Microbiology of Chinese Xuanwei ham production

Keywords

- Chinese Xuanwei ham
- Dry-cured ham
- Microflora
- Proteases
- Lipases

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Xuanwei ham of China is a popular dry-cured meat, but microbiology of its production is barely known. Surface microfloral changes accompanying production of Xuanwei ham are reported. Fifty hind legs of pigs were processed to dry-cured ham using an approximately 190 days long traditional process that involved salting, drying and fermentation as the major steps. Surface counts of microorganisms declined to low levels within 28 days as a consequence of salting. During the subsequent drying stage, microbial counts generally peaked within the first 40 days and subsequently declined progressively. Peak microbial counts were 4.7x10^8 cfu/cm^2 on the ham surface. Yeasts never became predominant. Moulds were dominant during the fermentation stage on the surface of the ham. A majority (>89%) of the bacterial isolates were staphylococci. Proteolytic and lipolytic activities were identified in many of the isolated bacteria and yeasts. Surface microorganisms involved in production of Xuanwei ham appear to be mainly staphylococci and species of *Penicillium* and *Aspergillus*. Yeasts appear to contribute, but to a lesser extent than bacteria and moulds. Understanding the progression of microbial changes during ham processing should allow production of ham batches of a consistent quality.

Xuanwei ham is a famed uncooked dry-cured ham of China. The ham is produced in Xuanwei, a city in the Yunnan Province, southwestern China. Xuanwei ham is characterised by its rose-red colour and a unique flavour. Nearly 20,750 t of Xuanwei ham are produced annually. The traditional production process uses hind legs of a local breed of pig. Cut and trimmed legs (i.e. 'green ham') that have been pressed by hand to remove blood are held under cool conditions for 24 h for ripening the meat. The legs are then salted by hand. This is followed by a 40-day drying stage and then by a 120-day fermentation stage. The total process takes nearly 190 days. Figure 1 illustrates the main stages of production of Xuanwei ham.

Although Xuanwei ham has been produced since the Qing Dynasty (1727 AD) (Yu et al., 2005), the chemical, physical and microbiological changes accompanying its production are only now being understood. Some of the quality attributes of Xuanwei ham have been linked to certain yeasts (Jiang et al., 1990), bacteria such as micrococci and staphylococci, and moulds that occur in the final product (Li et al., 2003). The information about the microbiology of Xuanwei ham is sometimes contradictory. For example, according to some sources (Jiang et al., 1990; Wang et al., 2006), moulds do not play a direct role in determining the quality of Xuanwei ham, but traditional producers and many consumers hold that high quality Xuanwei ham must have a 'green coat' of moulds on it. Similarly, moulds are believed to be essential contributors to flavour development in some European dry-cured hams (Lücke, 1986; Martín et al., 2004). The beneficial role of moulds notwithstanding, some moulds certainly produce mycotoxins in Xuanwei and other dry-cured hams (Wang et al., 2006; Rojas et al., 1991; Núñez et al., 1996a).

This work reports on changes in the surface microflora and the pH, water activity, and salt content of the meat during production of Xuanwei ham. Endogenous and microbial enzymes such as lipases and proteases are known to contribute to flavour development in many dry-cured hams (Toldra, 1998). Therefore, the proteolytic and lipolytic activities of some of the microorganisms isolated from ham were assessed and are reported. Microbiology of some of the other dry-cured hams has been discussed in the literature (Hinrichsen and Pedersen, 1995; Núñez et al., 1996b; Comi et al., 2004). Production of ham of a consistent quality requires a closely controlled progression of microbial species during its processing. Therefore, understanding the development of microbial ecology during ham production is essential so that the environmental conditions are modulated to achieve the desired progression of microorganisms.

Materials and methods

Source and processing of ham

Fifty hind legs (or green hams), 11±0.7 kg each, were harvested from locally slaughtered and skinned crossbred pigs (Yorkshire x Duroc x local Wujin) that had been raised in Kunming Gao-Shang-Gao pig farm, Kunming, China. The trimmed green hams were processed according to the traditional Xuanwei process. The production process involved: 1) cooling of hams in a cold room at 4 to 8 °C and 75% relative humidity (rH), for 24 h; 2) massaging the hams by hand with curing salt (NaCl with 0.15% $NaNO_3$) applied at 60 g/kg green ham for 10 min, then stacking the salted hams on the cement floor in a 20 m^2 air conditioned room kept at 5 to 6 °C and 72–79% rH. During holding in this room for 28 days, the ham pile was turned over once every 7 days; 3) washing the salted hams under running water; 4) drying the hams for 40 days by hanging the legs from bamboo supports in a 30 m^2 air conditioned room held at an average temperature of 13 °C and 56% rH; 5) fermentation of the hanging hams in the same room maintained at an average temperature of 20 °C and a relative humidity of 73%, for 120 days. Variations of temperature and relative humidity in the room used for ham production during 189 days of processing are shown in Figure 2.

Ham sampling and identification of microorganisms

At each sampling time, three replicate samples were taken from the lean surfaces of each of three randomly selected legs at the following stages of processing: before salting, during salting after 14 and 28 days, during drying after 20 and 40 days and during fermentation after 30, 60, 90 and 120 days. Microorganisms were enumerated on selective media specified. Samples were obtained from ten different positions by swabbing with a sterile gauze that had been dipped in a sterile solution of 0.9% w/v NaCl. For each sampling, a 9 cm^2 area was swabbed by covering the surface with a sterile sheet of stainless steel having a punched hole of 3 x 3 cm.

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Fig. 1: Various stages of production of Xuanwei dry-cured ham: a) green ham, b) salting, c) drying, d) fermentation, e) typical presentation of final product

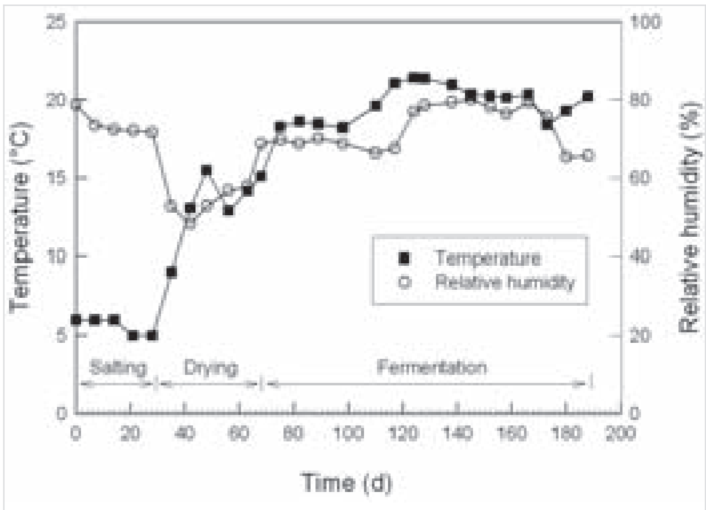

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Fig. 2: Temperatures and relative humidities in processing rooms during ham processing

Each swab was washed in sterile 0.9% w/v NaCl solution in a sterile blender. The samples were then appropriately diluted and plated on agars for recovery of colonies in accordance with the Chinese National Standard (2004). The values presented are the means of the three counts from each of the three hams sampled on each occasion.

The selective media prepared in 1 L distilled water were: Mannitol Salt Agar (MSA) for staphylococci (1 g beef extract, 75 g NaCl, 10 g mannitol, 10 g peptone, 20 g agar, pH 7.5); Malt Agar (MA) for moulds (20 g malt extract, 10 g peptone, 10 g glucose, 20 g agar, pH 6); Yeast Extract Peptone Dextrose agar (YPD) for yeasts (5 g yeast extract, 20 g glucose, 20 g peptone, 20 g agar, pH 5); de Man, Rogosa and Sharpe agar (MRS) for lactobacilli (5 g yeast extract, 5 g beef extract, 20 g glucose, 10 g peptone, 2 g K_2HPO_4, 20 g $CaCO_3$, 20 g agar, pH 6.2); Synthetic Defined Agar (SDA) for micrococci (50 g NaCl, 40 g glucose, 10 g peptone, 15 g agar, pH 5.5) and Nutrient Agar (NA) for total aerobic bacteria (5 g NaCl, 3 g beef extract, 10 g peptone, 20 g agar, pH 7.2) (CHEN, 1995). The incubation conditions according to CHEN (1995) were as follows: 37 °C, 2 d, aerobic, for MSA; 28 °C, 5 d, aerobic, for MA and YPD; 37 °C, 3 d, facultative (the plate was covered with a layer of the same medium before incubation), for MRS; and 37 °C, 2 d, aerobic, for SDA and NA. The NA plates were used for total bacterial counts only. The counts for staphylococci, lactobacilli and micrococci were made on selective media plates.

For microbial identification, 384 isolates were randomly selected and identified based on morphological and microscopic observation and standard biochemical tests. Tests used to identify yeasts were nitrate assimilation, glucose fermentation, urease activity as well as carbon and nitrogen assimilation tests. Tests used to identify bacteria were catalase, oxidase, glucose fermentation, anaerobic growth and growth with 7.5% NaCl. Specific tests were also carried out for species identification. These included phosphatase, gelatinase, casein hydrolysis and acid formation assays. Staphylococci and micrococci were distinguished using the biochemical tests of VILAR et al. (2000). The moulds were identified to genera or species level by their macro- and micro-morphological characteristics following the methods of SAMSON et al. (1995). All purified cultures were maintained on agar slants at 4 °C.

The dominant microorganisms were screened and tested for proteolytic and lipolytic activities by the agar plate method (BARNETT et al., 2000), using 2% casein and 8% skim milk or 4% pork fat and 1.2% olive oil as substrates. Plates for proteolytic and lipolytic activity assays were incubated at 37 °C and 28 °C, respectively, for 2 to 8 days. The diameters of colonies (r) and surrounding clear zones (R) were determined and expressed as the ratio R/r.

▶ Physico-chemical analyses

Five samples of *biceps femoris* (BF) and *semimembranosus* (SM) muscles were taken at each stage of ham processing from each of the five randomly selected hams, for determination of moisture content, water activity (a_w), sodium chloride content and pH. All analytical methods followed the Chinese National Standard GB/T 5009-2003 (2004). Moisture content was determined by drying a 3 g blended sample to constant weight, at 103±2 °C. Sodium chloride content expressed as w/w % was determined by potentiometric titration with $AgNO_3$ using an autotitrator. A 10 g blended sample mixed with 90 g distilled water was used for measuring the pH using a pH meter (HI 9025, Hanna Instruments, Italy). Water activity (a_w) was determined at 25 °C with an a_w-apparatus (SJN 5021, Jiang Ning Machine Factory, P.R. China).

Tab. 1: Bacteria and yeasts recovered from Xuanwei ham

Bacteria	Strains	%	Yeast	Strains	%
Marinococcus halophilus	4	3.6	*Trichosporon*	12	27.0
Micrococci	4	3.6	*Candida*	2	4.5
Tetracoccus	2	1.8	*Rhodotorula*	12	27.0
Pediococcus pentosaceus	2	1.8	*D. hansenii*	6	13.6
Staphylococcus xylosus	52	47.3	*Debaryomyces*	10	22.7
S. equorum	18	16.3	*Cryptococcus*	2	4.5
S. arlettae	16	14.5			
S. carnosus	8	7.3			
S. epidermidis	4	3.7			
Total	**110**	**100**	**Total**	**44**	**100**

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Results and discussion

▶ Microbial changes during processing

Green ham had average bacterial counts of 5.6x10^6 cfu/cm^2 on the surface. All microbial counts declined rapidly on salting and no microorganisms were detected by day 28 of the salting stage (Fig. 3). Clearly, the high salt concentration and low temperature (Fig. 2) proved inhibitory to all microbial growth. After salting, the temperature was gradually increased to about 13 °C and the

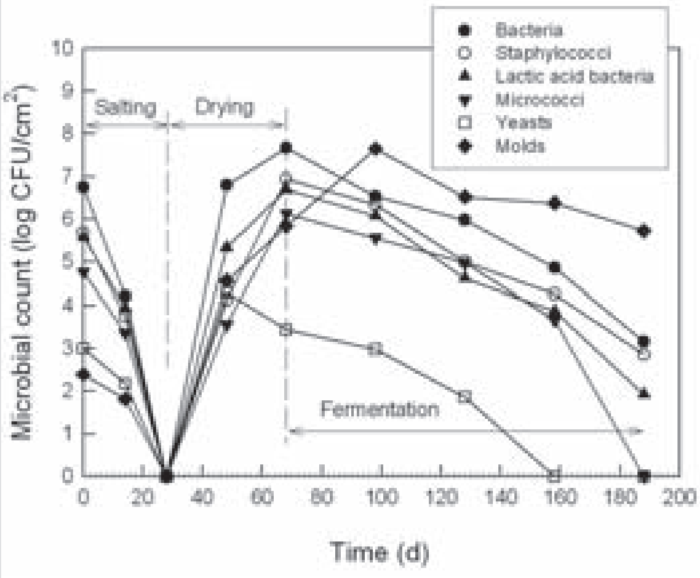

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Fig. 3: Microbial changes on ham surfaces during processing

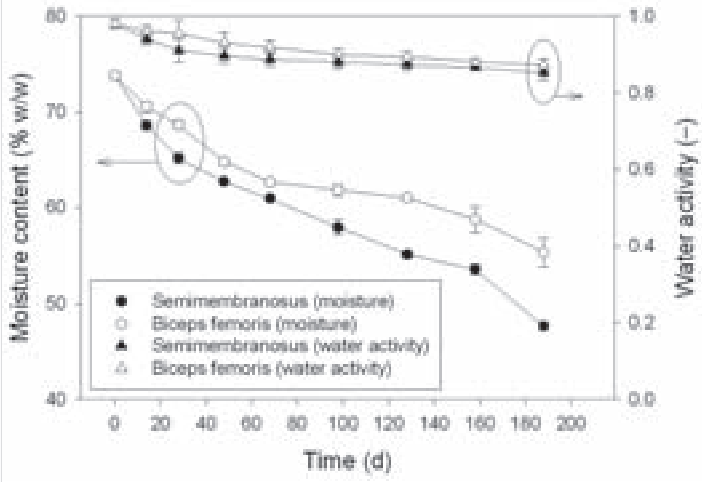

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Fig. 4: Changes in moisture contents and water activities of muscle tissues during ham processing

relative humidity was reduced to 48 to 69% during the 40-days drying stage (Fig. 2). During this period, surface counts of all microbes increased gradually (Fig. 3). Surface counts of all microorganisms except yeasts and moulds, peaked on day 68 (Fig. 3). Surface yeasts peaked on day 48 whereas the number of surface moulds peaked on day 98, during the fermentation stage (Fig. 3). During much of the drying and fermentation periods, the counts of yeasts were much lower than counts of bacteria and moulds (Fig. 3).

All microbial counts declined throughout the fermentation stage (Fig. 3). Yeasts and micrococci were not detected by the end of fermentation. During much of the fermentation stage, moulds predominated (Fig. 3). A relatively high count of lactic acid bacteria in the finished product (Fig. 3) is not unusual for dry-cured meats. For example, relatively high levels of lactobacilli have been documented in Italian-type dry-cured ham (HINRICHSEN and PEDERSEN, 1995) and the Spanish dry-cured lacón (VILAR et al., 2000).

These results are generally consistent with published information on Xuanwei- and Italian-type dry-cured hams. For example, in the final Xuanwei ham, staphylococci and several moulds have been found to be dominant on the exterior surfaces (LI et al., 2003). For Italian-type dry-cured ham, 75% of predominant microbes have been reported to be Gram-positive, catalase- and oxidase-negative cocci on the final, 365 days old product (HINRICHSEN and PEDERSEN, 1995). Similarly, only staphylococci, micrococci, yeasts and moulds have been found on the surfaces of Iberian dry-cured ham (NÚÑEZ et al., 1996b).

Changes in moisture content and water activity of ham muscles during various stages of processing are shown in Figure 4. Corresponding data for salt content and pH of the muscles are given in Figure 5. For both muscles, moisture content and water activity values were highest at the green ham stage (Fig. 4). Moisture content and water activity declined progressively during subsequent stages of processing (Fig. 4). At any stage of processing, the values of moisture content and water activity in the two muscles were similar. The decline in surface microbial counts (Fig. 3) correlated well with the decline in moisture content and water activity seen in Figure 4 during the fermentation stage of processing.

Tab. 2: Moulds recovered from Xuanwei ham

Penicillium	Number	*Aspergillus*	Number	Others	Number
P. cyclopium	39	*A. versicolor*	24	*Paecilomyces sp.*	19
P. simplicissimum	26	*A. glaucus*	18	*Mucor*	5
P. citreo-viride	20	*A. fumigatus*	15	*Rhizopus*	3
P. viridicatum	18	*A. albicans*	12	*Neurospora*	1
P. citrinum	15	*A. niger*	2		
P. chrysogenum	13				
Total penicillia	131	Total aspergilli	71	Total	28

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Reducing moisture content during curing (Fig. 4) caused a corresponding increase in the salt content of the tissue, to a final value of 8.7±0.1% w/w NaCl (Fig. 5). During processing, the muscle pH increased from an initial value of pH 5.92 for the green ham to a final pH of about 6.2 (Fig. 5), a behaviour that is consistent with that for other dry-cured hams.

During salting, temperature and rH were approximately 5 to 6 °C and 72 to 79%, respectively. This combination of low temperatures and high humidity controlled microbial growth and reduced activity of endogenous meat enzymes to prevent spoilage, but prevented rapid drying. This allowed penetration of curing agents into ham muscle. As the temperature was raised to 10–15 °C during drying and the relative humidity was reduced (Fig. 2), controlled microbial growth took place but slowed again as the ham dried and its water activity declined. During fermentation, the microbial population was reduced progressively as the ham dried further and, as consequence, its salt content increased. Changes in water activity are of course well known to control microbial growth. For example, most bacteria cease to grow at water activity of less than 0.9 (JAMES, 2001) although growth of moulds and yeasts persists to lower values of water activity.

▶ Identifying ham microorganisms

In total, 110 bacterial isolates were obtained from drying (60% of the isolates) and fermentation (40% of isolates) stages of the ham samples. Of these, more than 89% were staphylococci, e.g. *Staphylococcus xylosus*, *S. equorum*, *S. epidermidis* and *S. carnosus*. Micrococci, *Tetracoccus*, *Pediococcus pentosaceus* and *Marinococcus halophilus* were found at levels of 3.6%, 1.8%, 1.8% and 3.6%, respectively (Tab. 1). 44 isolates of yeasts were obtained from the drying (24 isolates) and fermentation (20 isolates) stages of ham processing. These results

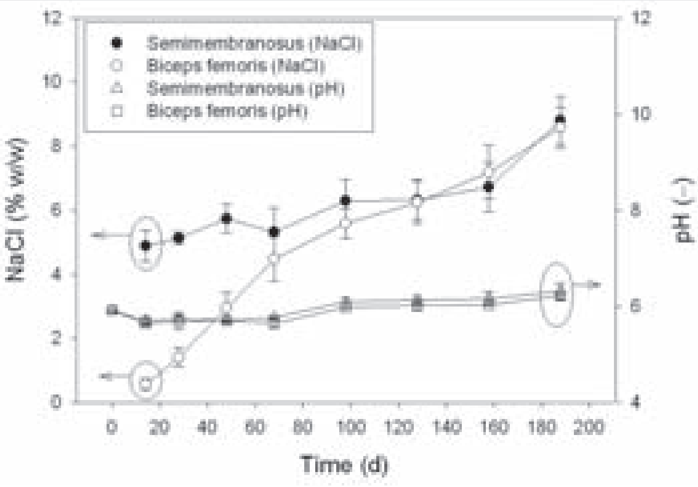

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Fig. 5: Changes in pH and NaCl contents of muscle tissues during ham processing

were similar to data that had been reported for other types of dry-cured hams (Núñez et al., 1996b; Carrascosa and Cornejo, 1991; Rodríguez et al., 1994; Simoncini et al., 2007).

Among the 230 mould isolates from fermenting hams were the following: *Penicillium* (57%), *Aspergillus* (31%), *Paecilomyces* (8%), *Mucor* (2%), *Rhizopus* (1.5%), and *Neurospora* (0.5%). *Penicillium* and *Aspergillus* were the predominant genera (Tab. 2). The isolated *Penicillium* spp. were identified as *Penicillium cyclopium* (30%), *P. simplicissimum* (20%), *P. citreo-viride* (15%), *P. viridicatum* (14%), *P. citrinum* (11%) and *P. chrysogenum* (10%). The following *Aspergillus* spp. were identified: *A. versicolor* (34%), *A. glaucus* (25%), *A. fumigatus* (21%), *A. albicans* (17%) and *A. niger* (3%). Penicillia and aspergilli have been reported to be the predominant mould genera on other dry-cured hams (Wang et al., 2006; Rojas et al., 1991; Núñez et al., 1996a), but *Eurotium* sp. appears to be the dominant mould isolate from Istrian dried ham (Comi et al., 2004).

Penicillium spp may adversely affect ham quality (Rojas et al., 1991; Northolt and Bullerman, 1982). For Xuanwei ham, a product that was comparable to the traditionally produced but without involving surface growth of moulds, has been produced (Wang et al., 2006). Moulds are of concern, because some produce the mycotoxins that have been found in the surface layers of various dry-cured hams (Núñez et al., 1996a). Mycotoxins generally do not seem to penetrate the ham further than 0.6 cm from the surface. According to Wang et al. (2006), about 16% of the moulds isolated from Xuanwei ham produce mycotoxins. Concern about mycotoxins notwithstanding, in a typical production process surface moulds, which are forming the 'green coat', certainly contribute to the maturation of the product and the flavour development, as evidenced by the consumer preference for the green coated product. Nevertheless, certain moulds are undesirable. Moulds rarely occur in the interior of a good quality product.

A total of 41 isolates, including 24 bacteria and 17 yeasts, was screened for proteolytic and lipolytic activities, as these can be important to flavour development in dry-cured hams (Toldra, 1998). Of the screened microorganisms, 16 bacteria and 14 yeasts demonstrated proteolytic capability. Lipase activity was demonstrated in 16 bacteria and 12 yeasts. Both lipase and protease activities were shown by 8 bacteria and 9 yeasts, i.e. 41% of the total microorganisms that were isolated. Enzyme activities were expressed as the ratio of the observed diameter (R) of the clear zone to the diameter of the colony (r), on an appropriate agar medium. A high value of this ratio indicated a high enzyme activity. The maximum R/r values for proteases were 3.00 and 3.53 for bacteria and yeasts, respectively. For lipases, the maximum R/r values were 4.67 and 5.50 for bacteria and yeasts, respectively.

Salt tolerant yeast *Debaryomyces hansenii* and the moulds isolated from Iberian dry-cured ham have been reported to have a strong proteolytic activity against myofibrillar proteins of raw pork (Rodríguez et al., 1994; Martín et al., 2001). Furthermore, a co-culture of *Penicillium chrysogenum* (Pg 222) and *D. hansenii* (Dh 345) isolated from dry-cured ham, has promoted protein hydrolysis in external muscle in dry-cured ham (Martín et al., 2004).

Conclusions

Chinese Xuanwei dry-cured ham has been produced since Qing Dynasty, but microbiological and physicochemical changes accompanying its production have been barely investigated. Variation in water activity, pH and salt content of ham as a consequence of the characteristic temperature and relative humidity profiling used in ham production, determine the types and numbers of microorganisms found on the ham at various stages of processing. Microbial population generally declines by day 28 of the salting stage but rebounds during subsequent drying stage. During fermentation, microorganisms decline progressively. Staphylococci are the most frequently occurring bacteria during various stages of ham processing. Among moulds, the genera *Penicillium* and *Aspergillus* are most dominant. Many of the microbial isolates found on the ham clearly possess proteolytic and/or lipolytic activities. An understanding of the microbial ecology during ham production should allow production conditions to be precisely profiled to favour the desired progression of species and suppression of the unwanted ones. This will allow the production of a safe ham of a consistently high quality.

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Literature references can be downloaded at *www.fleischwirtschaft.com/literature* and requested from the author or the editorial office, respectively.

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