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RELATIONSHIP BETWEEN THE STARCH GRANULE STRUCTURE AND THE TEXTURAL PROPERTIES OF HEAT-INDUCED SURIMI GELS

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Abstract

Starch, used as a textural additive in heat-induced surimi gel, influences the rigidity of the protein gel matrix and hence the gel strength according to its botanical characteristics. The present study focuses on the correlations existing between the textural properties of heat-induced surimi gels obtained by physical measurements and the characteristics of different commercial starches. The gelatinization temperature of starch was closely related to the expressible moisture, work to fracture, and elongation. Behaviour of starchy components during thermal processing and its relationship to fish protein gel matrix were studied by light and electron microscopy. These studies showed differences in starch swelling, amylose leaching, and amylopectin behaviour depending on water intake. Cryo-scanning electron microscopy revealed structural features which have never been observed by the classical cyto-techniques.

Key Words: Starch, modified starch, starch swelling, surimi gel, texture, microstructure, cytochemistry, image analysis.

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Introduction

A great interest in surimi-based products (surimi is a washed fish mince) has recently arisen in American and European countries. The development of surimi-based products exhibiting good texture requires texture optimization according to the functional properties of surimi and the nature of the additives. Starch is generally added to surimi-based products in order to enhance the gel strength (Okada and Migita, 1956). Besides its ability to modify the texture, the addition of starch lowers the cost of the products since more water is added to adjust the moisture of the final products. Little information is available to explain the effect of starch on gel strength and the relationship between the enhancing effect and the ultrastructure of the heat-induced surimi gel.

Wu *et al.* (1985) observed that the presence of starch increased the rigidity of the system to a degree dependent upon the starch type. The botanical origin of starch also influenced the rigidity and gel strength of a starch/surimi system. For example, Kim *et al.* (1987) noted that potato starch had a greater strengthening ability than wheat starch because it had a greater swelling power. Yamashita and Yoneda (1989) studied the influence of various starches (10% addition) in kamaboko at the heating temperatures at which the maximum gel strength was obtained. Initial gelatinization temperatures (85-90°C) were higher for potato, sweet potato, amylomaize and normal maize starches than those (75-80°C) observed in rice and waxy maize starches. Kim (1986) reported that expressible moisture and penetration force increased with an increase in the amylose fraction and that this was due to starch retrogradation. The effects of potato starch (reinforcing firmness, increasing elasticity, and reducing expressible water) were also measured by several authors (Chang-Lee *et al.*, 1989; Hastings, 1989; Hastings and Currall, 1989; Kim *et al.*, 1987; Ojima *et al.*, 1985), whereas Roussel and Cheftel (1988) obtained marked texture improvement (elasticity and rigidity) in sardine kamaboko gels with partial or total replacement of potato starch by egg white, soy protein isolate, or bovine serum albumin.

The ability to increase gel strength seems to be closely related to the ability of starch to swell. Starches

with high amylose contents or having high gelatinization temperature, which are used in surimi-based products, are less swollen and keep their granular form within the surimi gel during heat processing as shown by Okada (1963) and Kim *et al.* (1987). Yamazawa (1990) found that potato starch which has relatively strong gel-reinforcing effects was gelatinized thoroughly during heating to 90°C. The internal parts of the starch granules were observed to be completely disrupted by the heating process at a temperature above 110°C. However, the gel-reinforcing effect brought about by starch remained although the temperature of the heating process was above 90°C, where the structural disruption of starch granules was initiated.

Two possible explanations of how such starch swelling makes the gel more cohesive and firmer have been given by Lee and Kim (1986): increasing rigidity of the protein matrix may be the result of water transfer from the protein matrix to the swelling starch granules, and/or the formation of a large number of elastic globules through the gelatinization of the starch granules. Gelatinized starch globules would make the gel firm and elastic by acting as elastic masses within the protein matrix.

No detailed study has been reported on the effect of starch with respect to the swollen starch granule. The objective of the present study was, therefore, to compare the gel-strengthening effect of commercial starches in relation to their gelatinization behaviour and ultrastructural changes in the surimi gels.

Materials and Methods

Surimi was prepared from pout (*Trisopterus luscus*) caught off the Bay of Biscay (France). Following the procedure described by Verrez (1987), preparation of surimi was carried out at a pilot plant (SCOMA, Lorient, France) equipped with a complete production line.

Ten different commercial starches were incorporated into surimi to evaluate their gel-modifying abilities:

4 potato starches (approximately 25% amylose) from Roquette (Lestrem, France):

native potato starch (NPS);
Clearam PH10 (esterified and reticulated starch);
Clearam PGHV (acetylated starch); and
Cremalys 516 (emulsified and complexed starch).

5 cereal starches:

waxy maize (< 1% amylose) from Roquette;
normal maize (approximately 25% amylose) from Roquette;
amylomaize (approximately 70% amylose) from Roquette;
wheat (approximately 25% amylose) from Roquette;
rice (approximately 17% amylose) from Rémy Products (Wijgmaal-Leuven, Belgium).

1 leguminous starch:

smooth pea (approximately 35% amylose) from Woodstone (Saskatoon, Canada).

Preparation of heat-induced surimi gel

The thawed surimi was ground for 2 minutes with 2.5% salt (weight/weight, w/w surimi) and water to adjust the moisture level of the final product to approximately 80%, followed by addition of 5% starch (w/w surimi) and further grinding for 3 minutes at 3000 rpm using a refrigerated vacuum cutter (Stephan, Hameln, Germany).

For the ultrastructural study and physical analysis (color measurement, expressed moisture, rheological studies), a portion of the resulting paste was spread in an aluminium plate (18.5 x 12 x 3 cm^3) and cooked in a steamer (Thirode, Poligny, France) at 100°C for 30 minutes. Then the surimi gels were covered with a thin plastic film to prevent excess drying and stored overnight at 4°C. For determination of elongation, the other portion of the paste was extruded into a thin sheet (1.2 mm thick and 20 mm wide) and steamed at 100°C for 5 minutes, cooled, covered with a thin plastic film and stored at 4°C overnight. On the next day, the samples were equilibrated to room temperature before analyses.

Measurement of textural properties

The textural properties of gels, percent elongation, compressive force, percent of relaxation, and work to fracture, were measured using an Instron Universal Testing Machine (Model 6021, Instron Engg. Corp., Canton, MA). For the compressive force, percent relaxation, and work to fracture, cylindrical specimens (20 mm diameter x 20 mm length) were used.

Compression-relaxation: Compressive force at 50% deformation without failure was measured as an index of firmness of the gel (F). At the same time, percent relaxation was determined at 50% deformation, deformation maintained constant during 1 minute relaxation. The percentage of relaxation (%R) was expressed according to Stanley and Emmons (1977) as:

$$\%R = (F_{1\ min}/F) \times 100 \qquad (1)$$

where F and $F_{1\ min}$ were the forces recorded initially and after 1 minute relaxation, respectively. The results are an average of 10 replicates.

Penetration: A spherical plunger (5 mm diameter) was penetrated into the center of the cross gel cylindrical specimen (20 mm diameter x 20 mm length) at a speed of 20 $mm.min^{-1}$. Breaking stress and breaking distance were determined. Work to fracture (WF) in N.mm was defined as the product of breaking stress and breaking distance, and is equivalent to the Japanese gel strength measurement (Yamazawa, 1990). The results is an average of 12 replicates.

Percentage of elongation: A thin sheet of cooked extrudate (1.2 mm thick x 20 mm wide) was cut and attached to the Instron testing machine for an extending length of 4.0 cm. The test piece was stretched at a speed of 50 $mm.min^{-1}$ and the elongation length at the breaking point was measured. The results were express-

ed in percentage of elongation (%E). According to Kim and Lee (1987), this test is useful for predicting the tensile properties of an extrudate during the commercial process which involves various strenuous stretching steps. The result is an average of 10 replicates.

Measurement of expressible moisture

One gram of gel (minced through 5 mm diameter openings) was centrifuged on a small cylindrical filter paper (Gilson, Villiers le Bel, France) at about 2000 g for 10 minutes. The amount of moisture collected on the filter paper was calculated in terms of expressed moisture (EM) on a sample moisture content basis. EM is an average of 10 replicates.

Differential scanning calorimetry of starch

The gelatinization temperatures (peak temperature, Tp) of the different starches used in this study were determined by differential scanning calorimetry (DSC). Accurately weighed samples (8-25 mg) of starch, thoroughly mixed with distilled water to obtain a starch/water ratio of 1:2, were sealed in the Perkin-Elmer O-ring stainless steel capsules. Reference pan contained water. Samples were analyzed in a Perkin-Elmer DSC-4 at a heating rate of $5°C.min^{-1}$ over a temperature range of 10-120°C.

Color measurement

The whiteness of samples was measured with a HunterLab Colorquest spectrocolorimeter (HunterLab, Reston, VA) based on a white standard (L = 94.99, a = -1.06, b = 0.96). Whiteness was expressed in whiteness index (WI) according to the AATCC Test Method 110 as:

$$WI = Y + 800\ (xn - x) + 1700\ (yn - y) \qquad (2)$$

where Y, x and y were the CIE (Commision Internationale de l'Eclairage, International Lighting Commission) chromaticity co-ordinates of sample. For CIE illuminant D65 and the 2° standard observer: xn = 0.3138 and yn = 0.3309.

Microstructure study

Light microscopy: Small blocks (about 0.5 cm^3) of heat-induced gel were fixed in 4% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) for 2 hours at room temperature. The residual free aldehyde groups were blocked with 2,4-dinitrophenylhydrazine (DNPH) saturated in 15% acetic acid solution for 1 hour. The fixed samples were then dehydrated in a graded ethanol series, infiltrated with propylene oxide and embedded in Epon. Sections of 2 μm thickness were mounted on slides and Epon was eliminated by dipping the specimens in a specific alkaline solution (Maxwell, 1978) for 2 minutes and then rinsed successively with 100% methanol and running water. Starch was stained pink by first immersion in a 0.5% periodic acid solution for 8 minutes, followed by distilled water, then immersion in Shiff's reagent for 10 minutes in darkness (Periodic Acid Schiff = PAS). Specimens were rinsed 3 times in freshly prepared sulfurous acid for 2 minutes and then in running water. The prepared samples were examined under an Olympus (Vanox) microscope.

Transmission electron microscopy (TEM): Small rectangular strips of heat-induced gel were fixed in 4% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) for 1 hour at room temperature. The residual free aldehyde groups were then blocked with saturated DNPH in 15% acetic acid solution for 1 hour. The staining procedure for the carbohydrates (PATAg) was used before embedding: the fixed samples were stained by first immersion in a 1% periodic acid solution for 30 minutes, followed by saturated thiosemicarbazide for 24 hours and then immersed in a 1% silver nitrate solution for 3-4 days (Gallant, 1974). The stained samples were then dehydrated in an ethanol series, infiltrated with propylene oxide, and embedded in Epon. Sections of 0.1 μm thickness were mounted on copper grids previously coated with carbon and examined with a JEOL 100S TEM operated at 80 kV.

Scanning electron microscopy (SEM): Samples were frozen in nitrogen slush and fractured prior to observation in a JEOL JSM 840A using the cold stage (Hexland cryotrans CT 1500) according to the conditions recommended for starchy products (Sargent, 1988).

Image analysis: An image analysis system (PCSCOPE, i2S, Bordeaux, France) connected to a Tandon 286SX computer was used to measure volume fractions of starch granules in heat-induced surimi gels. Surfaces of the swollen starch granules observed in sections of surimi gels ranged from 8.10^3 μm^2 to 128.10^3 μm^2. Digitalized image scales were calibrated according to each magnification used. To evaluate the starch distribution (which is homogeneous in surimi gels), surfaces were extrapolated to volumes and can be expressed as a percentage of the spaces filled by the starch granules in the total volume of the gels. The result (%V) is an average of 5 replicates.

Statistical analysis

Correlation coefficients were calculated for 8 physical parameters. In order to summarize data, a principal component analysis (which is one of the multivariate statistical analyses) was applied.

Results and Discussion

Physical properties of surimi-starch gels: data and principal component analysis

The physical characteristics of surimi gels containing various starches are shown in Table 1. The gelatinization temperatures shown in Table 1 are based on gelatinization in the water-starch system at the maximum of the endothermic peaks. The acetylated potato starch (PGHV) gelatinized at a lower temperature (59.5°C) than the native potato starch (64.9°C) and esterified potato starch (PH10, 64.4°C). However, complexed amylose and emulsified potato starch (Cremalys 516) gelatinized at a higher temperature (70.9°C). Another uniqueness of Cremalys 516 is the presence of a second peak of low energy at 98°C, the endothermic peak of complexed

Table 1. Physical characteristics of heat-induced surimi gels with different kinds of starch.

	Native potato	Clearam PH10	Clearam PGHV	Cremalys 516	Normal maize	Waxy maize	Amylo-maize	Wheat	Rice	Pea
Peak temperature (Tp)*	64.9	64.4	59.5	70.9 and 97.8	70.8	72.7	92.8	64.0	76.8	70.3
Expressible moisture (EM)	6,9 (1.4)+	5.3 (1.1)	4.6 (0.3)	26.9 (2.3)	5.5 (1.0)	5.5 (0.8)	36.5 (1.5)	6.4 (0.8)	6.2 (0.5)	30.9 (1.3)
Elongation (%E)	158 (32)	153 (37)	171 (42)	66 (19)	126 (30)	157 (33)	123 (32)	231 (40)	183 (32)	-
Firmness (F)	10.7 (0.7)	11.2 (1.0)	12.6 (0.6)	10.4 (0.7)	9.8 (1.0)	9.2 (0.7)	6.7 (0.6)	7.1 (0.9)	8.0 (0.5)	8.7 (0.4)
Relaxation (%R)	58.8 (1.7)	58.6 (2.1)	57.2 (2.7)	57.6 (1.1)	60.3 (1.9)	59.2 (2.2)	53.6 (1.2)	60.2 (1.0)	61.7 (0.9)	58.3 (1.0)
Work to fracture (WF)	8.4 (1.3)	10.6 (0.9)	9.9 (1.5)	7.4 (1.4)	10.2 (1.2)	9.7 (1.4)	5.9 (0.8)	8.1 (0.6)	8.6 (1.5)	7.2 (0.8)
Volume density of starch (%V)	58.7 (2.6)	64.0 (1.6)	37.3 (7.0)	39.0 (9.6)	25.0 (4.5)	36.0 (4.9)	14.5 (2.4)	35.8 (5.4)	77.2 (5.7)	39.2 (16.7)
Whiteness index (WI)	17.6	19.8	16.9	21.5	23.9	17.2	19.3	26.3	24.3	20.4

*Corresponding to endothermic peaks in the water-starch (parts 2:1) system.
+Data in parentheses are standard deviations.

Table 2. Correlation coefficients among physical parameters (%E: % elongation; EM: expressible moisture; F: firmness; WF: work to fracture; %R: % relaxation; Tp: endothermic peaks temperature; %V: % volume density of starch; WI: whiteness index). The values are multiplied by 1000. Significance of correlation coefficients is given for 5% (882 and -885) and for 1% (-728, -733 and -779).

	EM	F	Tp	WI	%R	WF	%E	%V
EM	1000							
F	-404	1000						
Tp	**882**	-405	1000					
WI	-70	-542	91	1000				
%R	**-779**	24	-454	539	1000			
WF	**-885**	628	**-728**	-115	555	1000		
%E	-622	-240	**-733**	-269	455	249	1000	
%V	-502	-242	-311	77	588	338	287	1000

amylose. Amylomaize starch endotherm begins at 70°C and progresses up to 105°C.

Wootton and Bamunuarachchi (1980) reported that salt, sucrose, and sorbitol affected starch gelatinization. In the surimi gels as shown by Wu *et al.* (1985) and Yamashita and Yoneda (1989), gelatinization temperatures of the system "surimi-sucrose-sorbitol-salt and starch" shifted up to about 8 to 15°C. Thus, steaming the surimi gels at 100°C was enough to cover the range of starch gelatinization temperatures in the heat-induced gel.

The correlation matrix is shown in Table 2. The expressible moisture is positively correlated ($P < 0.01$) with the peak temperature and negatively with the work to fracture. This is in agreement with the results of Niwa *et al.* (1988a) and Yamashita and Yoneda (1989) who also found such correlations. The percentage of relaxation, which represents the index of elasticity, is negatively correlated ($P < 0.05$) with the expressible moisture. Gelatinization temperature is negatively correlated

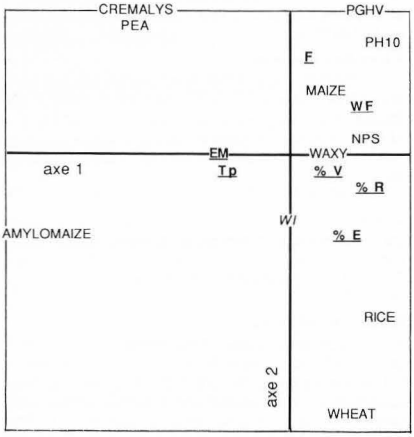

Figure 1. Samples on the first and second principal component plane. Starch abbreviations: NPS: native potato starch; PGHV: Clearam PGHV; PH10: Clearam PH10. Physical parameters are underlined (%E: % elongation; EM: expressible moisture; F: firmness; WF: work to fracture; %R: % relaxation; Tp: endothermic peaks temperature; %V: % volume density of starch; *WI*: whiteness index is a supplementary variable).

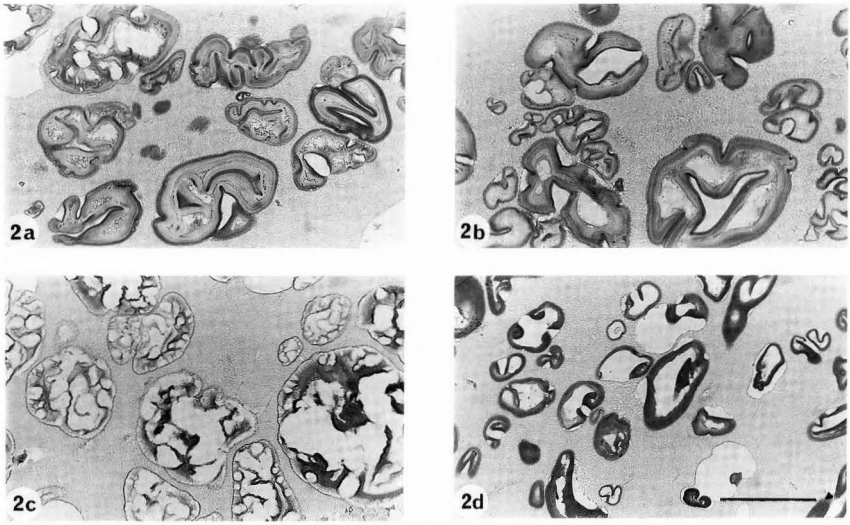

Figure 2. Light micrographs of surimi gels containing 5% potato starches: **a**: native starch; **b**: Clearam PH10; **c**: Clearam PGHV; **d**: Cremalys. Epon embedding before PAS staining. Bar = 100 μm.

($P < 0.05$) with the work to fracture and the percentage of elongation. So, when surimi gel is prepared with a starch that gelatinizes at high temperature, its texture characteristics are generally low, because of the lack of swelling.

Amylomaize starch brings a very low water-retention to the surimi gel. This result is also in agreement with the study of Kim (1986) who noticed that expressible moisture and penetration force increased with an increase in the amylose fraction due to increased retrogradation. But, in our case where the thermal treatment was at 100°C, the high EM for amylomaize is probably due to the low degree of swelling of the starch granules rather than to retrogradation of starch.

The total volume occupied by the starch granules after cooking does not show a correlation with the texture parameters (Table 2). On the other hand, Yamashita and Yoneda (1989) observed that whatever the size of potato starch granules (small, medium and large) and wheat starch granules (small and large), the physical properties (work to fracture, expressible moisture and whiteness index) of surimi gel containing 10% starch were relatively independent of the size of granules.

Original measurements on 10 samples (different types of starch) give a 10 x 7 data matrix (7 physical parameters considered) in which each sample can be represented by a point in a 7-dimensional space with parameters correlating with each other to varying degrees as shown in Table 2. As explained by Toda *et al.* (1971), by using the principal component analysis, it is possible to reduce the number of dimensions to a smaller number. Mathematically, the principal component analysis is equivalent to obtaining the latent roots and vectors of the correlation matrix among parameters.

The largest latent root of this matrix is 4.001, the second largest is 1.427 and the third largest is 0.926. These three components explain 90.8% of the total variance, i.e., 90.8% of the variance in the original 7-dimensional space is retained in the 3-dimensional space.

Parameters with a large contribution to the first component (57.1% of the total variance) are expressible moisture, peak temperature and work to fracture. Firmness and elongation contribute largely to the second component (36.8% of the total variance). By using the principal component analysis, the component scores of each sample can be calculated. They are plotted in Figure 1 where the X- and Y-axes represent the first and second components, respectively.

As can be seen clearly in Figure 1, a first group made up of Cremalys 516, pea, and amylomaize, easily distinguishes itself from the others by its high values for the first component (high values of expressible moisture, high peak temperature, and poor work to fracture). A second group, made up of waxy maize, native potato

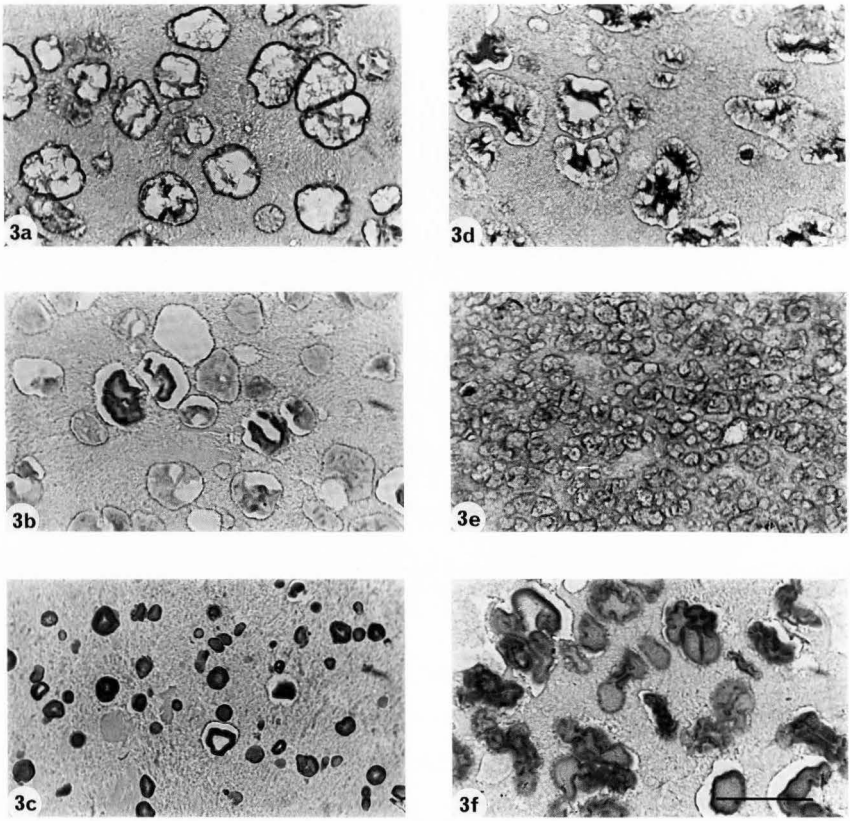

Figure 3. Light micrographs of surimi gels containing 5% cereal and legume starches: **a**: Waxy maize; **b**: normal maize; **c**: amylomaize; **d**: wheat; **e**: rice; and **f**: smooth pea. Epon embedding before PAS staining. Bar = 50 μm.

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starch, normal maize, Clearam PH10, and Clearam PGHV, cannot be differentiated easily on the basis of either the first or the second component. A third group, including rice and wheat, has high values for the second component (high elongation values).

These results show that the multivariate data can be used to make a classification. Starches can be classified into three groups according to their specific effects on the physical parameters of surimi gels. The gelatinization temperature, depending on the type of starch, is an important parameter (on the variability brought about by the addition of starch) of the textural properties of surimi gels.

Structural study

As Kim *et al.* (1987) observed through a microscope, gelatinization of the starch granules in heat-induced surimi systems was restricted. Figures 2 and 3 show that starch granules are generally uniformly dispersed in the gels. However, depending on the starch

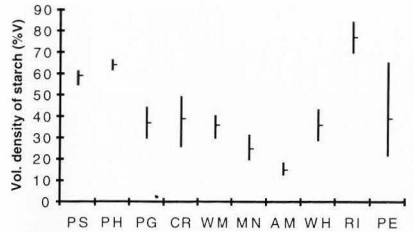


Figure 4. Representation of starch swelling rates in heat-induced surimi gels. Vertical bars represent minimum and maximum values and horizontal marks give levels of the mean values calculated from 5 replicates. Starch abbreviations: AM: amylomaize; CR: Cremalys; MN: normal maize; PE: smooth pea; PG: Clearam PGHV; PH: Clearam PH10; PS: native potato; RI: rice; WH: wheat; WM: waxy maize.

studied, some samples showed only a few variations in the measurements of the starch filling. With some of the others, and especially with pea starch, very important variations were observed from one field to another. Figures 2 and 3 are representative of the predominant aspects shown by each sample but most significant data are given in Table 1 where %V is the mean values of 5 image analysis. These mean values are marked by a bar on vertical lines given in Figure 4. For each sample, the minimum and maximum values are given by the limits of the vertical line.

Native potato starch (Fig. 2a) and esterified and reticulated potato starch PH10 (Fig. 2b) had some structural similarities. Starch granules in both samples appeared swollen and distorted. A thick layer of dense material remained at the periphery of the granules and also outlined a central gap. Between these denser parts, a granular material could be observed.

Structure of acetylated potato starch PGHV (Fig. 2c) appeared more alveolated with numerous vacuoles; conversely, emulsified and complexed potato starch Cremalys 516 (Fig. 2d) swelled more irregularly; the remaining dense material in the latter always appeared structured such as double brackets.

Maize starches also swelled differently according to the varieties. Waxy maize (Fig. 3a) showed a vesicular-like structure filling up the starch granules and, most of the time, more condensed material at the periphery of the granules. Normal maize starch granules (Fig. 3b), whose content was more homogeneous than the content of swollen waxy starch granules, developed few vacuoles. Amylomaize starch (Fig. 3c) was practically unswollen. Wheat starch (Fig. 3d) was composed of two populations of granules. After swelling, starch looked very particular, with specific star-shaped remnant material inside the granules. Rice starch was constituted of very small granules which swelled like the waxy maize starch. But, as seen on Figure 3e, there was much more expansion than with the other starches in the heat-induced gel. Smooth pea (Fig. 3f) gave the most heterogeneous gel, the volume occupied by granules varied widely from 22 to 65% (mean value 39%); structure of the swollen granules was similar to the starch granules of other legume starches and showed a festoon-like outline, a dense content, and a few vacuoles.

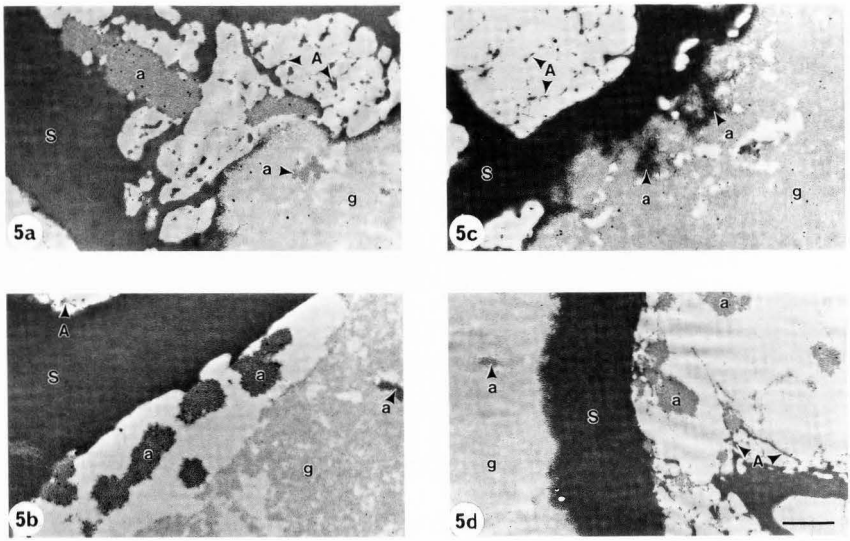
Ultrastructural study

Interactions between starch granules and kamaboko gel can be studied more accurately in a transmission electron microscope with help of contrasting methods developed for carbohydrates (Gallant, 1974). When water intake is sufficient, starch swells considerably and irreversibly, very deep structural and chemical transformations, as described by Leach *et al.* (1959) and more recently by Langton and Hermansson (1989), Autio (1990), Svegmark and Hermansson (1991), and Svegmark (1992), may occur. When there is not enough water intake to achieve the entire swelling, parts of starch granules do not swell so much and are apparently unchanged (darker material marked "S" in Figs. 5 and 6). These parts were thicker in native (Fig. 5a) and reticulated (Fig. 5b) potato starches, and their porosity was evidenced by minute clear areas. In the case of acetylated potato starch (Fig. 5c), they were thinner with a few pores in denser material; in addition, on the outside of the granules, deep indentations occurred which suggest material loss. Porosity was higher in emulsified and complexed potato starch granules (Fig. 5d). Compared to potato starches, and with exception for amylomaize (Fig. 6c), the other starch samples showed very thin peripheral dense layer (Figs. 6a and 6f); this peripheral material was absent in the other cases (Figs. 6b, 6d and 6e).

Inside the swollen starch granules, vacuoles were composed of numerous small vesicles (v) outlined by amylopectin network (A); it may also be seen with some small, somewhat spherical amylose precipitates (a); such precipitates, which were also observed in the protein matrix (g), were subsequent to amylose leaching.

Many times, white, empty areas were seen inside and around the starch granules observed under the light microscope. These areas are particularly well developed in Figures 3b, 3c and 3f. In wheat (Fig. 7a), native potato (Fig. 7b), or reticulated potato starch (Figs. 7c and 7d), fractures in cryo-SEM of surimi heat-induced gels revealed the presence of granular material filling in these areas. This material was probably solute material as it remained stable after deep freezing. Around the starch granules, these areas might result from retraction of the protein system during cooking or from shrinkage of starch granules when a part of the water intake absorbed for swelling returns to the protein gel.

As noticed with Figures 2 and 3, starch granules remained individual, randomly distributed inside the surimi gel and, with the exception to the very small and numerous rice starch granules (Fig. 3e), relatively well distant from each other. It was hypothesized (Lee and

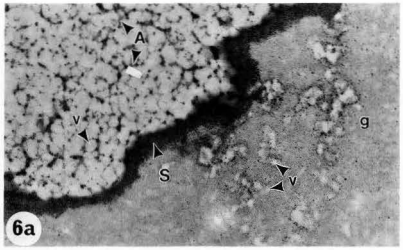


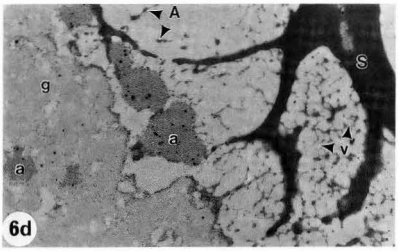
Figures 5 (above) and 6 (at right). Transmission electron micrographs of surimi gels containing 5% potato starches (**Figure 5**: **a**: native potato; **b**: Clearam PH10; **c**: Clearam PGHV; and **d**: Cremalys); and 5% cereal and legume starches (**Figure 6**: **a**: waxy maize; **b**: normal maize; **c**: amylomaize; **d**: wheat; **e**: rice; and **f**: smooth pea). PATAg staining before Epon embedding. a: amylose; A: amylopectin; g: heat-induced surimi gel; S: unswollen parts of the starch granule; v: vesicles. Bars = 1 μm.

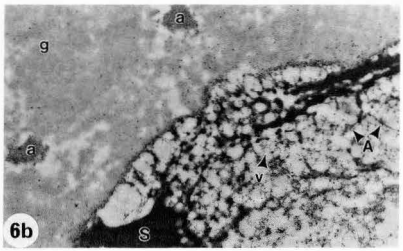
Kim, 1986) that starch granules could act as elastic globules (mass). In fact, as demonstrated here and depending both on the swelling power and the starch structure, part of starchy material leached out of the starch granules and led to the formation of intergranular connections. These carbohydrates were seen either as oligosides (black dots), vesicles (v) and/or amylose precipitates (a). Amylomaize, which is known to be resistant to swelling, leached out small amount of material (Fig. 8a), but staining showed also short-chain carbohydrates (o) inside the protein gel. Native potato starch (Fig. 8b), as well as most of other starch granules (Figs. 5 and 6) developed amylose precipitates (a) in addition to oligosides. These formations did not appear in the case of swollen waxy maize starch (Figs. 6a, 8c and 8d) which is free of amylose. On the contrary, vesicles (v) which formed inside the starch granules during swelling were detached from the granules and scattered in the protein gel (Figs. 8c and 8d). These detached vesicles bound one another.

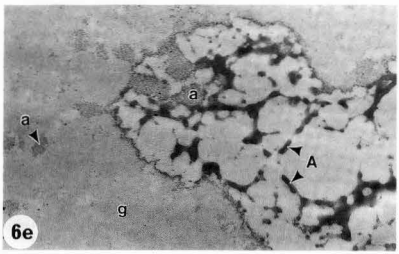
According to Okada (1963) and Kim and Lee (1987), the composite reinforcing effect of starch in the surimi gels may be explained by the following theory: the starch granules embedded in the protein gel absorb water from the matrix and compress the matrix as they swell during cooking. At the same time, the protein matrix loses moisture and becomes firmer.

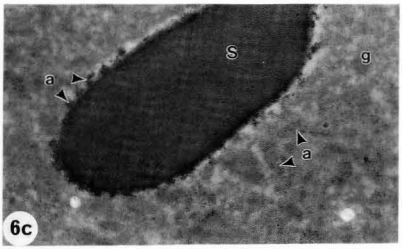
Niwa *et al.* (1988b, c) indicated that the swollen gelling substances would cause the elasticity to increase by decreasing the water content within the network structure, after all the contribution of the gelling substances to the muscular protein network structure seemed not always indispensable for the gel-strengthening action. The same authors also found that at the transition temperature measured by thermal scanning rigidity monitor (TSRM), a gel network system was already formed in the actomyosin sol accompanied by an increase in the rigidity of the system. When actomyosin-starch combinations are heated, the increase of rigidity is related to the starch granule swelling during gelatinization. That

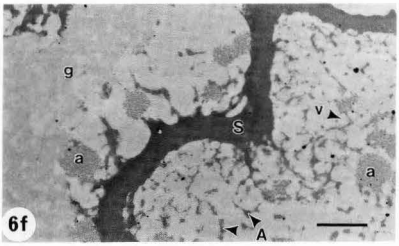










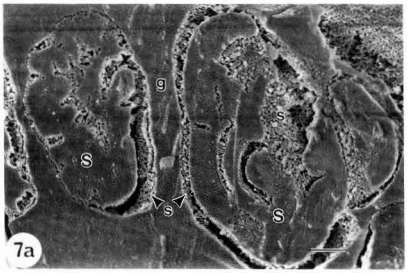


could be true up to a definite degree of swelling and Lee *et al.* (1992) suggest that maximum gel reinforcement by starch is achieved at moderate swelling of the starch granules. It seems that starches and proteins react separately during thermal processing. During the thermal gelation of proteins, water seems to be entrapped in the gel network in such a manner that water availability for starch gelatinization is limited. That could confirm the observation of Comer *et al.* (1986) that the formation of a starch matrix is not required for textural contribution from starch fillers in a meat protein system. It may be possible that carbohydrate leaching and binding has a real influence on the textural properties of heat induced surimi gels.

Conclusions

The results of this study indicate that the gelatinization temperature (which depends on the type and source of starch) was well correlated with the expressible moisture, work to fracture and elongation, but there was no correlation between texture properties and the volume fraction of partially swollen starch granules.

Starches can be classified into three groups according to their specific effects on the functional properties of surimi: rice and wheat for giving high elongation values for surimi/starch gels, waxy and normal maize, native potato starch, and modified potato starch (Clearam PH10 and PGHV) for their good gel-strength-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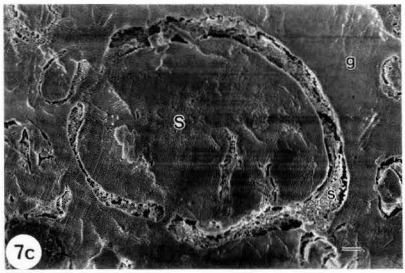
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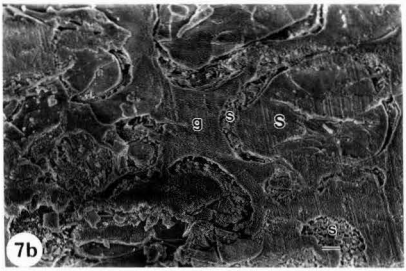
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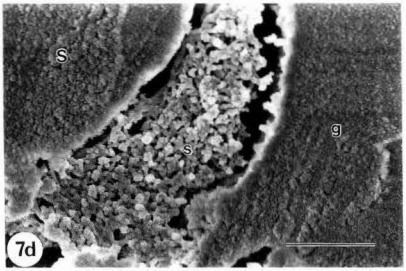
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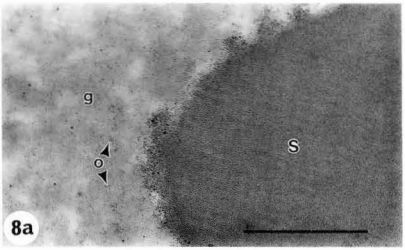
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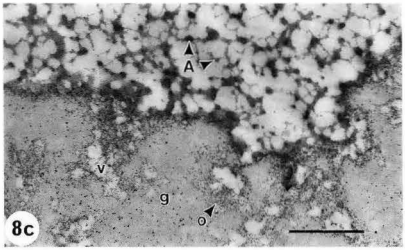
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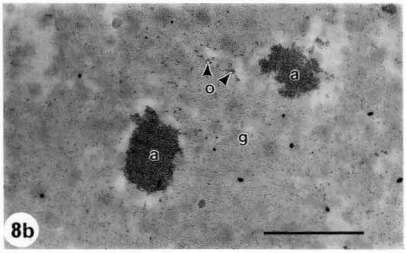
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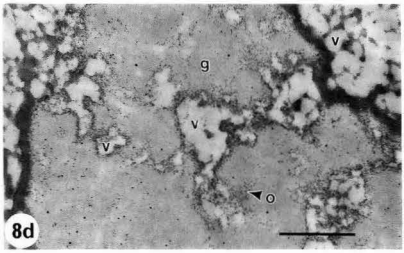
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Figure 7. Scanning electron micrographs of surimi gels containing 5% cereal and potato starches: **a**: wheat; **b**: native potato; **c** and **d**: Clearam PGHV. g: heat-induced surimi gel; s: solute; S: starch. Bar = 10 μm.

Figure 8. Transmission electron micrographs of surimi gels containing 5% cereal and potato starches: **a**: amylomaize; **b**: native potato; **c** and **d**: waxy maize. PATAg staining before Epon embedding. Focusing on the protein matrix shows interactions between surimi gel and carbohydrates which leached out the starch granules. a: amylose; A: amylopectin; g: heat-induced surimi gel; o: oligosides; S: heated starch; v: vesicles. Bar = 1 μm.

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ening effect and water retention capacity, and pea, amylomaize, and Cremalys 516 for their poor work to fracture, high expressible water and high gelatinization temperature.

We have shown from microscopical observations that some of the starchy material leached out of the starch granules. Depending on the nature of the starch, leached amylose or detached material may bind the starch granules in the intergranular space. Structures, which we called amylose and amylopectin by analogy with other studies, are macromolecular constituents which are known to be incompatible at temperatures above 90°C when the starch concentration is greater than 3%.

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Discussion with Reviewers

A.-M. Hermansson: Some of the conditions used for the texture measurement are questionable. Why was breaking stress measured during penetration of a 5 mm plunger and not as the stress at fracture during the tensile measurement, which is a more well defined test?

Authors: We have not determined a value of breaking stress as a textural property, but we have used the penetration test to determine the gel strength (breaking stress x breaking force). Gel strength results to energy necessary to break the gel and penetration measurement is a test generally used to determine the quality of surimi.

A.-M. Hermansson: Only one fish quality was used in the investigation. Would the same results have been obtained also with other fish raw materials?

Authors: The quality of surimi which depends on numerous factors such as quality of raw fish, fish handling, surimi processing and conservation and also on the species of fish used, influences the values of measured parameters. But the subject of our study was to determine the influence of the kind of starch in a surimi/starch product. Several authors have reported the same effects of the potato starch though they have not used the same quality and origin of surimi. So, we think that the differences of behaviour of starch (textural properties and microscopical observations) should be almost the same in another type of surimi.

A.-M. Hermansson: How do the authors differentiate between amylose (a), amylopectin (A), vesicles (v) and oligosides (o) in the TEM micrographs? What are the so-called vesicles composed of?

J.M. Kim: Can you explain what are those vesicles formed inside the starch granules during swelling and detached, and eventually scattered in the protein gel?

Authors: Theoretically, there are two different ways to differentiate components of each other in TEM micrographs: a) by cytochemical reactions when it is known that chemical functions may react specifically with markers or contrasting materials; b) by structural features when some components present particular shape, and especially when it can be associated to size. In the present study, we used the PATAg reaction (specificity for the α-glycol groups) by which the carbohydrate components were evidenced by silver atoms in the surimi gel. Fragments of starch may disrupt from the starch granules and can be easily recognized by same contrast density as in unswollen parts of the starch granules. Except for waxy maize starch (amylose free), masses of about 1 μm in diameter, which we called "amylose" (a), were always located inside the swollen starch granules and in the intergranular space. They probably precipitated as amylose-fatty acids complexes. Such structures were similar to leaching material that Svegmark and Hermansson (1991, 1993), and Autio (1990) observed with iodine, as mentioned in the text.

When amylose has leached out of the starch granules, the remnant material inside the starch granules appeared alveolated and was similar to "amylopectin" (A) that the same authors evidenced also with iodine in their studies. The structures called "amylopectin" in the present study showed denser and smoother contrast than structures called "amylose". For waxy maize starch, alveolation was particularly developed and detached from starch granules, giving rise to vesicles scattering in the surimi gel. Thus, we also called such alveoles (inside the swollen starch granules) vesicles. These vesicles should be formed by very thin external shell made of amylopectin; they probably contain sugars at a low degree of polymerization because they remain turgescent and keep some water from the surimi gel.

Inside the surimi gel, very thin silver alignments and/or isolated dots are seen in the micrographs. They are signs of the presence of carbohydrates and can be interpreted as short carbohydrate chains [oligosides (o) or dextrins].

J.M. Kim: What do you think about the thick or thin peripheral dense layers of the swollen starch granules?

Authors: There are several steps in starch swelling. Just below the swelling temperature, amylose leaches out of the starch granule. In the central part of the granules, hydrothermal breakdown occurs and soluble carbohydrate chains are formed. Then, a large amount of water penetrates the granule which swells from its center. At temperatures above 90°C, when there is excess water, the starch granule can swell considerably, the outer shell becoming thinner and thinner. It can disrupt, laying some ghosts in the gel or can solubilize totally. When there is not enough water, the starch granule cannot swell so much and the outer part remains larger. Peripheral dense layers of the swollen starch granules act as semi-permeable membrane. Svegmark and Hermansson (1993), who studied the effect of adding amylose to a high-swelling granular potato starch system, showed that the outer layer of the potato starch granules seemed to act as a restraint on the diffusion of amylose both into and out of the granules.