al., 2015b). 281

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283 **Proximate composition of chicken sausages**

Table 3 shows the proximate composition of chicken sausages as affected by varying levels of garlic and commercial chicory inulin powders. Fat, protein, ash and moisture contents in the sausage samples varied significantly with the incorporation of inulin powder into sausage formulations as a fat replacer, irrespective of the source of inulin.

Sausages with added inulin had lower fat contents than did the control sausages with no added inulin (p<0.05). Replacement of vegetable oil with inulin was the main reason for the observed low fat content in inulin incorporated sausages (Menegas et al., 2013). Additionally, with the increasing levels of inulin in formulations a significant decline in the fat content of sausages was observed, irrespective of the source of inulin. The use of inulin at 0.87%-30% in different types of sausages effectively reduced the fat content of products (Berizi et al., 2017;

Bis-Souza et al., 2018; Choi et al., 2016; Glisic et al., 2019; Huang et al., 2011; Keenan et al.,
2014; Méndez-Zamora et al., 2015; Menegas et al., 2013; Ö zer, 2019; Prapasuwannakul, 2018).

According to Table 3, an increase in moisture content was observed in sausages when the oil 296 content was gradually replaced with inulin, irrespective of the source of inulin (p < 0.05). 297 However, the effect is more prominent with commercial chicory inulin. The increment in 298 moisture content may be attributed to the capability of inulin to hold more water during 299 preparation and cooking process (El Zeny et al., 2019) due to the presence of hydrophilic groups 300 and the hygroscopic nature of inulin (Alaei et al., 2018). The findings of the present study are in 301 agreement with those reported by several other researchers on effect of substituting fat with 302 inulin on moisture levels in sausages (Berizi et al., 2017; Glisic et al., 2019; Menegas et al., 2013; 303 Özer, 2019). Furthermore, an inverse relationship between fat and moisture contents of sausages 304 formulated with and without inulin was previously observed (de Souza Paglarini et al., 2021; 305 Keenan et al., 2014; Méndez-Zamora et al., 2015; Šojić et al., 2011). 306

The increased level of substituting vegetable oil with inulin caused an enhancement of 307 protein content in the sausages, irrespective of the inulin source (p < 0.05; Table 3). However, the 308 effect was more prominent at an inclusion level of 3% of inulin in sausage formulations. 309 310 Similarly, studies conducted to evaluate the effect of inulin on quality parameters of different meat products revealed that inulin incorporated samples had higher protein contents than the 311 control samples with no added inulin (Glisic et al., 2019; Méndez-Zamora et al., 2015; Özer, 312 313 2019). The increase in protein content in sausages may be due to the substitution of oil with inulin which contained protein levels of 0.70 -1.31%. 314

Table 3 shows that the inclusion of garlic inulin in to chicken sausages up to 3% had no effect on ash content of chicken sausages as compared with the control sausages (p>0.05).

However, the ash contents were significantly higher in sausages with 2% and 3% commercial inulin as opposed to control samples and garlic inulin added sausages (p<0.05). Šojić et al., (2011) and Huang et al., (2011) stated that that inulin added sausages contained higher ash percentages than samples with no added inulin. This is attributed to the higher ash content of inulin powders which is arising from the plant sources.

322

323 Sensory analysis

Sensory quality attributes including flavour, colour and odour determine the consumer 324 preference for food products. Sensory scores given to different sensory attributes of chicken 325 sausages prepared substituting oil with inulin is presented in Fig. 2. The results indicate that 326 substituting the oil content with inulin did not affect the colour and juiciness of sausages 327 (p>0.05). However, significant differences were observed between treatments in relation to 328 texture, odour, flavour, fatty flavour and overall acceptability (p < 0.05). Hence, based on the sum 329 of ranks of sensory parameters (Data not shown) substitution of vegetable oil with inulin at 2% 330 was the best level to produce chicken sausage with better acceptability score, irrespective of the 331 inulin source. The increased level of inulin as a fat substitute in chicken sausages enhanced the 332 sensory scores for colour, appearance and texture (Alaei et al., 2018). In contrast, several studies 333 have revealed that inulin did not affect the acceptability of cooked sausages (Cáceres et al., 2004; 334 Luisa García et al., 2006), Chinese-style sausages (Huang et al., 2011) and dry-fermented 335 chicken sausages (Menegas et al., 2013). 336

Thus, based on the findings of the Experiment-1 it was possible to manufacture chicken sausages with 30.7% and 40.6% lower fat contents with higher product acceptability when vegetable oil was substituted with garlic inulin and commercial inulin at 2% level, respectively.

Therefore, these two formulations were selected with the control for analysis of meat quality parameters during frozen storage.

342

343 **Experiment-2**

344 Change in pH value under frozen storage

Table 4 represents the pH values of chicken sausages as influenced by different formulations 345 and storage period. There was a significant difference in pH values between the control and 346 inulin incorporated sausages (p < 0.05) until 21 d of storage. Sausages added with garlic inulin 347 (2%) showed higher pH values as opposed to control sausages and those added with commercial 348 inulin (2%) and this can be attributed to the slightly alkaline nature of garlic. In contrast, addition 349 of inulin and the reduction of fat content had no effect (p>0.05) on the pH of sausages (Mendoza 350 et al., 2001; Menegas et al., 2013; Muguerza et al., 2002) and minced meat (Furlán et al., 2014). 351 The pH values of sausages decreased over the 28-d frozen storage period, particularly in 352 control samples and garlic inulin added samples after 21 d (p<0.05; Table 4). This was probably 353 due to the metabolic activities of psychotropic bacteria and lactic acid bacteria that are 354 considered as the predominant microorganisms in meat products (Borch et al., 1996). 355 Furthermore, ingredients utilized in the formulation (Kim et al., 2011) and interactions between 356 the polysaccharides and polar and non-polar groups of proteins in food via electrostatic 357 association (Choi et al., 2010) may cause for pH reduction. In general, pH value of chicken 358 sausages ranged from 6.5 to 5.0 during a 28-d storage (Kotula and Wang, 1994). The pH values 359 reported in this study were within this range during the frozen storage. 360

361

363 Changes in water holding capacity under frozen storage

Water holding capacity of comminuted meats such as sausages is an important quality 364 attribute parameter particularly because the structure of tissues has already been destroyed during 365 the processing of such products (Lawrie and Ledward, 2006). Table 5 shows the water holding 366 capacity of experimental sausages during frozen storage period. Substitution of vegetable oil 367 with inulin had no effect on the water holding capacity of chicken sausages. In contrast, Méndez-368 Zamora et al. (2015) found that inulin as a fat replacer at 15% and 30% decreased the water 369 holding capacity of low-fat frankfurter compared to the control. However, the same quality 370 attribute was decreased with the increasing storage period in all experimental sausages. This can 371 be attributed to the decrease in pH values observed during the frozen storage as lower pH 372 denatures myofibrils and decrease the water holding capacity (den Hertog-Meischke et al., 1997). 373

374

375 Changes in colour values under frozen storage

Changes in colour values of sausages were weekly determined for 28 d under frozen storage 376 (Table 6). All three colour values of sausages were significantly affected by the frozen storage 377 period (p < 0.05). In this regards, the lightness (CIE L*) value of control sausages and those 378 enriched with garlic inulin decreased throughout the frozen storage (p < 0.05). In contrast, the 379 redness (CIE a*) and yellowness (CIE b*) values increased significantly with increasing storage 380 period, irrespective of the source of inulin. Menegas et al., (2013) reported that sausages became 381 darker during storage; CIE a* value increased (p<0.05) meanwhile CIE L* and CIE b* remained 382 unchanged (p>0.05) during refrigerated storage. 383

With respect to the effect of inulin enrichment in sausages on color values, sausages incorporated with garlic inulin and commercial inulin had lower CIE L* values than control

sausages until d 7 (p<0.05). Further, the same trend was detected in sausages enriched with garlic 386 inulin as opposed to control until d 28 (p<0.05). The reduced lightness values in inulin-added 387 low-fat sausages can be attributed to the decrease in the shine that is usually provided by fat 388 (Cáceres et al., 2004; Menegas et al., 2013; Nowak et al., 2007). Cáceres et al. (2004) further 389 revealed that the whitish translucent gel formed by inulin has no dominant color. The findings of 390 391 the current study are similar to those of the previous studies by Berizi et al. (2017) and Öztürk and Serdaroğlu (2017) who observed lower lightness values in inulin-enriched meat products, 392 emulsion type sausages and chicken meat balls, as opposed to those without enrichment, 393 respectively. In contrast, inulin addition significantly increased the CIE L* values in Chinese 394 style sausages compared to control (Huang et al., 2011). 395

Šojić et al., (2011) found correlations between the CIE L* value and fat content (r = 0.99; 396 p < 0.001) and also between the CIE a* value and fat content (r = -1.00; p < 0.001). Furthermore, 397 Menegas et al., (2013) noticed a higher redness value in sausage formulation with inulin 398 compared with that with standard oil content. Therefore, an increase in redness value was 399 expected with the decrease in the fat level of sausages. However, significantly higher redness 400 values in inulin-added reduced-fat sausages as opposed to control sausages were only observed 401 402 in d 1. From d 14 onward control sausages had higher redness values than inulin-added sausages (p<0.05). Recently, de Souza Paglarini et al., (2021) observed lower redness values in inulin gel-403 incorporated Bologna sausages as opposed to control sausages. 404

Addition of inulin into sausage formulation had no effect on the yellowness of the product at d 1 (p>0.05). From d 7 onward CIE b* value was significantly influenced by the level and source of inulin. In general, sausages added with commercial chicory inulin recorded higher yellowness values than those added with garlic inulin (p<0.05). Comparable yellowness values were

reported previously in meat products prepared with and without inulin (Berizi et al., 2017;
Prapasuwannakul, 2018).

411

412 Textural properties of sausages samples

Texture profile analysis revealed that incorporation of inulin into sausage formulations 413 significantly reduced the hardness (maximum force required to compress the sample) and 414 chewiness (work to masticate the sample for swallowing) (p < 0.05). Control sample showed a 415 significantly higher hardness value (1603.7 g) compared with sausages incorporated with 416 commercial inulin (1165.0 g) and garlic inulin (1237.0 g). Chewiness value for control sample 417 (47.3 mJ) was significantly greater than that of sausages incorporated with commercial inulin 418 (32.4 mJ) and garlic inulin (39.0 mJ). These results are comparable to those obtained by other 419 authors for frankfurters, pork bologna and beef burger incorporated with dietary fiber (Grigelmo-420 Miguel et al., 1999; Mansour and Khalil, 1997; Shand, 2000). Reduced-fat meat products 421 incorporated with inulin were softer than those having conventional levels of fat (Cáceres et al., 422 2004; Choi et al., 2016; Glisic et al., 2019; Özer, 2019; Prapasuwannakul, 2018) which may 423 reduce the amount of force required to chew samples for swallowing. 424

However, inulin enrichment did not change other texture parameters of sausages such as springiness, cohesiveness, adhesiveness and gumminess (p>0.05; Data not shown). Similar results were reported by Choi et al. (2016) for restructured sausages incorporated with chicory fiber as a fat replacer. Garcia et al. (2006) reported that differences observed in the texture properties of meat products depend on the type and level of inulin used. When inulin was incorporated as a gel in meat products, textural parameters changed only at a higher level of incorporation (7.5% or more) resulting softer sausages irrespective of the fat content. Therefore,

the addition of lower level of inulin in this study (2%) might be the reason for comparable values
observed for gumminess, cohesiveness, adhesiveness, and springiness of different sausage
samples.

435

436

Changes of lipid oxidation with the storage time

To investigate the effect of inulin inclusion on the lipid oxidation of sausages, TBARS values of the sausages were measured. Regardless of the level and source of inulin utilized the TBARS values of sausage samples increased significantly with increasing frozen storage (Table 7). Domínguez et al., (2019) stated that lipid oxidation in meat and meat products are influenced by storage time; with increasing time the possibility of radicals to cause damage to lipids increases. In addition, the release of iron from heme-proteins gets accelerated with long storage periods and it catalyzes multiple reactions in the initiation and propagation phases of lipid oxidation.

In addition, both garlic inulin and chicory inulin-added sausages showed lower TBARS values than control sausages throughout the storage period. This could be due to the differences in fat content of the experimental sausages, as shown in the Table 3. It has been previously reported that TBARS values near 0.5 mg kg⁻¹ sample are indicative of the beginning of oxidation, whereas those above 1.0 mg kg⁻¹ sample suggest advanced oxidative status (Reitznerová et al., 2017). In the present study, all experimental sausages had TBARS values lower than sensory rancidity detection (< 1.0).

451

453 Conclusion

This study was conducted with the aim of evaluating the sensory and physicochemical 454 properties of inulin incorporated low-fat chicken sausages. Overall, the findings showed that fat 455 content of the sausages can be reduced from 13.67% (Control sample) to 4.47-4.85% by 456 457 incorporating water-based inulin gel to the product formula at the level of 3% inulin. However, 2% garlic inulin contributed to better sensory qualities such as flavour and overall acceptability. In 458 addition, protein, ash and moisture contents of the sausage samples were increased when inulin 459 percentage was increased in the formulation. Hence, garlic inulin can be used to successfully 460 substitute fat in sausage production without altering the meat quality parameters. 461

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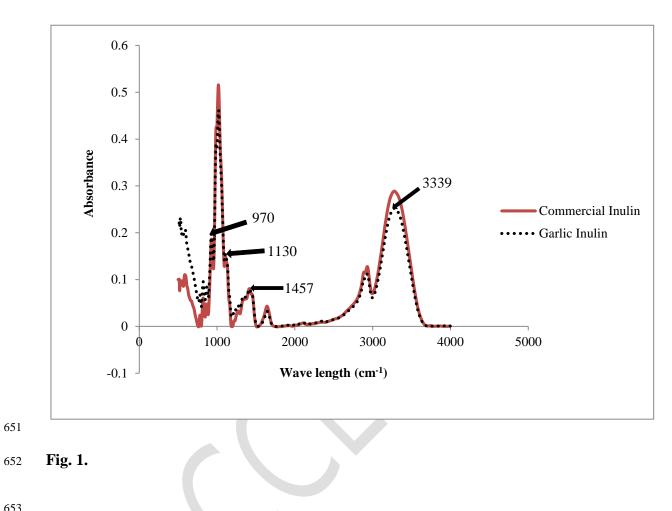
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Figure Legends

- **Fig. 1.** FTIR spectra of garlic inulin and commercial inulin
- **Fig. 2.** Radar chart for sensory evaluation to select the best sausages samples.

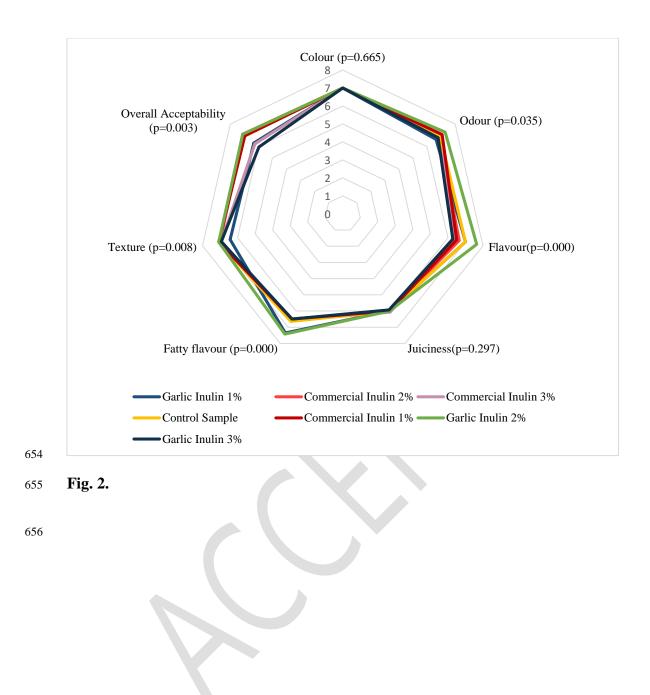
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657 List of Tables

Formulations (% of ingredients)*						
Control	GI 1%	GI 2%	GI 3%	CI 1%	CI 2%	CI 3%
71	71	71	71	71	71	71
3	2	1	0	2	1	0
0	1	2	3	0	0	0
0	0	0	0	1	2	3
6	6	6	6	6	6	6
15	15	15	15	15	15	15
1.5	1.5	1.5	1.5	1.5	1.5	1.5
1.5	1.5	1.5	1.5	1.5	1.5	1.5
0.5	0.5	0.5	0.5	0.5	0.5	0.5
1.2	1.2	1.2	1.2	1.2	1.2	1.2
0.15	0.15	0.15	0.15	0.15	0.15	0.15
0.15	0.15	0.15	0.15	0.15	0.15	0.15
	71 3 0 0 6 15 1.5 1.5 1.5 0.5 1.2 0.15	Control GI 1% 71 71 3 2 0 1 0 0 6 6 15 15 1.5 1.5 0.5 0.5 1.2 1.2 0.15 0.15	ControlGI 1%GI 2% 71 71 71 3 2 1 0 1 2 0 0 0 6 6 6 15 15 15 1.5 1.5 1.5 1.5 1.5 1.5 0.5 0.5 0.5 1.2 1.2 1.2 0.15 0.15 0.15	ControlGI 1%GI 2%GI 3% 71 71 71 71 3 2 1 0 0 1 2 3 0 0 0 0 6 6 6 15 15 15 1.5 1.5 1.5 1.5 1.5 1.5 0.5 0.5 0.5 1.2 1.2 1.2 0.15 0.15 0.15	ControlGI 1%GI 2%GI 3%CI 1% 71 71 71 71 71 71 3 2 1 0 2 0 1 2 3 0 0 0 0 0 1 6 6 6 6 15 15 15 15 1.5 1.5 1.5 1.5 1.5 1.5 1.5 1.5 1.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5 1.2 1.2 1.2 1.2 0.15 0.15 0.15 0.15	ControlGI 1%GI 2%GI 3%CI 1%CI 2%717171717171713210210123000000126666615151515151.50.50.50.50.50.51.21.21.21.21.21.20.150.150.150.150.150.15

Table 1. Percentage ingredients used for the production of low-fat chicken sausages

659 *GI-garlic inulin; CI-Commercial inulin

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Constituents (%)	Garlic inulin powder	Commercial inulin powder	SEM ¹	
Moisture	5.10 ^A	3.62 ^B	0.369	
Ash	0.94 ^A	0.68^{B}	0.059	
Crude protein	0.70^{B}	1.31 ^A	0.154	
Crude fat	0.55 ^B	0.67^{A}	0.029	
Inulin	63.28 ^B	73.43 ^A	2.36	

Table 2. Chemical constituent composition (%) of garlic and commercial inulin powders

^{A-B} Values in the same row with different superscripts differ significantly (p < 0.05).

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⁶⁶³ ¹Standard error of mean (n=10).

665	Table 3. Proximate composition of chicken sausages as affected by different sources and
666	levels of inulin.

Constituents	Formulations ¹							SEM ²
(%)	Control	GI 1%	GI 2%	GI 3%	CI 1%	CI 2%	CI 3%	-
Fat	13.67 ^A	12.89 ^{AB}	9.47 ^c	4.85 ^D	11.97 ^B	8.12 ^C	4.47 ^D	0.779
Moisture	63.33 ^D	66.11 ^{CD}	67.10 ^{BC}	69.74 ^B	69.20 ^{BC}	73.59 ^A	74.30 ^A	0.846
Ash	1.01 ^C	1.12 ^C	1.17 ^{BC}	1.20 ^{BC}	1.19 ^{BC}	1.38 ^B	2.24 ^A	0.087
Protein	9.65 ^c	9.97 ^{BC}	10.24 ^{ABC}	10.76 ^A	10.09 ^{ABC}	10.24 ^{ABC}	10.44 ^{AB}	0.086

^{A-D} Values in the same row with different superscripts differ significantly (p < 0.05).

⁶⁶⁸ ¹GI- Garlic inulin; CI- Commercial inulin.

⁶⁶⁹ ²Pooled standard error of mean (n=21).

670

Table 4. Effect of different inulin sources on the pH value of low-fat chicken sausages
during storage.

673

Period	Formulations			SEM ¹
	Control	Garlic inulin	Commercial inulin	
Day 1	6.18 ^{Ay}	6.36 ^{Ax}	6.16 ^{Ay}	0.032
Day 7	6.14 ^{Ay}	6.34 ^{Ax}	6.05 ^{Az}	0.043
Day 14	6.12 ^{Ay}	6.24 ^{ABx}	6.04 ^{Az}	0.029
Day 21	6.10 ^{ABx}	6.12 ^{BCx}	6.02 ^{Ay}	0.016
Day 28	5.96 ^{Bx}	6.10 ^{Cx}	6.00 ^{Ax}	0.045
SEM ²	0.023	0.030	0.022	

⁶⁷⁴ ^{A-C} Values in the same column with different superscripts differ significantly (p < 0.05).

^{x-z}Values in the same row with different superscripts differ significantly (p<0.05).

⁶⁷⁶ ¹Pooled standard error of mean (n=9).

⁶⁷⁷ ²Pooled standard error of mean (n=15).

678

Table 5. Effect of different inulin sources on the WHC (%) of low-fat chicken sausages
during storage.

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Period	Formulations			SEM ¹
	Control	Garlic inulin	Commercial inulin	SEM
Day 1	91.83 ^{Ax}	91.64 ^{Ax}	90.42 ^{Ax}	0.624
Day 7	87.30 ^{ABx}	86.18 ^{Bx}	86.50 ^{Bx}	0.538
Day 14	85.43 ^{BCx}	83.57 ^{Bx}	83.50 ^{BCx}	0.451
Day 21	81.04 ^{CDx}	78.80 ^{Cx}	80.96 ^{Cx}	0.555
Day 28	78.14 ^{Dx}	76.04 ^{Cx}	77.24 ^{Dx}	0.636
SEM ²	1.371	1.494	1.245	

^{A-D} Values in the same column with different superscripts differ significantly (p < 0.05).

⁶⁸³ ^{x-z} Values in the same row with different superscripts differ significantly (p<0.05).

 1 Pooled standard error of mean (n=9).

 2 Pooled standard error of mean (n=15).

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Table 6. Effect of different inulin sources on the CIE colour values of low-fat chicken
 sausages during storage.

Period	Formulations			SEM ¹
	Control	Garlic inulin	Commercial inulin	012101
CIE L*				
Day 1	68.87 ^{Ax}	65.75 ^{Ay}	65.97 ^{Ay}	0.513
Day 7	68.30 ^{Ax}	65.41 ^{Ay}	65.86 ^{Az}	0.450
Day 14	65.98 ^{Bx}	63.26 ^{By}	65.74 ^{Ax}	0.450
Day 21	65.49 ^{Bx}	63.17 ^{By}	65.50 ^{Ax}	0.404
Day 28	64.73 ^{Cy}	63.18 ^{Bz}	65.21 ^{Ax}	0.308
SEM ²	0.436	0.315	0.121	
CIE a*				
Day 1	11.51 ^{Cy}	12.07 ^{Cx}	11.73 ^{Cxy}	0.104
Day 7	12.61 ^{Bx}	12.71 ^{Bx}	12.73 ^{Bx}	0.028
Day 14	14.09 ^{Ax}	13.55 ^{Ay}	12.88 ^{Bz}	0.181
Day 21	14.24 ^{Ax}	13.60 ^{Ay}	13.67 ^{Ay}	0.108
Day 28	14.26 ^{Ax}	13.67 ^{Ay}	13.86 ^{Axy}	0.099
SEM ²	0.297	0.172	0.206	

CIE	b*
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Day 1	32.71 ^{Cx}	32.75 ^{Cx}	33.31 ^{Cx}	0.140
Day 7	33.83 ^{By}	33.46 ^{By}	34.34 ^{Bx}	0.137
Day 14	36.45 ^{Ax}	34.83 ^{Ay}	34.58 ^{By}	0.299
Day 21	37.13 ^{Ax}	34.85 ^{Ay}	36.93 ^{Ax}	0.382
Day 28	37.35 ^{Ax}	34.91 ^{Ay}	37.27 ^{Ax}	0.413
SEM ²	0.507	0.245	0.419	

⁶⁸⁹ ^{A-C} Values in the same column with different superscripts differ significantly (p < 0.05).

⁶⁹⁰ ^{x-z} Values in the same row with different superscripts differ significantly (p < 0.05).

⁶⁹¹ ¹Pooled standard error of mean (n=9).

⁶⁹² ²Pooled standard error of mean (n=15)

	Formulations		
Control	Garlic inulin	Commercial inulin	SEM ¹
0.056 ^{Cx}	0.039 ^{Ey}	0.036 ^{Cy}	0.003
0.071 ^{Cx}	0.053 ^{Dy}	0.046 ^{Cz}	0.004
0.136 ^{Bx}	0.088 ^{Cy}	0.089 ^{By}	0.009
0.171 ^{Bx}	0.130 ^{Bxy}	0.099 ^{By}	0.012
0.226 ^{Ax}	0.154 ^{Ay}	0.145 ^{Ay}	0.014
0.017	0.012	0.011	
	0.056 ^{Cx} 0.071 ^{Cx} 0.136 ^{Bx} 0.171 ^{Bx} 0.226 ^{Ax}	$\begin{array}{cccc} 0.056^{Cx} & 0.039^{Ey} \\ 0.071^{Cx} & 0.053^{Dy} \\ 0.136^{Bx} & 0.088^{Cy} \\ 0.171^{Bx} & 0.130^{Bxy} \\ 0.226^{Ax} & 0.154^{Ay} \end{array}$	$\begin{array}{c ccccc} 0.056^{Cx} & 0.039^{Ey} & 0.036^{Cy} \\ \hline 0.071^{Cx} & 0.053^{Dy} & 0.046^{Cz} \\ \hline 0.136^{Bx} & 0.088^{Cy} & 0.089^{By} \\ \hline 0.171^{Bx} & 0.130^{Bxy} & 0.099^{By} \\ \hline 0.226^{Ax} & 0.154^{Ay} & 0.145^{Ay} \\ \end{array}$

Table 7. Effect of different inulin sources on TBARS values (MDA mg/kg) of low-fat
 chicken sausages during storage.

⁶⁹⁵ ^{A-E} Values in the same column with different superscripts differ significantly (p < 0.05).

⁶⁹⁶ ^{x-z} Values in the same row with different superscripts differ significantly (p<0.05).

⁶⁹⁷ ¹Standard error of mean (n=9).

 698 ²Standard error of mean (n=15).

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