Encapsulation of Lactic Acid in Starch by Extrusion for using as pH Regulated Binder of Meat Products

Geun-Pyo Hong, Yeun-Sul Lee, Ji-Yoo Baek¹, and Mi-Jung Choi^{1*}

Department of Food Science and Biotechnology of Animal Resources, Konkuk University, Seoul 143-701, Korea

¹Department of Molecular Biotechnology, Konkuk University, Seoul 143-701, Korea

Abstract

This study was carried out to investigate the encapsulation of lactic acid in starch matrix for the application into emulsified sausages. For the encapsulation of lactic acid in starch, the extrusion method was applied, by the different extrusion pressure level. The particle size and morphology of lactic acid containing starch granules and the rate of release of lactic acid from those granules were determined by using Mastersizer[®], a scanning electron microscopy, and electrical conductivity. The size varied slightly depending upon the extruder pressure and influenced entrapment efficiency. Lactic acid was released more slowly, when the extruder had fewer holes, which meant higher extrusion pressure, than when the extruder had more holes. Extruder pressure is therefore critical for producing finer granules that can retain lactic acid longer, during the processing of meat products.

Key words: starch, lactic acid, encapsulation, extrusion, emulsified sausages

Introduction

During the last decade, the consumption of nutritionally enriched products has been increasing, which, along with the demand that such products be functionally enhanced, has led to great interest in encapsulation technology. The objective of encapsulation is to protect fragile substances from high temperatures, light, and other adverse conditions by enveloping those substances with such materials as biodegradable synthetic polymers and natural biopolymers, and thus maintaining the substances in stable and soluble form (Choi *et al.*, 2009; Choi *et al.*, 2010). Encapsulation techniques have been developed using nano- or micro-technology to keep the encapsulated particles as small as possible, however, its application in meat products has been limited, constrained by disadvantages such as low content of ω -3 fatty acids and by the need to ensure controlled release of acidic compounds. Bologna-type sausages supplemented with pre-emulsified polyunsaturated fatty acid (PUFA) n-3 to obtain the desired microstructure have been evaluated for their sensory

attributes (Cáceres *et al.*, 2008) and chicken meat products prepared with fish oil and activated carbon have been evaluated for their physico-chemical characteristics (Park *et al.*, 2002).

Lactic acid, an essential ingredient of sausages, is particularly critical to the final pH of the product after ripening (Spaziani *et al.*, 2008). European consumers in the Mediterranean region reject the strong acid flavors associated with quickly ripened sausages and have a predilection for mildly acidic sausages. According to Pérez-Alvarez *et al.* (1999), the properties of lactic acid in dry-cured sausages determine the activity of the microbial flora, which comprises mostly lactic-acid bacteria. Therefore, controlled release of lactic acid during ripening is crucial to obtaining high-quality meat products.

Carbohydrates such as starch or gum are generally added as fillers to increase bulk, to delay drying, to lower formulation costs, to change the texture of the product, and to make the product more stable when frozen (Hong *et al.*, 2008; Totosausa and Pérez-Chabelab, 2009), and this use of carbohydrates has increased with the development of low-fat meat products (Choi *et al.*, 2008, Hoe *et al.*, 2006). Among the several forms of carbohydrates, starch is particularly suitable as a coating to encapsulate a given active substance to protect lipids from oxidation and to entrap volatile flavors (Heinemann *et al.*, 2005;

*Corresponding author: Mi-Jung Choi, Department of Molecular Biotechnology, Konkuk University, Seoul 143-701, Korea. Tel: 82-2-450-3048, Fax: 82-2-455-1044, E-mail: choimj@konkuk.ac.kr

Yuliani *et al.*, 2006). It has many other benefits as well such as cheap price, easily available in food, and compatible with different encapsulation techniques. These techniques include the use of starch or modified starch, e.g., helical inclusion complexes of flavor compounds with amylase (Itthisoponkul *et al.*, 2007; Wulff *et al.*, 2005), emulsion by homogenization (Li *et al.*, 2009), and spray drying (Buffo and Reineccius, 2000). Starch undergoes structural alterations including gelatinization, melting, swelling, fragmentation, and crystallization, especially during extrusion, depending on moisture content, pressure, temperature, and shearing force (Totosausa and Pérez-Chabelab, 2009).

We encapsulated lactic acid with potato starch using extrusion at different pressures and studied the rate of release of lactic acid from the encapsulated form and the effect of that rate on the pH of the final product.

Materials and Methods

Materials

Lactic acid was purchased from Kanto Chemical Co. Inc. (Japan), commercial-grade potato starch from Cheju Starch (Korea), and monoglyceride, as an emulsifier, from Quest International (The Netherlands). All chemicals were used without further purification.

Encapsulation of lactic acid with starch by extrusion

Potato starch (3% w/v) and lactic acid (1% v/v) were mixed with distilled water for 10 min at 350 rpm at 25°C; 1% w/v of monoglyceride was added and the mixture stirred at 350 rpm for 10 min, and then heated to 100°C for 30 min, by which time the starch was gelatinized. The mixture was cooled to 50°C before forcing it through the

extruder. The laboratory-made extruder consisted of a twin screw extruder and an output tube with varying number of holes. For preparing microparticles, the extrusion was performed for 1 h at 50°C. During this process, we measured the pressure level during extrusion. The pressure varied with the number of holes (1, 5, 10, 20, 40, or 80 holes) in the output tube of the extruder as follows: $P1$ (one hole) = 64.1×10^3 N/m², $P5 = 47.6 \times 10^3$ N/m², $P10 = 33.5 \times 10^3$ N/m², $P20 = 16.8 \times 10^3$ N/m², $P40 = 8.4 \times 10^3$ N/m², and $P80 = 4.2 \times 10^3$ N/m² (Table 1).

Standard curve for the amount of lactic acid as measured by pH

The amount of lactic acid was measured by using a pH meter (Corning pH meter 440, USA). Lactic acid solutions of various strengths (0 M to 1 M) were prepared with distilled water to obtain a standard curve for lactic acid concentration and electric conductivity as shown in Fig. 1 and Eq. (1):

$$Y = 11.464 \ln X + 251.94 \quad (R^2 = 0.996) \quad (1)$$

where X is the lactic acid concentration (mol) and Y is the electrical conductivity (mV).

Determination of entrapment efficiency (EE)

After entrapping the lactic acid droplets within starch granules during gelatinization, the suspension was centrifuged at 5000 rpm in a test tube (Kubota, Japan). The supernatant was collected and its electrical conductivity was measured using a pH meter. The pellet was washed with distilled water three times, re-suspended in distilled water, and centrifuged once again to collect the free lactic acid. The amount of lactic acid entrapped within the starch

Table 1. Effect of extrusion pressure on size and entrapment efficiency of starch granules (mean size was 31.53 ± 1.21 μ m)

Abbreviation	Number of holes in the extruder	Pressure (N/m ²)	Mean size (μ m)	Entrapment efficiency (%)
P1	1	64.1×10^3	$29.42 \pm 2.42^{a*}$	82.44 ± 2.51^a
P5	5	47.6×10^3	27.76 ± 2.12^b	84.73 ± 3.21^a
P10	10	33.5×10^3	25.98 ± 0.46^b	70.99 ± 1.01^b
P20	20	16.8×10^3	23.56 ± 0.93^c	70.23 ± 3.20^b
P40	40	8.5×10^3	24.23 ± 1.56^c	71.94 ± 2.42^b
P80	80	4.1×10^3	26.09 ± 1.07^b	74.22 ± 2.47^c

*Means $\pm$ S.D. (n = 3)

^{a-c}Means with the same letter within a same column are not significantly different ($p > 0.05$).

Fig. 1. Standard curve of electric conductivity as a function of lactic acid concentration fitted with exponential function.

granules as a percentage of total lactic acid used was calculated using Eq. (2):

$$\text{Entrapment efficiency (\% EE)} = \frac{[(\text{Total amount of lactic acid (mol)} - \text{measured amount of free lactic acid (mol)}) / (\text{total amount of lactic acid (mol)})] \times 100}{(2)}$$

Measurement of particle size

Particle size distribution was determined by diffusion using Mastersizer[®] a light-scattering particle size analyzer (MAM 5005, Malvern Instruments Ltd., UK). The sample to be analyzed was placed into the suspension fluid at a high dilution level. We used distilled water as the suspension fluid.

Rate of release of lactic acid

Starch granules (10 mL samples) prepared at various extrusion pressures and washed free lactic acid were placed into conical test tubes without shaking. After storage at 25°C for 24 h, the amount of lactic acid released from the granules was measured using a pH meter. The sensor of the pH meter was retained within the sample to monitor the rate of release of lactic acid using Eq. (1).

Scanning electron microscopy

The surface structure of the nanocapsules (granules) prepared conventionally and with high hydrostatic pressure was examined using a scanning electron microscope (E-1010, Hitachi Science System Ltd., Japan). Freeze-dried starch-lactic acid complexes were cut into rectangular forms (5 mm×3 mm×2 mm) and an ion sputter was used to coat the samples with gold.

Statistical analysis

The data were analyzed for ANOVA using SAS ver. 9.1 (SAS Institute, USA). The differences among the means were compared using Duncan's multiple range tests. The correlations between independent variables and measured values were calculated as Pearson's correlation coefficients. Each treatment was replicated three times.

Results and Discussion

Effect of preparation process on entrapment efficiency

The amount of lactic acid was measured at different stages of the process: after hydration (mixing with water and starch), after heating the mixture to 100°C, and after cooling it to 50°C. At each step, free lactic acid was removed

by centrifuging and the amount of lactic acid entrapped within the granules was measured and entrapment efficiency (EE) calculated using Eq. (2) (Fig. 2).

Overall, extrusion pressure had no effect on EE irrespective of the method of preparation. After hydration and the subsequent extrusion, EE was approximately 28%. After heating at 100°C, as much as 98% of the lactic acid remained trapped inside the starch granules. As the starch swells and gets partially dissolved during heating, lactic acid is trapped within the network of gel formed by the melting starch granules (Conde-Petit *et al.*, 1998). However, as the matrix cooled, the entrapped amount was reduced to 70% irrespective of the extrusion pressure. During heating and the subsequent cooling, the swollen starch granules are spontaneously transformed into the typical double-helical crystals of amylose, a process referred to as retrogradation. The turbid appearance of the solution of lactic acid-starch complex and the formation of a precipitate upon cooling were the visible manifestations of retrogradation. However, 28% of the lactic acid was not entrapped within the starch granules after cooling. A practical implication of this observation is that given the processing temperature during the manufacture of meat products, it is possible that lactic acid would be released owing to starch gelation.

Effect of extrusion pressure on particle size and entrapment efficiency

The pressure at which the granules were extruded, which varied with the number of holes in the extruder tube, affected the particle size distribution and EE of the granules. Particles of potato starch were approximately

Fig. 2. Lactic acid content of starch granules after hydration, after heating to 80°C, and after cooling to 4°C. Means $\pm$ S.D. (n = 3). Values within a column marked with different letters are significantly different at $p < 0.05$.

31.5 μm in diameter before the addition of lactic acid and the monoglyceride. After passing through the extruder, the particles of potato starch loaded with lactic acid were to 23-29 μm in diameter depending on the pressure: the higher the pressure, the smaller the particles, probably as a result of the increase in temperature as the mix was forced through the small holes under high pressure by the adiabatic expansion through high pressure at small number holes. Our results are in good agreement with those obtained by Yilmaz *et al.* (2001), who reported that the average size of oil droplets in starch matrices decreased at higher screw speeds, increasing the melt temperature and decreasing throughput.

Entrapment efficiency was influenced by the pressure of extrusion. At higher pressures, encapsulation was more efficient. It is noteworthy that when the particle size increased after extrusion, EE was higher. The natural granular structure of starch is destroyed by hydrothermal treatment during food processing. Heating with excess water leads to swelling of starch granules and to a more or less pronounced leaching of amylose. Amylose has a helical structure, and its leaching creates a cavity at the centre: lactic acid enters to fill the cavity, spontaneously forming a lactic acid-starch complex. Thus the amount of lactic acid within a granule determines the size of the lactic acid-starch complex and, in turn, its EE. Moreover, higher pressure increases the temperature, which increases the extent of swelling, which, in turn, results in larger particles and higher EE (Conde-Petit *et al.*, 2006). This outcome can be explained thus: potato starch is rich in amylopectin, and its helical structure resists the process of complex formation (Na and Kim, 2008) owing to the long side chains or branches; however, such a structure also increases its capacity to trap lactic acid when adequate pressure during extrusion raises the temperature, which serves to overcome the resistance offered by amylose.

Rate of release of lactic acid

The standard curve obtained by plotting lactic acid concentration against electrical conductivity (Fig. 1) was used for fitting the regression using Eq. (1). The effect of the number of holes (and therefore that of the pressure of extrusion) on the rate at which lactic acid was released from the complex is shown in Fig. 3. Granules formed from extruders with 1 hole or 5 holes released lactic acid more slowly than those formed with extruders with more than 5 holes. Thus, extruding at low pressure produced slow-release granules. In the case of P1, at 2000 s, the conductivity remained steady at 215 mV; in contrast, in

Fig. 3. Electrical conductivity (A) and concentration (B) of lactic acid as a function of time.

P5 was it increased from 215 mV to 222.5 mV. Of all the treatments, P40 resulted in the fastest release rate (Fig. 3B). However, there was no relationship between particle size and the rate of release (Table 1).

From these results, we calculated the release rate in terms of the total amount of retained lactic acid (Fig. 4). The release rate of lactic acid was increased with pressure and more rapidly with time. Granules resulting from P1 had retained 60% of their lactic acid content after 20 h whereas those from P5 had released half of the content after 10 h, and could retain only about 30% after 20 h. Granules resulting from P10 to P80 had released half of the content after 5 h; the rate of release went on increasing until there was no more acid to be released. Henrist *et al.* (1999) studied the effect of extrusion temperature on the rate at which starch loaded with a drug released the drug into water and found that higher extrusion temperature led to slower release *in vitro*.

Higher extrusion temperatures resulting from higher extrusion pressures affected the rate of retrogradation. The higher content of amylopectin molecules and the separation of amylose and amylopectin during heating ensure

Fig. 4. Lactic acid release profiles of starch granules prepared at different extrusion pressures.

faster re-arrangement of amylopectin molecules on cooling, which makes the swollen granules or amylopectin-rich zones more rigid after longer ageing times (Keetels *et al.*, 1996). Therefore, the lactic acid-starch complexes prepared at high pressures release lactic acid more slowly over time because it is encased within a firmer network of starch.

Ultrastructure of lactic acid-starch complex

The surface of potato starch granules before any treat-

Fig. 5. SEM images of potato starch granules (A) and lactic acid-starch complex without monoglyceride (B).

ment was smooth and the granules showed a wider range of sizes (Fig. 5A). The granules were typically spherical

Fig. 6. SEM images of lactic acid-starch complexes formed at different extrusion pressures. A, P10: $33.5 \times 10^3 \text{ N/m}^2$; B, P20: $16.8 \times 10^3 \text{ N/m}^2$; C, P40: $8.4 \times 10^3 \text{ N/m}^2$; D, P80: $4.2 \times 10^3 \text{ N/m}^2$.

or ovoid with a diameter of 20-30 μm . The surface of the lactic acid-starch complex was also smooth (Fig. 5B). On the other hand, extruder pressure and adsorption of monoglyceride on the surface had a marked effect on the texture of the surface and the size of the granules (Fig. 6). The adsorbed monoglyceride played a significant role as an emulsifier in meat products such as emulsified sausages. Extrusion at higher pressure also resulted in larger granules.

Conclusions

Potato starch is a promising material for encapsulating lactic acid in the form of granules and controlling the rate at which it is released from the granules. The pressure at which the granules are extruded during their manufacture determines the rate of release. The size of the granules varied slightly depending upon the extruder pressure, and granules of different sizes differed in the efficiency with which they trapped lactic acid. Furthermore, processing steps such as hydration, swelling, and cooling also affect EE and the rate at which lactic acid is released: when the extruder had fewer holes, which results in greater extrusion pressure, the granules released lactic acid more slowly compared to the granules from the extruder with more holes. In conclusion, extruder pressure is crucial to the manufacture of granules of optimum size that can retain lactic acid for longer periods during the process of producing meat products. Controlling the rate of release of lactic acid is particularly important to avoiding sudden changes in pH during the process. Our study makes it possible to predict the release of lactic acid in the manufacture of real meat products and also demonstrates the potential of monoglycerides adsorbed on the surface of the starch-lactic acid complex in ensuring more stable emulsified meat products.

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(Received 2012.2.29/Accepted 2012.3.20)