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Effect of pumpkin seed powder on the chemical and bacteriological properties of refrigerated aged chicken cuts

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Abstract

This study was conducted to evaluate the effect of adding 5% pumpkin seed powder (PSP) per kg of meat on the chemical and bacteriological characteristics of aged Isa Brown chicken meat. Fifty-one 70-week-old female birds, slaughtered according to Islamic rites, were used. Breast and thigh cuts were taken and divided into three groups: the first group (negative control, T1) was plucked and skinned (without hot water); the second group (positive control, T2) was plucked and skinned using scalding water; and the third group (T3) was treatment 1 but the cuts were mixed with 5% PSP per kg. The treatments were compared after 1 and 7 days of storage at 4°C. The results showed a significant decrease in pH values ($P \leq 0.05$) in the breast and thigh samples of treatment T3 after 1 and 7 days of storage. It was also observed that moisture, protein, and ash content showed a significant increase ($P \leq 0.05$) in samples treated with 5% pumpkin seed powder compared to control samples. Similarly, treatment of cuts with pumpkin seed powder showed a significant decrease in fat content ($P > 0.05$). Biological testing showed a significant decrease in colony formation (CFU) of total aerobic and chemoreceptor bacteria in the T3 treatment compared to the control groups (T1 and T2). Overall, the results indicate that pumpkin seed powder preserved the quality of poultry meat during refrigerated storage, thus extending shelf life and reducing spoilage. Therefore, natural preservatives can be used to improve meat quality and safety, meeting consumer demand for healthy and sustainable meat.

Keywords: *Older chickens, cucurbits, cold-loving bacteria, food spoilage*

1. Introduction

Chicken meat is one of the most important sources of animal protein with high nutritional value, due to its easy digestibility, high content of essential amino acids needed by the body, and low-fat content compared to some red meats. Despite the widespread consumption of chicken meat, utilizing older hens after their egg-laying period is an important step in enhancing food security and reducing economic waste (Choe and Kim 2020; Jayasena et al. 2013) [1, 2].

Due to their long rearing cycle, exhausted laying hens have tough meat with a high protein content, low fat content, and are rich in beneficial omega-3 polyunsaturated fatty acids. These characteristics give them unique nutritional value and high economic value (Ezugwu et al. 2023) [3]. Given the increasing global consumption and demand for poultry meat, processed chicken meat, including exhausted chicken meat from hens that have reached the end of their egg-laying life, is among the most consumed products (Zhu et al. 2022) [4]. Tenderizing the meat of laying hens after the end of their productive life is essential to improve its commercial value and consumer acceptance. Therefore, a number of diverse tenderizing strategies are employed by the meat industry, ranging from mechanical and chemical to biological (Ezugwu et al. 2023) [3]. One such strategy involves the use of cucumycin, a serine protease extracted from fruits of the Cucurbitaceae family, which includes pumpkin, melon, cucumber, cantaloupe, and watermelon. This plant proteolytic enzyme is characterized by its high pH and temperature tolerance and oxidative stability (Antão and Malacata 2005) [5]. Koltsov and Danilin (2021) [6] indicated that pumpkin seeds, as a byproduct of pumpkin cultivation, are characterized by their mineral and essential oil content. Several varieties were studied, and their chemical composition was determined. Sodium content ranged from 5.07 to 17.6 mg/100g, potassium from 5.04 to 7.07 mg/100g, calcium from 1.56 to 1.59 mg/100g, magnesium from 1.25 to 3.23 mg/100g, iron from 6.9 mg/100g, copper from 0.38 to 0.69 mg/100g, and zinc from 4.8 to 7.4 mg/100g. Some varieties also contained a high percentage of linoleic acid (52 to 63%), followed by oleic acid (11.7 to 27.3%). Overall, the results suggest that pumpkin seeds are important in the production of functional foods and biologically active additives. Butternut squash has been found to contain phenolic compounds and antioxidants that contribute to reducing oxidative stress in the body. The use of dried pumpkin seed powder as a preservative in chicken meat pieces has been explored and found to extend shelf life up to 12 days (Rani et al. 2024; Kamiloglu et al. 2024) [7, 8]. This study aimed, therefore, to evaluate the effect of adding 5% pumpkin seed powder (PSP)/kg of meat on the chemical and bacteriological characteristics of aged Isa Brown chicken meat [7, 8]. This study aimed, therefore, to evaluate the effect of adding 5% pumpkin seed powder (PSP)/kg of meat on the chemical and bacteriological characteristics of aged Isa Brown chicken meat.

2. Materials and Methods

Sample Collection and Preparation

The study was conducted in accordance with the Tikrit University Research Policy Guidelines on Animal Ethics and Welfare and was approved by the Animal Experiments Committee under serial number [AgTUA-027-2026]. Female birds of the species *Isa Brown* were purchased from the Sahari Karbala Poultry Company. (51) birds (70 weeks old) were used. They were slaughtered according to Islamic rites, and the breast and thigh meat were taken and divided into three treatments, with 10 pieces of breast meat and 10 pieces of thigh meat allocated to each treatment. the first group (negative control, T1) was plucked and skinned (without hot water); the second group (positive control, T2) was plucked and skinned using scalding water; and the third group (T3) was treatment 1 but the cuts were mixed with 5% PSP per kg. The treatments were compared after 1 and 7 days of storage at 4°C.

Preparation of Butternut Squash Seed Pulp Powder

Butternut squash fruits were obtained from a private farm in Samarra, Salah al-Din Governorate. Mature fruits with a firm skin and a uniform orange color were selected, and the fruits were free from cracks and mold. The seeds were collected and washed, then dried in a hot air dryer at 45-50°C until a final moisture content of 4-6%. The seeds were then peeled by hand, and the pulp was washed to remove any remaining skins. The pulp was then oven-dried at 40°C until completely dry, and finally ground into a fine powder using a laboratory mill. The powder was stored in airtight containers away from sunlight.

Study's measurements

Biochemical indicators:

The pH of the pectoral and thigh muscles was determined individually (Xiong et al., 1993) [9]. Five grams of synthesized minced meat were used in 100 ml of distilled water, and the pH of a 10 ml sample was measured after 5 minutes of rest using a Chinese-made Biobase pH meter. The moisture content of the samples was also determined according to the American Society of Agricultural Chemistry (AOAC, 2008) guidelines [10]. Clean, pre-dried flasks of known weight were used. Ten grams of the homogenized sample were placed in each flask and dried in a convection oven at 100°C until the weight stabilized. The ash content was determined according to the method described in A.O.A.C. (2008) [10]. Five grams of the sample were used in a pre-dried ceramic bowl of known weight, which was then dried in an electric oven at 100°C to remove moisture. The bowl was subsequently transferred to an incineration furnace at 600°C until a white or gray powder was obtained.

The protein content was also estimated using the Kjeldahl method for determining total nitrogen (A.O.A.C. (2008) [10]). Two grams of the sample were placed in a digestion tube with 10 mL of 95% sulfuric acid and two drops of perchloric acid (HClO4). Digestion continued until the solution became clear. The molecule was then distilled by adding 10 mL of 0.1 N sodium hydroxide. The released ammonia was collected in a 50 mL flask containing 25 mL of 2% boric acid, two drops of Bromocresol Green indicator, and Methyl Red indicator. This mixture was then quenched with 0.05 N HCl. The amount of acid required to change the indicator color from green to red was calculated, and the crude protein content was calculated using the following equation:

$$\text{Protein\%} = \frac{HCl\ consumed\ (ml) \times Normality\ (0.05) \times 0.014 \times 6.25}{sample\ weight\ (g)} x100$$

Given that 6.25 is the nitrogen coefficient/100g of protein

The indicators also included estimating the fat percentage in frozen and fresh meat samples according to the AOAC (2008) method [10] using a Soxhlet apparatus. One gram of dried, ground meat was weighed and placed in a thimble of the apparatus (of a known weight). 200 ml of the solvent (diethyl ether) was added, and the extraction process lasted approximately 4-6 hours. The solvent was collected from the apparatus, the thimble was removed, and the sample was placed in an electric oven for 30 min at 80°C to remove any remaining solvent from the flask. The dried thimble was then weighed after cooling, and the fat percentage was calculated using the following equation:

$$\text{Fat\%} = \frac{burn\ bowl\ weight\ (post\ extraction - before\ extraction)}{sample\ weight\ (g)} x100$$

Experimental Design and Statistical Analysis

Treatments were distributed using a two-factor (2×2) experiment implemented with a completely randomized design (CRD) with 3 replicates. Significant differences between means were compared using Duncan's (1955) polynomial test [11]. The SAS 2012 software was used for statistical analysis of the data according to the following mathematical model: *Yijk= m+ti+pj+ tp(ij)+eijk* Where:

Yijk = Observational values K for treatment i and item j

μ = Overall mean of the studied trait

Ti = Effect of treatment (since the experiment included the effect of 2 treatments)

Pj = Effect of storage period (since the experiment included the effect of 2 storage periods)

tp(ij) = Interaction between treatment and storage period

eijk = Normally distributed random error with a mean of zero power e ≤ 2

Bacteriological assay

The biological test included estimating the total number of aerobic bacteria. One milliliter of the fifth dilution ($1x10^{-5}$) treatment was transferred to sterile Petri dishes. Nutrient agar medium was added, mixed, and allowed to solidify. The dishes were incubated upside down at 35°C for 24 hours. Afterward, the colonies of the growing bacteria were counted using a colony-counting slide [12]. The total number of cryophilic bacteria was also estimated, as one milliliter of the well-diluted samples was placed in sterile glass Petri dishes. This was then mixed with nutrient agar medium. After mixing, the dishes were allowed to solidify and incubated upside down at 4°C for 72 hours. The colonies were counted using a colony-counting device, as previously described [12].

3. Results and discussion

All Table (1) 4°C shows that the pH value was 6.54 and 6.81 in the refrigerated control samples T1 and T2, with a significant decrease (P≤0.05) in the pH of the T3 brisket samples. Refrigerated thigh meat showed significant differences (P≤0.05) between treatments, particularly after seven days of storage at 4°C, reaching 6.17, 6.86, and 5.91 for samples T1, T2, and T3, respectively. A significant decrease (P ≤ 0.05) was observed in meats treated with 5% pumpkin seed powder. This is likely attributed to the pumpkin's ascorbic acid content and the presence of terminal acid residues in glycogen molecules (Perez, 1997) [13]. It is evident that the presence of proteolytic enzymes in pumpkin seeds leads to the release of amino acids, resulting in a change in pH, as well as the breakdown of some glycogen and the formation of lactic acid due to the activity of ATP-ase enzymes. These results are consistent with those of Serdaroğlu et al. (2018) [14], who tested four different beef patty formulations. In these tests, lean meat was replaced with a pulp and seed mixture at 0%, 2%, 3%, and 5%. The pH values of the beef patties ranged from 5.67–5.71 and 5.86–5.92 for the raw and cooked samples, respectively. Therefore, the acidity and alkalinity of the raw materials incorporated into meat products are crucial for the final pH value and, consequently, the functional properties of the product.

Table1. Effect of treatment with pumpkin seed powder on pH and moisture content in aged chicken cuts after 1 and 7 days of cold storage at 4°C

Treatment	Body part Tested	pH		Moisture %	
		Day 1	**Day 7**	**Day 1**	**Day 7**
T1	**Breast**	6.56 ±0.025 a	6.54 ±0.010 a	73.497 ± 0.028 a	73.782 ± 0.016 a
	Thigh	6.72 ±0.033 a	6.17 ±0.043 b	71.187 ± 0.011 b	72.099 ± 0.040 b
T2	**Breast**	6.81 ±0.015 a	6.72 ±0.030 a	75.307 ± 0.037 a	75.385 ± 0.031 a
	Thigh	6.86 ±0.041 a	6.74 ±0.036 a	72.213 ± 0.021 b	72.357 ± 0.033 b
T3	**Breast**	5.78 ±0.040 b	5.64 ±0.045 b	74.999 ± 0.026 a	75.117 ± 0.024 a
	Thigh	6.01 ±0.040 b	5.91 ±0.032 b	72.015 ± 0.027 b	72.005 ± 0.022 b

*Treatments T1, T2 and T3 are for negative control where chicken plucked and skinned (without hot water), positive control as they were plucked and skinned using scalding water, and T3 the cuts were mixed with 5% PSP/ kg, respectively. Values that are followed by same letter(s) within a column are not different according Duncan's multiple range test (P≤0.05)

Regarding the effect of the factors on the product's moisture content, the results (Table 1) show that the refrigerated breast samples recorded a moisture content of 73.497% ± 0.028% in treatment T1 (manual feather and viscera removal without scalding), compared to a higher moisture content of 75.307% ± 0.037% in treatment T2, where hot water (53°C) was used for feather removal. The treatments did not differ during the storage periods. Breast meat samples (T3) containing pumpkin seed pulp powder recorded moisture content of 74.999% and 75.117% after one day and seven days of refrigerated storage, respectively, with no difference in the other treatments. The moisture content of the chicken thigh samples (Table 1) recorded lower levels than those recorded in the breast samples, with values of 71.187% and 72.015% in control T1 and T3, respectively. Rani et al. (2024) [7] indicated that adding pumpkin seed powder and chia seed powder to chicken meat increased moisture content and improved stability during storage due to the plant material's fiber and protein content and its ability to retain water. It was also observed that the moisture content of chicken meat stored in the refrigerator for 1, 7, and 1 days was 72.94%, 72.04%, and 70.72%, respectively (Al-Jubouri 2020) [15].

results indicate that the protein percentage in chicken breast samples showed significant differences (P≥0.05) between treatments, with the lowest value in treatment T1 (20.01%) compared to the highest value in T3 (22.02%) after one day of storage. T3 also recorded the highest protein percentage (22.20%) after seven days of storage (Table 2). Meanwhile, the protein percentage in thigh meat ranged between 17.55% and 18.85% for samples T1 and T3, respectively. The protein percentage in meat from older poultry treated with pumpkin seed powder was consistently higher than in the other treatments, regardless of the cleaning method (hot or cold). [17].

Table2. Effect of treatment with pumpkin seed powder on the protein and fat content in aged chicken cuts after 1 and 7 days of cold storage at 4°C

Treatment	Body part Tested	Protein %		Fat %	
		Day 1	**Day 7**	**Day 1**	**Day 7**
T1	**Breast**	20.01 ± 0.050 b	20.55 ± 0.050 b	2.00 ± 0.000 ab	2.15 ± 0.050 a
	Thigh	17.05 ± 0.044 a	17.55 ± 0.051 b	4.10 ± 0.100 c	4.35 ± 0.050 bc
T2	**Breast**	21.39 ± 0.052 b	20.45 ± 0.050 b	1.89 ± 0.006 b	2.05 ± 0.051 ab
	Thigh	17.50 ± 0.050 b	17.85 ± 0.041 ab	3.00 ± 0.000 d	3.35 ± 0.050 d
T3	**Breast**	22.02 ± 0.057 ab	22.20 ± 0.055 a	0.35 ± 0.002 d	0.40 ± 0.003 d
	Thigh	18.0 ± 0.053 a	18.85 ± 0.055 a	7.00 ± 0.000 a	7.35 ± 0.051 a

*Treatments T1, T2 and T3 are for negative control where chicken plucked and skinned (without hot water), positive control as they were plucked and skinned using scalding water, and T3 the cuts were mixed with 5% PSP/ kg, respectively. Values that are followed by same letter(s) within a column are not different according Duncan's multiple range test (P≤0.05)

These results are consistent with those of de Melo et al. (2024) [16], where the use of pumpkin seed flour at a rate of 5% and 10% w/w improved the nutritional parameters of beef burgers, with protein levels of 18.04% and 19.86% respectively, compared to the control group's 17.61%. Similarly, the addition of sumac seed powder to broiler feed at a rate of 2 g/kg feed resulted in an increase in protein content to 21.30% (Hasan et al. (2024).

The results showed that the fat percentage in chicken breast samples was 2.00% in T1 and 1.89% in T2. However, the fat percentage in chicken breast meat in treatment T3 decreased significantly (P≤ 0.05) to 0.35 and 0.40 after one day and seven days of storage, respectively. As for thigh meat, there were significant differences (P≥ 0.05) between treatments T1 and T2, with values of 4.35 and 3.35. Fat levels increased in treatment T3, reaching 7.00 and 7.35 after one and seven days of storage, respectively. It is noted that the study's results partially agree with Yüncü-Boyacı et al. (2025) [18], who found that chicken meat products treated with pumpkin seed powder showed a decrease in fat content. This suggests that the dietary fiber in the powder reduces the percentage of fat in chemical analysis. On the other hand, the addition of rosemary and carnosic acid to chilled minced beef led to a significant reduction (p>0.05) in fat percentage compared to the control group (Al-Alwani, 2017) [19]. Ali et al. (2019) also showed that fish burgers prepared with mashed pumpkin and mashed potatoes had a lower fat percentage than the control groups.

Table 3, which deals with the Regarding the effect of treatments on ash content, it was observed that chicken breast samples treated with pumpkin seed powder (T3) showed a significant increase (P ≤ 0.05) in ash content to 1.65%, compared to T1 (1.15%) and T2 (1.35%), after one day of treatment. At seven days, T3 also recorded the highest ash content (1.55%). Similarly, the results showed that thigh samples exhibited the highest ash content in treatment T3, reaching 2.02% and 2.09% after 1 and 7 days of refrigerated storage, respectively, a significant difference compared to the other treatments (Table 3). The significant increase (P < 0.05) in ash content in meat treated with pumpkin seed powder is attributed to the direct contribution of the minerals present in the seed powder and the relative dilution effect resulting from the reduced fat content. Improved water retention capacity contributed to increased retention of water-bound minerals and protein within the muscle tissue, which was reflected in the higher ash content compared to the control sample. The high content of pumpkin seeds is also attributed to sodium (5 mg), calcium (5.6 mg), iron (0.938 mg), magnesium (74.3 mg), phosphorus (26.1 mg), and copper (0.19 mg). These results are consistent with a study by Serdaroğlu et al. (2018) [14], which observed that cooked beef patties treated with pumpkin seed pulp powder had a higher ash content compared to the control (p<0.05), likely due to the high mineral content of the pumpkin mixture. The high mineral content of pumpkin pulp, which increases the ash content in meat, may contribute to treating deficiencies in certain minerals (iron, zinc, selenium, iodine, copper, chromium, and manganese) that play vital roles in the body, particularly thyroid metabolism, antioxidant activity, and immune system function (Vural et al., 2020) [21].

Table3. Effect of treatment with pumpkin seed powder on chicken cuts content of ash after 1 and 7 days of cold storage at 4°C

Treatment	Body part tested	Ash %	
		Day 1	**Day 7**
T1	**Breast**	1.15 ±0.050 a	1.15 ±0.058 a
	Thigh	1.25 ±0.050 a	1.35 ±0.050 a
T2	**Breast**	1.35 ±0.058 b	1.35 ±0.058 b
	Thigh	1.50 ±0.051 a	1.58 ±0.052 b
T3	**Breast**	1.65 ±0.052 a	1.55 ±0.057 a
	Thigh	2.02 ±0.053 b	2.09 ±0.051 b

*Treatments T1, T2 and T3 are for negative control where chicken plucked and skinned (without hot water), positive control as they were plucked and skinned using scalding water, and T3 the cuts were mixed with 5% PSP/ kg, respectively. Values that are followed by same letter(s) within a column are not different according Duncan's multiple range test (P≤0.05)

Bacteriological assay

Table (4) shows that the total bacterial count in breast meat was relatively high in treatment (T1), which recorded (6.55) bacteria/100 g of meat after one day of refrigerated storage, compared to significantly lower counts in treatment T2 (hot water cleaning), which recorded bacterial counts of (6.38-6.07) bacteria/100 g after 1 and 7 days, respectively. Meanwhile, the average total bacterial count decreased in treatment (T3) in meat treated with pumpkin seed powder, recording 5.90 and 5.60 after 1 and 7 days of refrigerated storage. This is attributed to the cooling effect, which shocks some thermophilic and moderately warm bacteria. It was also observed that in the leg meat of treatments T1 and T2, bacterial counts were recorded at 6.57 and 6.16 bacteria per 100 grams of meat, respectively, after 1 and 7 days. This was significantly higher than in treatment T3, where the bacterial counts did not exceed 6.17 and 6.07 bacteria per 100 grams of meat for the same storage periods. The marked decrease in the total bacterial count in the brisket and leg meat treated with 5% pumpkin seed powder is likely due to the powder's content of phenolic compounds and active ingredients with antimicrobial activity. Additionally, the powder lowered the pH and improved water retention, thus reducing the availability of free water necessary for bacterial growth. Furthermore, the antioxidant properties of pumpkin seed powder contribute to slowing down fat breakdown, creating a less favorable environment for microbial growth compared to the control sample. These results confirm previous studies (SOHAIL and MASUD 2014) [22] where Cucurbita pepo seed oil showed high nutritional components, including proteins, minerals and unsaturated fatty acids, especially linoleic acid and oleic acid, in addition to its inhibitory efficacy against Gram-positive Staphylococcus aureus bacteria.

Table4. Effect of treatment with pumpkin seed powder on total bacterial count of aerobic and cryophilic bacteria in aged chicken cuts after 1 and 7 days of cold storage at 4°C

Treatment	Body part	Aerobic bacteria (CFU g^{-1})		Cryophilic bacteria (CFU g^{-1})	
	Tested	**Day 1**	**Day 7**	**Day 1**	**Day 7**
T1	**Breast**	6.55 a	6.20 bc	6.50 a	5.50 b
	Thigh	6.57 a	6.23 b	6.50 a	5.69 d
T2	**Breast**	6.38 b	6.07 cd	6.30 b	5.30b
	Thigh	6.50 a	6.16 b	6.30 b	5.42 de
T3	**Breast**	5.90 d	5.60 e	5.30 b	5.06 c
	Thigh	6.17 bc	6.07 bc	6.00 c	5.10 e

*Treatments T1, T2 and T3 are for negative control where chicken plucked and skinned (without hot water), positive control as they were plucked and skinned using scalding water, and T3 the cuts were mixed with 5% PSP/ kg, respectively. Values that are followed by same letter(s) within a column are not different according Duncan's multiple range test (P≤0.05)

Regarding the effect of treatments on the number of cryophilic bacteria, the results (Table 4) indicate that the number of cryophilic bacteria in breast meat was highest in treatments T1 and T2, ranging from 6.50 to 5.30 bacteria/100g of meat, compared to lower numbers in treatment T3, which recorded 5.30 and 5.06 CFU/100g of meat after 1 and 7 days of storage, respectively. Thigh meat showed cryophilic bacteria numbers ranging from 6.50 to 6.30 CFU/100g of meat in treatments T1 and T2, which decreased after 7 days to 5.69 and 5.42 CFU/100g, respectively. A significant decrease was also recorded in treatment T3 (5% pumpkin seed pulp powder), which recorded 6.00 and 5.10 CFU/100g after 1 and 7 days, respectively.

These results confirm what was stated in the study (Amin et al. 2018) [23] which showed that the oil extracted from the seeds of the local pumpkin (Cucurbita maxima Linn.) showed antibacterial activity against E. coli and Shigella sonnei. They also confirm the crucial role of good cooling, slaughter hygiene, and storage conditions, especially the effectiveness of the logistics cold chain, in controlling microbial contamination (Musa et al. 2024) [25].

4. Conclusion

The results showed that pumpkin seed powder demonstrated a clear ability to lower the pH value in the treated aged chicken meat compared to the absence of the powder. This indicates that pumpkin seed powder possesses active compounds with an acidifying and antimicrobial effect, which positively impacts meat stability and quality. There was an increase in moisture content in the pumpkin seed powder treatment, attributed to increased water retention resulting from the interaction of phenolic compounds and plant fibers with meat proteins. This led to improved functional properties of the aged chicken meat. The addition of pumpkin seed powders also increased the crude protein content, demonstrating the contribution of the powder's plant protein components and its role in reducing protein loss during processing and curing. The study recorded a higher ash content in the treatments containing pumpkin seed powder, indicating the powder's richness in minerals such as zinc, magnesium, and iron, which may contribute to enhancing the nutritional value of the treated meat. Pumpkin seed powder also resulted in a lower fat content compared to the treatments that did not include it. This is attributed to the potential effect of the seed components in inhibiting fat deposition or the binding of its fibrous components to fat, thus reducing its proportion in the meat. The positive changes in the characteristics of the meat samples indicate that pumpkin seed powder could be a promising natural additive for improving the quality and nutritional value of aged chicken meat while reducing reliance on artificial additives. This study confirms the potential to utilize spent laying hens—those after their egg-producing lifespan and transform them into a product of improved quality and higher marketability using natural plant-based additives, thereby contributing to reducing economic waste and promoting sustainability in the food industry.

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