Encapsulation of Gac Powder Extract and Its Application in Low-Nitrite Chicken Sausage

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***Abstract*—The aril and pulp of gac fruit were vacuum dried at 60°C prior to extract with ethanol using microwave assisted method. The extract was encapsulated with maltodextrin using spray drying techniques. The inlet temperatures were varied from 140, 160, and 180 °C, while the ratio of extract:Maltodextrin solution were varied at 1:5, 1:10, and 1:20. The moisture content, water activity, lycopene content, and radical scavenging ability were monitored to optimize the drying condition at inlet temperature of 160 °C and the ratio was at 1:5. The encapsulated gac powder extract (EGPE) at this condition was used as incorporated in chicken sausage prepared at low-nitrite level (25ppm) prior evaluating the quality. The results showed that EGPE could reduce cooking yield slightly. However, it could improve the redness and yellowness of sausage as well as maintain this characteristic during storage at 10 °C for a week. EGPE could also inhibit microorganisms indicated by a reduction of total plate count. Moreover, the lipid oxidation of sausage, determined by TBARS value, was also retarded when EGPE was applied. These results suggested that EGPE could be functional ingredient in chicken sausage at low nitrite level.**

***Index Terms*—Gac powder extract, encapsulation, Low-nitrite sausage, antioxidant, lycopene**

I. Introduction

Gac (*Momordica cochinchinensis spreng*) is an indigenous plant available in tropical countries especially Southeast Asia, including Vietnam, Thailand, China, and India. Its fruit is harvested when the skin turned to be orange or red in normally autumn to winter [1]. Gac fruit has numerous bioactive compounds contributing to health benefit. It is utilize as natural colorant in Asian-traditional dishes, especially in Vietnamese culture. The fruit can be separated into 4 parts (peel, pulp, aril, and seed). The aril part of fruit is the most attractive due to high carotenoids contents [2]. In addition, high fatty acid content in aril seemed to be interested since high essential fatty acid content e.g. oleic acid and linoleic acid were found in aril oil [3]. Extraction of aril oil from gac fruit has been done by either cold press or solvent extraction. These methods could recover both carotenoids and fatty acids. This fraction has also been processed and commercialized in different forms e.g. frozen gac aril or gac oil capsule, and dried gac powder [4]. However, aril constitutes only a low proportion compare to other parts. Therefore, seeking for bioactive compounds from other part is worth trying to maximally utilize gac resource. It has been reported that the pulp of gac contains a considerable number of carotenoids and should be processed together with aril part [5]. In addition, there are other phenolic compounds presenting in the pulp of gac fruit [2]. Those compounds were identified as flavnoids, phenolic acid, and hydroxycinnamic acid. Therefore, extraction of active compounds from both aril and pulp of gac fruit could be the alternative choice to maximally utilize the gac resource for being functional ingredients in food and related industries. However, there is no study documented regarding processed gac aril and pulp into dried powder.

Extraction of active compounds from gac fruit fraction has been done in different ways. The common solvents, water and hexane, were applied in order to get gac oil from aril part. However, extraction of the pulp would be more effective when ethanol was applied. In addition, the extraction process will be shorter when microwave assisted technology was introduced. Therefore, extraction of aril and pulp of gac fruit would be the promising process to get the active compounds for further application.

It has been reported that both fatty acid and carotenoids in gac fraction are also susceptible to degradation from oxidation, which accelerated from heat and light. Therefore, keeping the extract in the powder would be the alternative way to stabilize these compounds during storage. Several drying techniques were applied and those techniques play different roles on maintaining the carotenoids contents. It was noted that the loss of carotenoids content was depended on how high of the temperature [6]. Particularly, spray drying is suitable for processing the gac fruit extract into dried powder but the loss of bioactive compound was documented at high temperature (> 200°C). Fortunately, application of maltodextrin as the wall material was found to be prevented to degradation of carotenoid during encapsulation of gac fruit aril. However, encapsulation of aril and pulp of gac fruit with maltodextrin using spray drying has not been performed. In addition, the optimum condition for encapsulation should be optimized. In addition, utilization of encapsulated gac fruit extract in meat product is feasible to retard lipid oxidation as well as showing potential to be natural colorant and antimicrobial agent in low nitrite sausage.

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Normally, nitrite was added into sausage for developing of desirable color through the formation of nitrosyl hemochrome. The reaction between nitrite and fat or protein in meat product also resulted in desirable flavor. Moreover, inhibition of microbial growth and also prevention of toxin production of some microorganism (*Clostridium botulinum*) by nitrite is well known [7]. However, high amount of nitrite left over in meat product may also generate the toxic substance, namely nitrosamine. This compound is recognized as the carcinogen, which showing the potential to induce cancer. Therefore, the limited amount of nitrite in meat product is regulated. According to codex regulation, the residue nitrite in meat product is limited at only 85 ppm. Therefore, the limited amount of nitrite may not enough to perform all functions and alternative ingredient would be search out for synergistic function at the low level of nitrite.

Therefore, the aim of this study was to optimize the condition for encapsulation of gac aril and pulp extract with maltodextrin using spray drying. In addition, the feasibility to applied this encapsulated gac extract in low nitrite chicken sausage was also evaluated.

II. Materials and Methods

A. Materials

Gac fruits were collected as the ripened one from Nong Khai Province, Thailand. The fruits were cleaned and collected only the aril and pulp as gac fruit sample. This sample was dried to be gac powder (GP) using the vacuum drier (Binder GmbH, Tuttlingen, Germany) at 60°C for 48 h until the moisture content was constant at approximately 5% and used as gac powder.

The skin and visible fat were trimmed out from chicken breast. Thereafter, the flesh one was ground before dividing into a portion of 200 g. These samples were packed in vacuum condition prior to store at -20°C until used.

B. Gac Powder Extraction and Encapsulation

Gac powder was extracted with absolute ethanol using the microwave aid technique according to the method described previously [8]. The ratio of GP: ethanol was controlled at 5:100. The household microwave (GE107Y, Samsung, Malaysia) was used to heat the mixture at 180 W for 90 s at the working frequency of 2450 MHz. The extracted mixture was centrifuged at 1,000 × g for 10 min and the supernatant was collected to filter through filter paper. The filtrate was subjected to evaporator (R-200, Buchi, Switzerland) to remove solvent until obtaining an ethanolic extract before mixing with maltodextrin solution (DE 20%) by controlling that ratio of extract:maltodextrin at 1:5, 1:10, and 1:20. In order to perform spray drying, the total solid of slurry mixture was controlled at 30.0%. The slurry was fed into drying chamber of spray dryer (B-290, Buchi, Switzerland) at the speed of 12-14 mL/min. The inlet temperatures were varied at 140, 160, and 180 °C. The drying air flow rate and compressor air pressure were controlled at 600 L/h and 4 bar, respectively. The dried powder was used as an encapsulated GP extract (EGPE). It was kept under dark condition until used.

C. Characterization of EGPE

Total carotenoid content. Sample (1 g) was dissolved in hexane (10 mL) and left at dark condition for 15 h. Then, the sample was centrifuged at 6000 xg for 20 min before determining the absorbance at 503 nm using spectrophotometer. The lycopene content was calculated according to the equation 1:

$$Lycopene\ (\frac{g}{L}) = \frac{A_{503}\times 536.9}{17.2\times 10000} \quad (1)$$

DPPH radical scavenging ability. Sample (1 g) was dissolved in hexane (10 mL) before analyzing for the DPPH radical scavenging ability. Sample solution (0.5 mL) was mixed with ethyl acetate (1.5 mL) and DPPH solution (1.5 mL) before incubating in the dark condition for 30 min. Then, the sample was read for absorbance at 515 nm (A sample). The control was prepared by taking off sample from the reaction cocktail and used as A control. The DPPH radical scavenging ability was calculated according the equation 2:

$$\text{DPPH radical scavenging ability (\%)} = \left(\frac{A\ control - A\ sample}{A\ control}\right) x100 \quad (2)$$

Moisture content.

Moisture content was determined by oven drying at 105 °C until constant weight of sample was obtained.

Water activity.

The water activity (aw) of encapsulated powder was evaluated using aw analyzer based on infrared based analysis.

D. Preparation of Low-nitrite Chicken Sausages

TABLE I. Chicken Sausage Recipe for Evaluating the Effects of Inclusion of EGPE on the Quality of Low Nitrite Sausage

Ingredients (%)	Treatments		
	CTR (25N)	25N+EGPE	REF(150N)
Chicken breast mince	60	60	60
Fat	20	20	20
Ice	18	18	18
NaCl	1.5	1.5	1.5
Sodium nitrite	0.0025	0.0025	0.0150
Sodium tripolyphosphate	0.4	0.4	0.4
EGPE	0	1	0

The model sausage of low-nitrite sausage (25 ppm nitrite) was prepared using the formulation in Table I. Effect of EGPE at 1.0% was evaluated in comparison with control (low-nitrite sausage without EGPE) and reference (150 ppm nitrite). The minced chicken breast was chopped with a quarter amount of ice for 30 s using hand-type food mixer (Bowl Rest™ mixer, Hamilton Beach, N.C., U.S.A). Salt, sodium nitrite, and ice were added before chopping for 1 min. Minced back fat and ETPE was finally added and continuing chopping for 1

min to obtain the batter with temperature below 16°C. The batter (40 g) was loaded into centrifuge tubes (50 mL) before removing trapped air by centrifugation (200 × g for 30 s). All tubes were heated at 80 °C for 30 min before cooling down in ice-water for 15 min. All cooked sausages were kept in cold room over night before determining the characteristics.

E. Analysis of Low-nitrite Chicken Sausage

1) Cooking yield

The weight of sausage after cooking was recorded. The cooking yield was calculated relative to an original weight (sample weight before cooking) and expressed as percentage.

2) *Storage stability of sausage under air package*.

Sausage samples were packed in polystyrene bag and sealed individually with air before keeping in an incubator controlled at 10°C. Sample were taken to monitor the changes of characteristics every week as described below:

3) Color

Color values of sausage were measured using the Hunter color meter (Color reader, CR-10, Minolta, Japan). Color values were reported as Hunter *L*, *a*, and *b* for lightness, redness, and yellowness, respectively.

4) Thiobarbituric acid reactive substances (TBARS).

The TBARS value was analyzed in sample without erythorbate in order to evaluate the ability of ETPE on oxidative inhibition. The homogenized sample (2 g) was mixed with 17 mL of trichloroacetic acid solution (2.5%) and 3 mL of TBA solution (1%) before heated at 90 °C for 30 min. After cooling, upper phase was taken for 5 mL to mix with 5 mL of chloroform and centrifuge at 200 g for 5 min. Then, the upper phase (3 mL) was taken to mix with petroleum ether and centrifuged again at the same condition. Finally, lower phase was taken to measure the absorbance at 532 nm using spectrophotometer (UV-1601, Shimadzu, Japan). Standard curve of malondialdehyde used to calculate the TBARS value and expressed as mg malondialdehyde/kg sample.

5) Total microbial count

Total microbial count was used to determine aerobic microorganisms in sausage sample using spread plate technique. The sausage sample was homogenized aseptically and taken (10 g) to mix with sterilized water (90 mL). Serial dilution was made before taking sample for 0.1 mL to spread on plate count agar (2 plates /dilution). All plates were incubated at 37 °C for 24 h. The colony appeared on plate was recorded as colony forming unit/ g of sample (CFU/g).

6) *Statistical analysis*

A significant difference at confidential level ($P<0.05$) was considered for mean values, which was based on the ANOVA analysis using the PASW statistics 16 (SPSS Inc., Chicago IL, USA).

III. Results and Discussion

A. Effect of Maltodextrin Ratio

The suitable ratio between extract and wall material, which is maltodextrin has been evaluated. It was found that the lycopene content in dried powder was remained at the highest level when the maltodextrin was mixed at the ratio of 1:5 (Table II). This results was slightly different from previous study, which indicated that addition maltodextrin at 10% provide the highest ability to retain either carotenoids or antioxidant activity during drying gac oil at 120° C [2]. The different might be due to different active compounds in the extract and also inlet temperature. Our results let us hypothesized that encapsulation of gac powder extract at the ratio to maltodextrin at 1:5 would be able to produce the powder with ability to inhibit lipid oxidation.

TABLE II. DPPH Radical Scavenging Ability and Lycopene Content of EGPE at Different Maltodextrin Ratio

Wall material ratio	DPPH (%)	Lycopene (μg/g)
1:5	22.15 ± 2.15^{a}	0.71 ± 0.31^{a}
1:10	15.53 ± 1.19^{b}	0.37 ± 0.17^{b}
1:20	21.15 ± 4.29^{a}	0.13 ± 0.03^{c}

Mean ± SE was averaged from 2 replications
Different letter indicates the statistical difference at $P < 0.05$

This hypothesis was confirmed by highest radical scavenging ability of sample at the ratio 1:5 as shown in the Table II. The important role of maltodextrin on protection of carotenoids during spray drying was also documented [9]. Therefore, the optimum ration between extract and maltodextrin would be 1:5 and this condition was used to optimize the effect of inlet temperature.

B. Effect of Inlet Temperature

In order to find the optimum condition for encapsulation the extract from gac fruit powder, the suitable temperature for drying was optimized by varied from 140-180°C. It has been found that the moisture content and water activity of sample decreased when the inlet temperature was increased (Table III). This suggested that higher temperature provide more rapid evaporation of water during drying. At higher inlet temperatures, there is a greater driving force for water removal from atomized feed. This is because of the high temperature difference between drying air and atomized feed. This would be explained why high temperature resulted in a reduction of moisture content. However, moisture content and water activity of sample spray dried at 160 or 180°C were not different statistically. This suggested that these temperatures provided the stable powder at the same degree.

TABLE III. Water Activity and Moisture Content of EGPE At Different Inlet Temperature

Inlet Temperature (°C)	a_w	Moisture (%)
140	0.31 ± 0.06^{a}	3.35 ± 0.29^{a}
160	0.28 ± 0.05^{b}	2.84 ± 0.43^{b}
180	0.28 ± 0.03^{b}	2.04 ± 0.10^{b}

Mean ± SE was averaged from 2 replications
Different letter indicates the statistical difference at $P < 0.05$

The data in Table IV showed the high inlet temperature resulted in a reduction of DPPH radical scavenging ability although the lycopene content in increased. This suggested that lycopene in the sample spray dried at 160 and 180 °C may not in the active form. A reduction of carotenoids after spray drying at high temperature at 200 °C was also reported [6]. It has been reported that high temperature could transform the trans-lycopene to be cis-configuration. Then, the radical scavenging ability could be reduced. In addition, radical scavenging ability of encapsulated gac powder extract may not contribute from only carotenoids but also from phenolic compounds. These, phenolic compounds could be destroyed at high temperature as well. Therefore, encapsulated extract at as low temperature as possible would be the strategy to maintain active compounds in gac powder extract. Based on the study, the suitable inlet temperature would be 160 °C when the stable material and high lycopene content as well as the radical scavenging ability. Therefore, encapsulated gac powder extract spray dried at inlet temperature 160 °C at the ratio of extract:maltodextrin at 1:5 was selected for further analysis.

TABLE IV. DPPH RADICAL SCAVENGING ABILITY AND LYCOPENE CONTENT OF EGPE AT DIFFERENT INLET TEMPERATURES

Inlet Temperature (°C)	DPPH (%)	Lycopene (μg/g)
140	21.05 ± 4.89[a]	0.26 ±0.11[b]
160	19.37 ± 4.63[b]	0.45 ±0.43[a]
180	18.77 ± 3.86[b]	0.49 ±0.36[a]

Mean ± SE was averaged from 2 replications
Different letter indicates the statistical difference at $P < 0.05$

C. Effect of EGPE on Low-nitrite Sausage

1) Cooking yield

The technical production parameter of low-nitrite sausage was evaluated from the cooking yield. This result showed that the cooking yield of sausage was slightly reduced when compare with other treatments (Table V). This suggested that inclusion of maltodextrin in the sausage may play any negative effect on emulsion forming or gelation of emulsion during cooking of sausage sample. This suggested that inclusion of encapsulated gac powder extract in emulsion sausage would be possible in the limited amount.

TABLE V. COOKING YIELD OF LOW-NITRITE SAUSAGE IN CORPORATED WITH ENCAPSULATED GAC POWDER EXTRACT

Sausage type	Cooking yield (%)
CTR (25N)	98.13±0.42[ab]
25N+EGPE	97.65±0.14[b]
REF (150N)	98.73±0.11[a]

Mean ± SE was averaged from 2 replications
Different letter indicates the statistical difference at $P < 0.05$

2) Storage stability

The storage stability of low-nitrite chicken sausage was evaluated at 10 °C in the air package, which is represented the commercial storage condition. Thereafter the qualities of sausage were evaluated.

3) Effect of EGPE on color

Generally the color of sausage is one of the most crucial attribute due to reflecting the first impression of the consumer. Moreover, the nutritional quality may also relate to the food color, especially carotenoid containing product. In this study, encapsulated gac powder extract was introduced in order to improve or maintain the color of sausage at low nitrite level. The color values including lightness, redness, and yellowness were monitored during storage and the value of lightness was shown in Table VI. It can be seen that the lightness value of sausage in gradually decreased when the encapsulated gac powder extract was added. It also showed as the lowest value compared to control and reference sample. This might be due to the presenting of dark pigment in sample. The samples were considered as bright color although the lightness of treatment is slightly lowered. During storage for one week, the lightness of treatment was still maintained at the high level suggesting the stable color upon adding food ingredient.

TABLE VI. LIGHTNESS OF CHICKEN SAUSAGE WITH EGPE AT LOW NITRITE LEVEL DURING STORAGE AT 10 °C/1 WEEK

Sausage sample	Storage time (day)	
	0	7
CTR (25N)	86.6±0.36[a]	85.3±0.07[a]
25N+EGPE	83.9±0.11[b]	82.7±0.21[b]
REF (150N)	85.9±0.05[a]	85.1±0.24[a]

Mean ± SE was averaged from 2 replications with 6 measurements
Different letter indicates the statistical difference at $P < 0.05$

The redness of sample also showed the similar trend as found in lightness. Addition of encapsulated gac powder extracted resulted in an increase of redness. The redness of sample with EGPE was also found at the higher level of reference sample although the nitrite was added for 150 ppm. This suggested that encapsulated gac powder extract could provide desirable color in sausage at even the low-level of nitrite. This would likely relate to the presenting of carotenoids, particularly lycopene encapsulated in maltodextrin as the wall material. This encapsulation technology seemed to be the effective technique to stabilize the pigment during storage since the redness of sausage was maintained at the high level for a week (Table VII). This suggested that encapsulated gac powder extract played the synergistic effect as natural colorant in sausage.

TABLE VII. REDNESS OF CHICKEN SAUSAGE WITH EGPE AT LOW NITRITE LEVEL DURING STORAGE AT 10 °C/1 WEEK

Sample	Storage time (day)	
	0	7
CTR (25N)	4.8±0.22[c]	4.8±0.10[b]
25N+EGPE	8.7±0.04[a]	8.8±0.11[a]
REF (150N)	5.1±0.15[b]	4.8±0.07[b]

Mean ± SE was averaged from 2 replications
Different letter indicates the statistical difference at $P < 0.05$

The yellowness of sausage was also evaluated as the results presented in Table VIII. It can be noticed that the yellowness of sample added with encapsulated gac powder extract showed highest value. This results were corresponded with a reduction of lightness concomitant with gradually increase in yellowness in treatment sample. The stable of yellowness was also observed as found in lightness and redness. This strongly indicated the stable

of coloring pigment in the encapsulated powder. Based on the color measurement, encapsulated gac powder extract show the potential for being natural colorant in sausage production.

TABLE VIII. YELLOWNESS OF CHICKEN SAUSAGE WITH EGPE AT LOW NITRITE LEVEL DURING STORAGE AT 10 °C/1 WEEK

Sample	Storage time (day)	
	0	7
CTR (25N)	6.9±0.09[b]	7.8±0.23[b]
25N+EGPE	20.2±0.23[a]	20.4±0.40[a]
REF (150N)	6.8±0.07[b]	8.0±0.10[b]

Mean ± SE was averaged from 2 replications
Different letter indicates the statistical difference at P < 0.05

4) The microbial count of sausage

The number of total microorganism in sausage was monitored to evaluate the shelf life of product during storage at 10 °C. Total microbial number were not detected in the first day of storage due to thermal pasteurization of sample at 80 °C over 30 min. However, this condition could not destroy all microorganisms in the sample. Therefore, the left over microbes could be growth during storage (Table IX). After keeping at cold storage for a week, it can be seen that total microorganism was counted for over 5 log cycle in the control sample (with only nitrite at 25 ppm). It can be clearly noted that addition of encapsulated gac powder extract in this sausage could reduce the number of microorganisms for one log cycle. This suggested that the encapsulated powder also showed the potential for being antimicrobial agent as well as natural colorants. The antimicrobial effect of gac fruit has been documented for either gram negative or gram positive bacteria [4]. The ethanolic extract of gac aril could inhibit the growth of *Micrococcus luteus*. In addition, ethanolic extract was found to obtain extract with higher inhibition efficiency than water extract [10]. The results in this study suggested that encapsulation technique could be able to maintain the microbial inhibition. This would be benefit for extending the shelf life of sausage although the ability may not be superior when compared with the high nitrite content at 150 ppm in reference sausage. However, low nitrite content would be more preferable to the consumer rather than adding high amount of nitrite.

TABLE IX. TOTAL MICROBIAL COUNT (LOG CFU/G) OF CHICKEN SAUSAGE WITH EGPE AT LOW NITRITE LEVEL DURING STORAGE AT 10 °C/1 WEEK

Sample	Storage time (day)	
	0	7
CTR (25N)	ND	5.3
25N+EGPE	ND	4.5
REF (150N)	ND	ND

ND = Not detected at 2 fold dilution
Different letter indicates the statistical difference at P < 0.05

5) Effect on lipid oxidation

Lipid oxidation is important reaction generating undesirable characteristics in sausage as the rancidity. The oxidation of lipid is initiated by radical formation of unsaturated fatty acids accelerated by metal ion or light. Thereafter, the peroxide would be formed as intermediate products before further degrading to volatile compounds such as aldehyde and ketone. It is classified as chemical deterioration of food product and it must be prevented in food product containing high fat content e.g. sausage. The lipid oxidation can be monitored by detecting the secondary products of oxidation and this technique is suitable to follow the reaction during storage. The reaction between thiobarbituric acid and aldehyde are well known for this application. The TBARS values of sausage after storage for 0 day were not different (P<0.05) and were found at such a low number (Table X). This is because fatty acids in all samples were not degraded. However, this value gradually increased in control sample.

TABLE X. TIOBARBITURIC ACID REACTIVE SUBSTANCE (MG MDA/KG) OF CHICKEN SAUSAGE WITH EGPE AT LOW NITRITE LEVEL DURING STORAGE AT 10 °C/1 WEEK

Sample	Storage time (day)	
	0	7
CTR (25N)	0.019±0.002	0.127±0.006[a]
25N+EGPE	0.018±0.001	0.071±0.008[b]
REF (150N)	0.014±0.004	0.014±0.005[b]

Mean ± SE was averaged from 2 replications
Different letter indicates the statistical difference at P < 0.05

This suggested that low nitrite content was not enough to inhibit oxidation although it is enough for developing the desirable color. A reduction of TBARS value was clearly found in sample with encapsulated gac powder extract. This suggested that bioactive compounds presented in the powder would be able to inhibit the lipid oxidation. This also corresponded with the DPPH radical scavenging ability of encapsulated gac powder extract presented in Table II and Table III. Those compounds would likely be carotenoids (lycopene and carotene), which are mainly found in the aril part of gac fruit. Moreover, phenolic compounds presented in either aril or pulp fractions were reported [2]. These phenols could scavenge the radical effectively and would likely able to terminate the oxidation during propagation step. Therefore, the oxidation of lipid was finally inhibited. Spray-dried gac aril powder showed radical scavenging ability against DPPH and ferric-reducing antioxidant powder (FRAP) reactions [11]. The results suggested that radical scavenging ability based on in vitro assay was sufficient to evaluate the effectiveness of compound for further application in food system. This would be the alternative strategy to produce the image of meat product from carcinogenic food to be healthier.

IV. CONCLUSIONS

Extraction of gac powder (aril and pulp) with ethanol as solvent using microwave assisted technique yielded an extract containing photochemical. This extract could be encapsulated with maltodextrin using spray drying method. The optimal condition for encapsulation was achieved by controlling the inlet temperature at 160° C and the ratio between extract:maltodextrin was fixed at 1:5. Applying this condition provided the encapsulated powder containing high lycopene content and radical scavenging ability. This powder could be the natural

colorant, antimicrobial agent, and antioxidants in low-nitrite sausage made from chicken.

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