

Light and Scanning Electron Microscopy of Wheat - Paste Dough and Bread

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ABSTRACT. Light (LM) and scanning electron (SEM) microscopy were used to follow the changes that occur during dough mixing and baking and to compare the structure of wheat and mixed wheat - date paste doughs and bread. Date paste (4, 8 and 12%) was used as a partial replacement for wheat flour in the bread dough formula.

The well developed gluten protein in doughs containing 4% date paste or 3% sucrose were identified by microscopic examination. Rupture and discontinuities in the 12% date paste supplemented doughs were also apparent.

Crumb examinations indicated gelatinization. The association between starch and gluten protein and the liberation or separation of the starch granules from the protein matrix could be seen. Whereas adding 4% date paste to the bread formula improved the bread crumb structure. 12% paste had an adverse effect.

Including sucrose in local bread formulae is known to improve the product. There is no sugar industry in Saudi Arabia and in most other date producing countries, and sucrose or other sugar sources must be imported. Readily available dates are a good source of sugars (78%), dietary fiber (7%), minerals and trace elements (Yousif *et al.* 1991a). Hence it seems prudent to utilize dates as a sugar substitute and/or as fermentation substrate in bread formulae.

The structure of the bread dough and crumb has been the subject of numerous studies (Pomeranz 1976, Angold 1979, Evers 1979).

Previous investigations by light, scanning and transmission electron microscopy (Sandstedt *et al.* 1954, Khoo *et al.* 1975, Lopez and Bushuk 1983, Pomeranz *et al.* 1984, Pomeranz and Meyer 1984, Bechtel, 1985) showed that dramatic changes took place in the structure of both dough and bread as a result of including large amounts of additives in the basic water - wheat flour dough. Supplementation of bread dough with single cell protein (Evans *et al.* 1977) thickened gluten, masked starch granules contours and enhanced gluten rupture. On the other had, adding emulsifiers, improved gluten sheeting. The addition of soya flour, salt, shortening and sugar to water - flour doughs altered the dough structure. Sugar, in particular, aided dough development by reducing the space around starch granules and changing the protein from a network to a sheet - like system (Fretzdorff *et al.* 1982). More recently, Moss (1985) used LM and SEM to study the effect of wheat germ on dough gluten development. Increased levels of germ in wheat flour produced doughs with over developed gluten which, after baking, gave breads with a coarse texture and low volume.

Chabot *et al.* (1979) compared various sample preparation procedures for SEM analysis of bread. They included several commonly - used methods of drying and fixation, followed by chemical dehydration: their effects on the ultrastructure of white bread were evaluated. The method of specimen preparation profoundly influenced bread crumb structure. The freeze drying - technique was the best since it altered least the structure breakdown.

No information is available on the effect of date paste on the structure of dough and bread. Accordingly, the objective of this study was to use LM and SEM to follow changes that take place during dough mixing and baking and to compare the structure of wheat and mixed wheat - date paste doughs and bread.

Materials and Methods

Flour: A commercially milled wheat bread flour was obtained from Allied Mills (Uxbridge, London). It is sold under the trade name of Sovereign flour. Some of the flour characteristics (at 14% mb) were: extraction rate, 75%; protein, 12.4%; moisture, 13.2%; ash, 0.54%; damaged starch, 28% Farrand: mixing time, 2.33 min and water absorption, 63.7%.

Date paste: Dates of the Ruzeiz variety were purchased from Nadic Date Factory (Alhasa, Saudi Arabia). The dates were cleaned, destoned and soaked in tap water for 5 min, allowed to drain for 10 min and ground in a meat grinder. Some of the

Table 1. A sample of baking test record showing the basic formula and the physical properties of the bread dough and the loaf produced

	Dough	Control (CI)	4% Date Paste	8% Date Paste	12% Date Paste	Control + 3% Sucrose (CII)
Basic formula (amount and description of ingredients)	Flour (g)	1000	960	920	880	970
	Salt (g)	18	18	18	18	18
	Yeast (g)	25	25	25	25	25
	Fat (g)	7	7	7	7	7
	Water (ml)	600	580	550	500	580
	Improver* (mg)	100	100	100	100	100
	Date Paste (g)	-	40	80	120	30g sucrose
	Total Wt (g)	1650	1630	1600	1550	1630
	Watts/hr/Input	18.10	17.90	17.60	17.10	17.90
	Flour temperature (°C)	22	22	22	22	20
	Water temperature (°C)	35	25	25	25	25
	Mixing time (secs)	314	324	360	367	310
	Dough temperature (°C)	30	30	30	32	31
	Dough weight (g)	1632	1605	1557	1520	1602
	Scaling weight (g)	460	460	460	460	460
	1st mould at (hr)	10.25	10.54	11.15	11.34	8.55
	2nd mould at (hr)	10.36	11.04	11.25	11.44	9.05
	Intermediate time (min.)	10	10	10	10	10
	Proof temperature (°C)	31	31	31	31	31
	Final proof commenced (hr)	10.27	11.07	11.27	11.45	9.06
	Finished (hr)	11.10	11.42	12.04	12.30	9.43
	Proof time (min)	33	35	37	45	37
	Proof temperature (°C)	41	42	42	42	41
	Mean proof height (cm)	11	11	11	11	11
	Oven temperature (°C)	230	230	230	230	230
	Set in oven at (hr)	11.10	11.42	12.05	12.32	9.43
	Baking time (min)	25	25	25	25	25
	Loaf height (cm)	13.1	14	13.9	12.6	13.2
	Oven spring (cm)	2.1	3	2.9	1.6	2.2
	Loaf volume (ml)	1465	1585	1565	1440	1540
	Specific volume (ml/g)	3.67	3.97	3.88	3.50	3.92

*Ascorbic acid

date paste characteristics (on dwb) were: total sugar calculated as 78.2%: ash 3%: and dietary fiber 7%. The paste had a moisture of 18.8%, pH 5.96 and water activity of 0.66.

Baking procedure: Doughs and bread were prepared according to a modified Chorleywood Bread Process (Jain 1975), The baking formula (Table1) included: 1000g flour, 18g salt, 25g fresh compressed yeast, 7g fat, 100mg ascorbic acid, water as needed (the required amounts of water, after adjusting their temperature, as determined by the research water absorption meter were added). Doughs were prepared with 3% sucrose or 0, 4, 8 or 12% date paste based on flour.

Combiend ingredients were mixed with an energy input of 11 Watt/hour/kg dough. The dough was scaled to 460g samples and moulded using a small commercial moulder. Dough samples were proofed for 10 min at 30°C, moulded, panned and proofed at 43°C and 80% RH until the pieces attained the height of 11cm, and were baked at 230°C for 25 min.

Microscopy samples: Small pieces of fresh dough (immediately after mixing) and bread crumb (cut immediately after baking) were frozen by immersion in liquid nitrogen. The frozen samples were freezedried at - 70°C.

Light microscopy technique: Samples for light microscopy were fixed using 4% phosphate buffered gluteraldehyde at pH 6.8 (Moss 1985). Samples were embedded into molten wax by a gentle vacuum infiltration. Sections (8 um thick) were cut using a microtome, mounted on gelatinized slides, dewaxed and stained with iodine to identify starch (Cook 1974) and with xyloidine ponceau to identify protein (Pomeranz and Shellenberger 1961). The sections were observed under model K. Zeiss photomicroscope and photographed.

Scanning electron microscopy technique (SEM): For SEM, the samples were mounted onto SEM stubs with a special M- glue (Agar Aids, Essex, England) and were sputter coated with gold. The preparations were viewed and photographed in a JSM - 258 scanning microscope at an accelerating voltage of 15 kv.

Results

Light microscopy of freshly mixed dough: The effects of adding sucrose or date paste on the freshly mixed wheat bread dough structure are illustrated in Figs. (1-4). A strong interaction between the starch granules (S) and the protein matrix (PM) is evident. Both small (B) and large (A) granules participate in the protein - starch association. Figures 2 and 3 also show that the protein matrix (PM) of the 3% sucrose and 4% date paste containing doughs is more developed than that of the

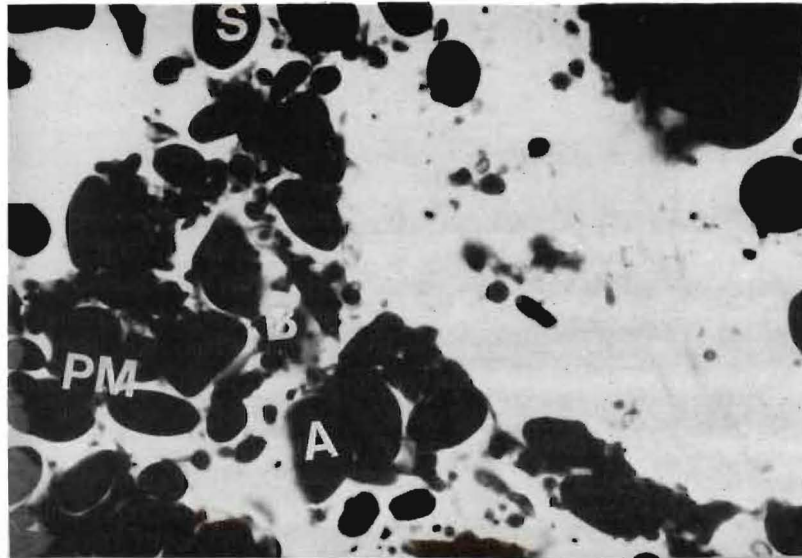


Fig. 1. Light micrograph of the sugar-free control dough (magnification = 320 x).
S = starch; PM = protein matrix; B = small starch granule; A = large starch granule

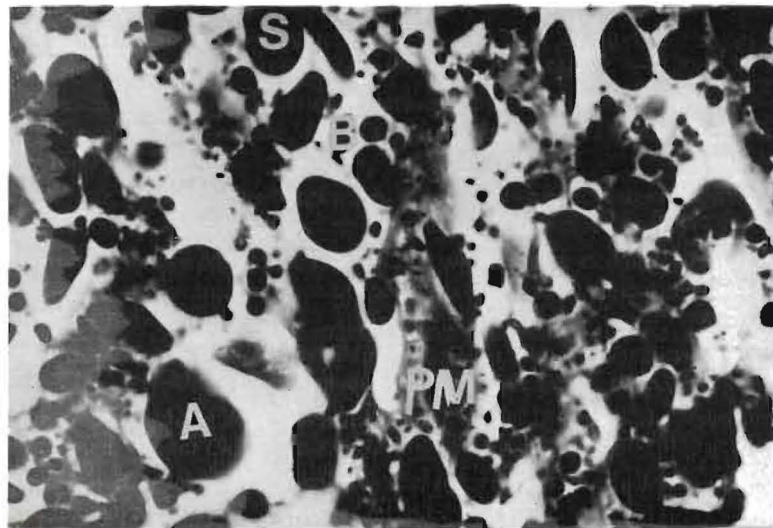


Fig. 2. Light micrograph of sucrose (3%) control dough (magnification = 320 x).
S = starch; PM = protein matrix; A = large starch granule; B = small starch granule



Fig. 3. Light micrograph of 4% added date paste dough (magnification = 400 x).

S = starch; PM = protein matrix; A = large starch granule; B = small starch granule

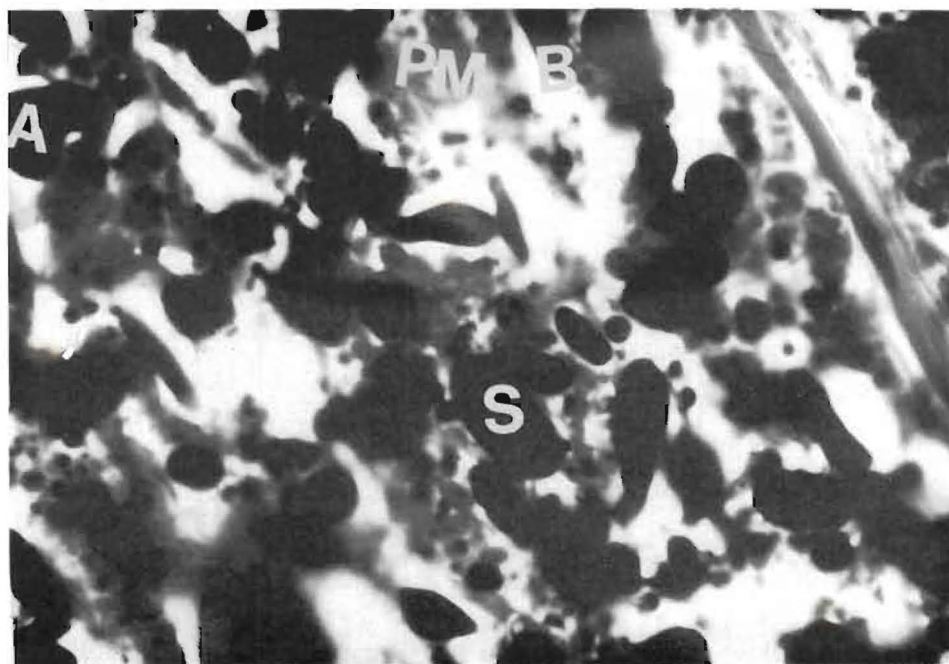


Fig. 4. Light micrograph of 12% added date paste dough (magnification = 400 x).

S = starch; PM = protein matrix; A = large starch granule; B = small starch granule

sugar-free control dough (Fig. 1). The less intensely stained protein and its improved continuity and sheetability are evident. Figure 4. Which represents the light micrograph of the 12% added date paste raw dough shows a more intensely stained protein, indicating discontinuity and rupture of the protein matrix. Scanning electron microscopy of raw dough: Examination of the freshly mixed sugar free control reveals a mass of starch granules bound by a continuous gluten matrix (Fig. 5). Whereas in some areas the gluten formed a thin film (CN) that conformed with the shape of the starch granules (continuous network), in other areas the gluten was thicker and had a sheet-like character (SL). Discontinuities in the protein matrix (PD) could also be seen in some areas in Fig. 5.

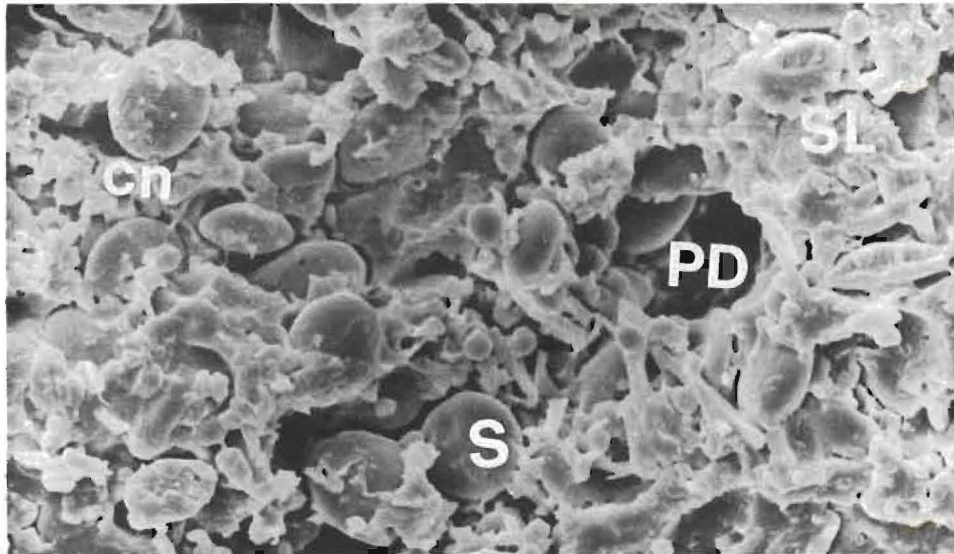


Fig. 5. Scanning electron micrograph of the sugar-free control dough (magnification = 750 x).
cn = gluten thin films; SL = sheet-like gluten; PD = protein discontinuities; S = starch

Gluten in doughs containing 4% added date paste or 3% sucrose developed into a continuous smooth network (CN) which conformed to the surface contours of the starch granules (Fig. 6 and 7). The gluten sheet (GS) was extremely thin and translucent, in some areas starch granule silhouettes were visible beneath the protein veil. These improvements in gluten sheetability, continuity and cohesiveness over the sugar-free control (Fig. 5) might be due to the sugar effect present in these doughs and its role in controlling the balance between extensibility and elasticity.

The greatest difference (*vs* the control) in the freshly-mixed dough structure can be seen in the dough containing 12% date paste (Fig. 8). The gluten structure was severely altered by increasing the level of date paste up to 12%. The gluten

sheets were very thin in some areas (TN) and thick in other areas (TC) and lost their smooth appearance (see control in Fig.5).

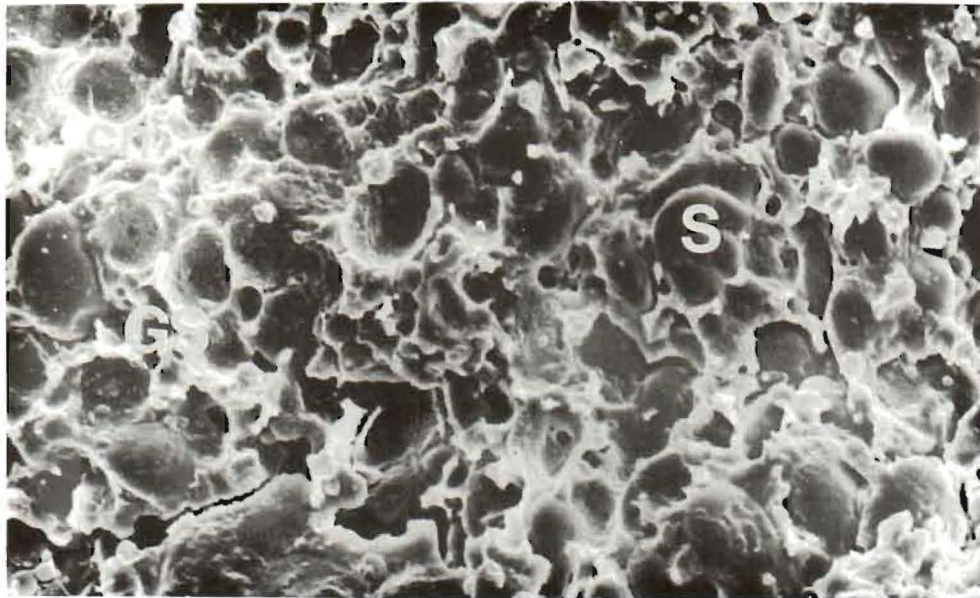


Fig. 6. Scanning electron micrograph of the sucrose (3%) control raw dough (magnification =750 x).
cn = gluten continuous network; GS = gluten sheets; S = starch



Fig. 7. Scanning electron micrograph of 4% added date paste dough (magnification = 750 x).
cn = gluten continuous network; GS = gluten sheets; S = starch

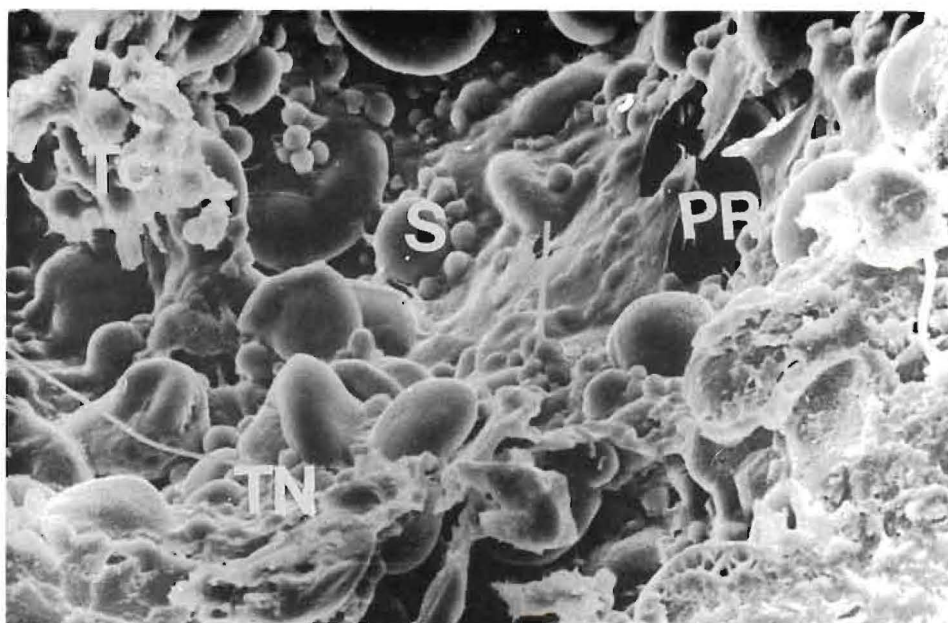


Fig. 8. Scanning electron micrograph of 12% added date paste dough (magnification = 750 x).

S = starch; TN = gluten thin sheets; TC = gluten thick sheets; PR = protein rupture

Breakdown of the gluten sheet (Fig.8) and loss of its integrity were also characteristic of the raw dough containing 12% date paste. The most severe ruptures in the gluten sheet (PR) occurred at the starch - protein interface, indicating the adverse effect of the high levels of date paste on the balance of elasticity and extensibility of the dough and resulting in a dough which is too slack and sticky and very difficult to handle.

Light microscopy of the bread crumb: Figures 9-12 demonstrate the effect on the microstructure of the bread crumb of partial replacement of wheat flour with 3% sucrose, 4% or 12% date paste.

Both small (B) and large (A) starch granules were integral parts of the crumb structure (Fig 9 and 10). Although many starch granules (particularly small ones) remained intact (I), partially or even complete gelatinization (GL) distorted the shapes of the other granules.

Figures 9-12 further indicate that the degree of gelatinization in the bread was not uniform. It is apparent from Fig. 12 that starch granules in the 12% date paste crumb show the least degree of gelatinization, compared with the other treatments (Fig. 9, 10 and 11).

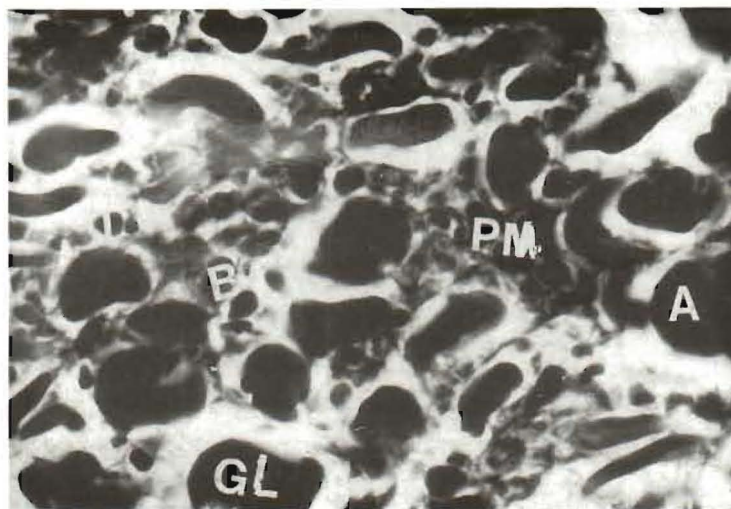


Fig. 9. Light micrograph of the sugar-free control crumb (magnification = 320 x).
A = large granule; B = small granule; PM = protein matrix;
I = intact granule; GL = gelatinized starch

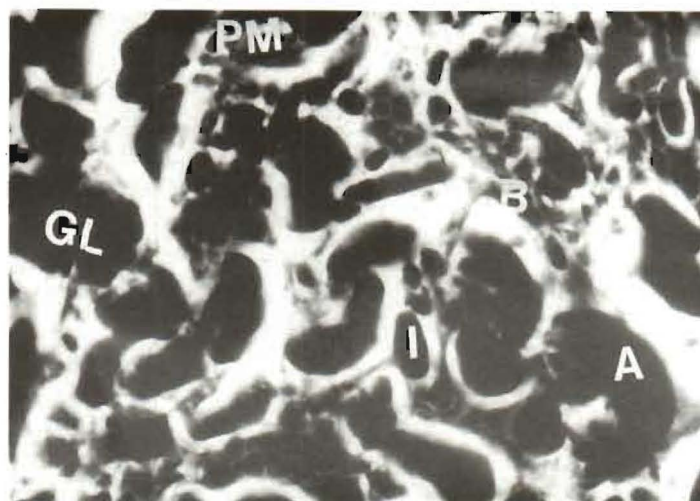


Fig. 10. Light micrograph of the sucrose (3%) control crumb (magnification = 320 x).
A = large granule; B = small granule; PM = protein matrix;
I = intact granule; GL = gelatinized starch

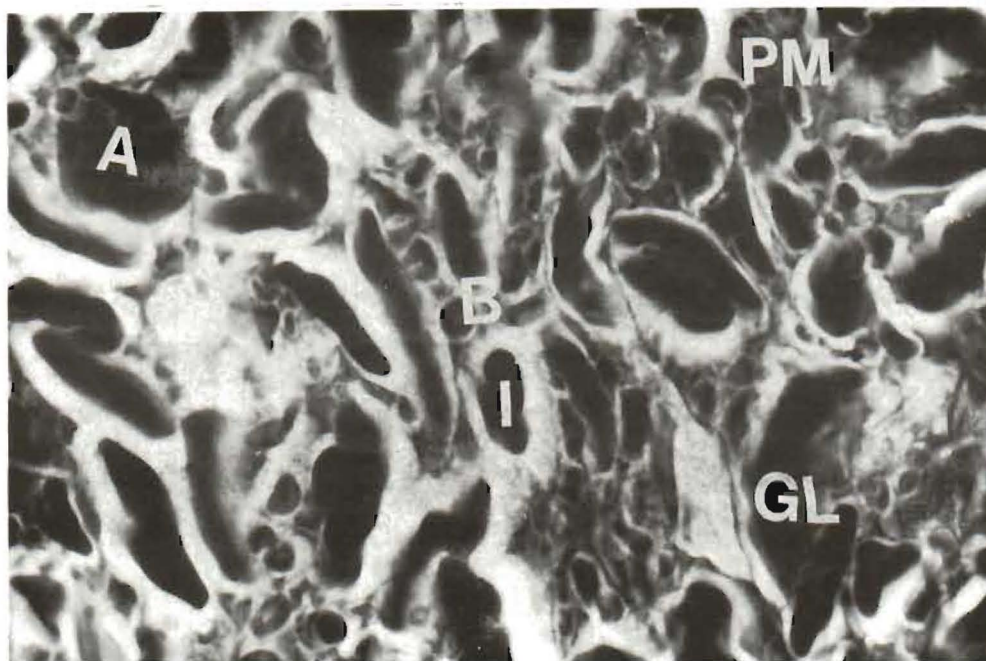


Fig. 11. Light micrograph of the 4% added date paste crumb (magnification = 400 x).
A = large granule; B = small granule; PM = protein matrix;
I = intact granule; GL = gelatinized starch

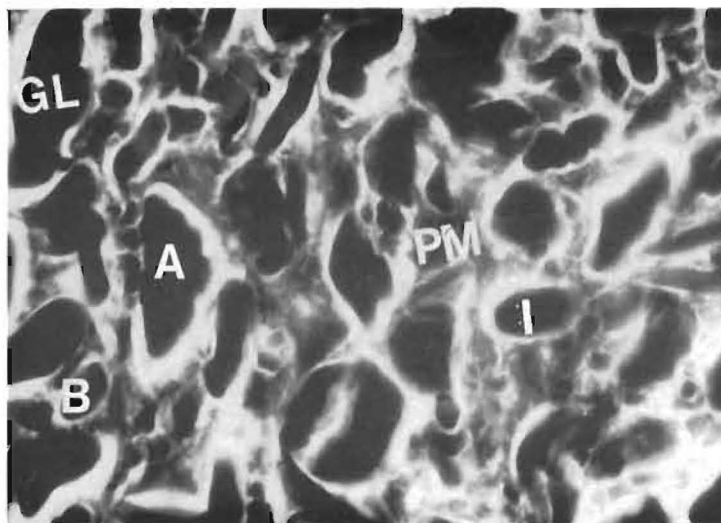


Fig. 12. Light micrograph of the 12% added date paste crumb (magnification = 400 x).
A = large granule; B = small granule; PM = protein matrix;
I = intact granule; GL = gelatinized starch

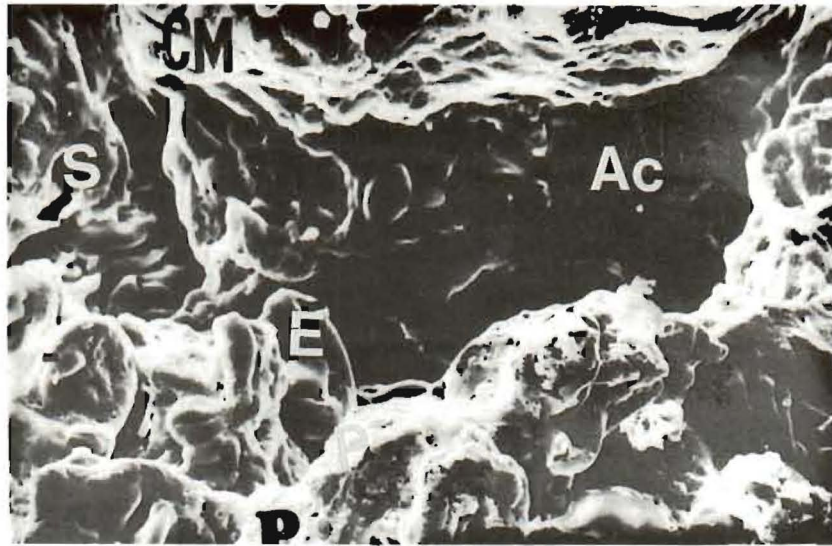


Fig. 13. Scanning electron micrograph of the sugar-free control crumb (magnification = 750 x).
 S = starch; P = protein; AC = gas cell;
 CM = veil-like protein and underlying starch forming cohesive mass;
 E = signs of enzymatic digestion on the surface of starch granule

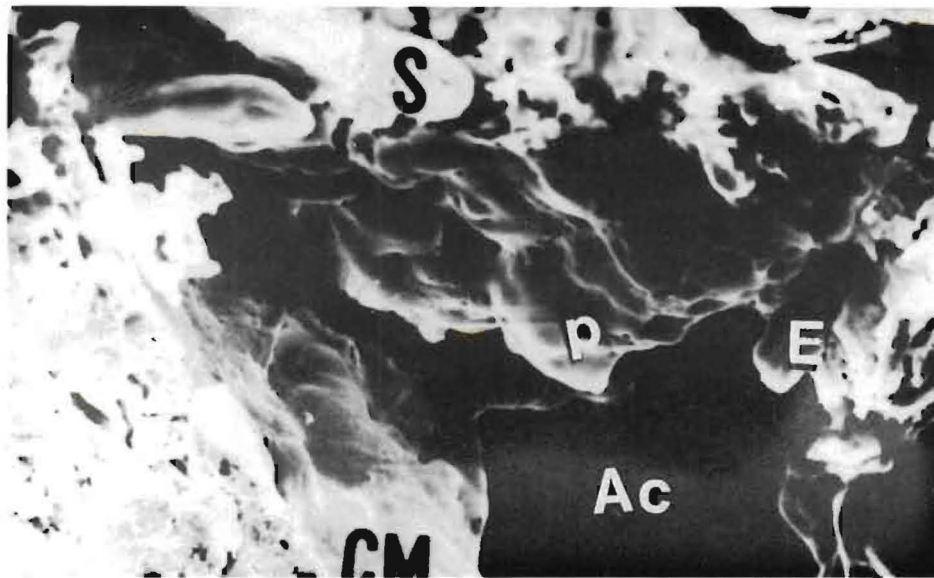


Fig. 14. Scanning electron micrograph of the sucrose (3%) control crumb (magnification = 750 x).
 S = starch; P = protein; AC = gas cell;
 CM = veil-like protein and underlying starch forming cohesive mass;
 E = signs of enzymatic digestion on the surface of starch granule

Scanning electron microscopy of bread crumb: The effect of replacing wheat flour with 4% or 12% date paste, or 3% sucrose (sucrose control) on the ultrastructure of the bread crumb is shown in Fig. 13 to 16.

As can be seen from the micrographs of the bread crumb (Figs. 13-15), large gas cells (AC) with thin walls are characteristic of wheat bread crumb. It is also worth noting that the starch (S) and protein (P) structure in bread crumb is essentially different from that in the dough. The protein bread crumb matrix appears swollen and fused (Fig. 13). In some areas, veil-like protein and underlying starch form a cohesive mass (CM). Furthermore, enzymatic digestion (E) of many starch granules creates interesting patterns within the granules (Figs. 13 and 16).

The starch-protein bridge over the gas cells in Fig. 15 reflects the strong interaction between starch and protein in the bread crumb. The thick protein strand (PS) in Fig. 16 indicates that the protein in the 12% date paste - containing dough is not completely developed and lacks continuity, as has been shown before (Fig. 8 and 12).

As in the light microscopy study, a wide variation in the extent of gelatinization can be noted between the bread crumb containing 12% date paste (Fig. 16) and the other breads (Fig. 13-15). Less gelatinization and liberation of starch granules (LS) from the protein matrix can be noted in the 12% date paste bread crumb.

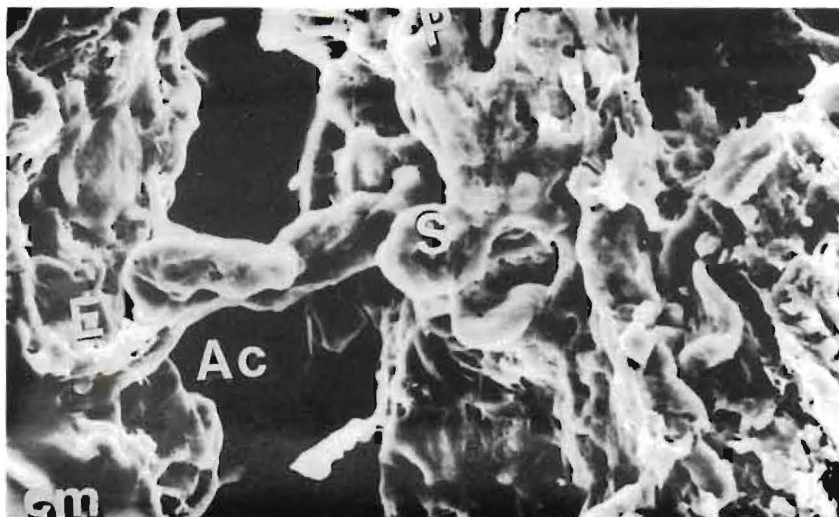


Fig. 15. Scanning electron micrograph of bread crumb containing 4% added date paste (magnification = 750 x).

S = starch; P = protein; AC = gas cell;

CM = veil-like protein and underlying starch forming cohesive mass;

E = signs of enzymatic digestion on the surface of starch granule

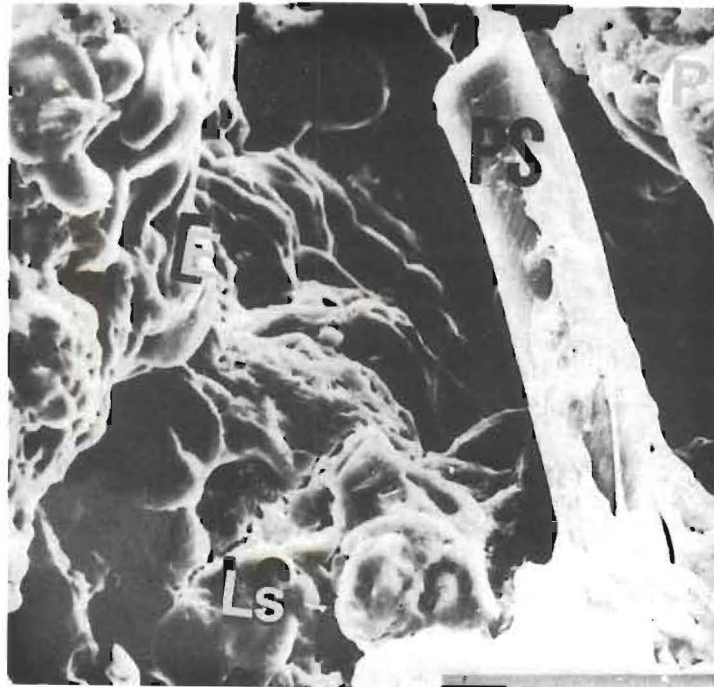


Fig. 16. Scanning electron micrograph of bread crumb containing 12% added date paste (magnification = 750 x).

LS = liberated starch; P = protein; PS = protein strand;
E = signs of enzymatic digestion on the surface of starch granule

Discussion

Two microscopy techniques, light (LM) and scanning electron microscopy (SEM) were used to follow changes in the structure of date paste supplemented dough and bread. Examination by the two microscopic techniques yielded consistent results. SEM identified modifications in the gluten protein film, the effect of enzymes on the starch granules, and the nature of the interaction between starch and protein. LM identified the interference of cell wall constituents on the gluten protein matrix, the extent of gluten protein development (*via* the intensity of staining), and the degree of starch gelatinization.

According to Moss (1972), that the extent of gluten development in bread dough can be identified by the staining intensity. Figures 1 to 4 indicate that 4% date paste or 3% sucrose enhanced gluten development. Many investigators (Khoo *et al.* 1975, Evans *et al.* 1977, Pomeranz *et al.* 1984, Moss 1985, Bechtel 1985) used SEM to

identify the nature of gluten development by observing the gluten film properties (such as length of the sheets, film thickness rupture or discontinuities). In this study, well developed gluten in doughs containing 4% date paste or 3% sucrose can be estimated from Figures 6 and 7. Rupture and discontinuities in the 12% date paste dough are apparent in Fig.4.

The bread crumb could be examined better, than the dough, by LM and SEM. Gelatinization is evident (Fig. 9 to 16). The association between starch and gluten and the liberation or separation of the starch granules from the protein matrix can be seen.

Finally, the microscopic examination is in good agreement with the rheological properties (Yousif *et al.* 1991b) and the test baking results (Table 1). The microscopic examination confirmed the improvement in dough and bread structure resulting from replacement of flour by 4% date paste or 3% sucrose. The microscopic examination also demonstrated the deleterious effect of high levels of date paste on gluten film properties and on the bread crumb structure.

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استخدام المجهر الضوئي والألكتروني لدراسة تأثير اضافة عجينة التمور

على عجينة ولبابة الخبز

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يقدر الفائض من التمور السعودي وخاصة تمر الدرجة الثانية في بعض المناطق كالجوف والقطيف وبيشة بحوالي مائتي ألف طن سنوياً . وعليه فقد تبني مركز أبحاث النخيل والتمور بجامعة الملك فيصل بالاحصاء برنامجاً بحثياً طموحاً لإستغلال هذا الفائض من التمور عن طريق تطوير منتجات جديدة كعجينة التمور وادخال هذه العجينة في العديد من الصناعات الغذائية كصناعة الخبز .

تبين من دراسة سابقة أن اضافة عجينة التمور كبديل جزئي للطحين في صناعة الخبز قد أدى إلى تحسين الصفات الريولوجية لعجينة الخبز وكذلك إلى تحسين صفات جودة الخبز . وللتعرف على الدور الذي تقوم به عجينة التمور في صناعة الخبز فقد استخدم كل من المجهر الضوئي والألكتروني لدراسة تأثير

إضافة عجينة التمور بنسب مختلفة (٤ ، ٨ ، ١٢ ٪ من وزن الدقيق على تركيب عجينة الخبز الطازجة والمتخمرة وعلى لبابة الخبز .

أمكن باستخدام الفحص المجهرى سواء الضوئى أو الألكترونى التعرف بسهولة على التحسن فى خواص الجلوتين فى خلطات الخبز المحتوية على ٤ ٪ عجينة تمور أو على ٣ ٪ سكروز ، فى حين اتسم جلوتين خلطات الخبز المحتوية على ١٢ ٪ عجينة تمور بالتمزق وعدم الإستمرارية .

أظهر الفحص المجهرى للبابا الخبز حدوث عملية الجلتنة للنشا وأمكن تقييمها بسهولة ، كما بين الفحص أيضاً الأرتباط بين النشا والجلوتين وانفصال وتحرر حبيبات النشا . لقد كان واضحاً أن إضافة عجينة التمور بنسبة ٤ ٪ إلى خلطة الخبز قد أدى إلى تحسين تركيب لبابة الخبز ولكن رفع نسبة العجينة إلى ١٢ ٪ قد أضر بتركيب اللبابا .