EFFECT OF YEAST PROTEIN CONCENTRATE ON BREADMAKING

EFFECT OF YEAST PROTEIN CONCENTRATE AND DRIED WHOLE YEAST ON EXTRUDATES PROPERTIES

ISOLATION OF FERMENTATION STIMULANTS FROM YEAST PROTEIN CONCENTRATE

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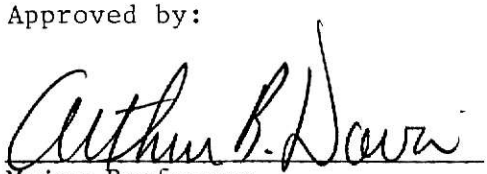

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GENERAL INTRODUCTION

The international production of yeast for human consumption started in Germany about 1910 with brewer's yeast which was separated from brewed beer, washed, debittered, and dried (Reed 1981). In the 1960's, recognition of a world wide shortage of protein led to interest in non-meat protein sources. Yeast was found to be one of the better sources for the following reasons:

1. Acceptance: Yeast has been consumed by humans for centuries in such food as bread and beer (Reed, 1981; Waslien, 1975).
2. Favorable economics of yeast production (Mateles and Tannenbaum, 1968; Sinskey et al, 1975).
3. Relatively small waste disposal problems (Waslien, 1975; Vananuvat, 1977; Lipinsky et al, 1970).
4. High efficiency in converting carbohydrates into protein (Vananuvat, 1977; Lipinsky et al, 1970).
5. Yeast is easily modified genetically to meet our needs (Waslien, 1975).
6. Independent of climate (Waslien, 1975; Vananuvat, 1977).

The chemical composition of yeast has been extensively studied. Detailed chemical composition of various yeasts grown on different media have been tabulated in several reviews (Reed, 1981; Waslien, 1975; Mateles and Tannenbaum, 1968; Vananuvat, 1977; Lipinsky, 1970; Sinskey et al, 1975). Chemical composition of yeast varies with specific variety, condition of growth, type of propagation, nutrient medium, growth stage, length of storage and storage temperature (Reed, 1981; Waslein, 1975; Martini et al, 1979; Ingledew et al, 1977; Mateles and Tannenbaum, 1968; Sinskey et al, 1975).

Average proximate composition of yeasts is about 45% protein (N x 6.25), 4% to 7% fat, 26 to 36% carbohydrates, and 5 to 10% ash (all values on dry basis) (Waslien, 1975). Fifteen to 20% of the nitrogen is from nucleic

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acids (Reed, 1981). Values given in different reviews vary due to production condition and yeast variety.

Yeasts contain all essential amino acids. They are especially high in lysine, the first limiting amino acid in cereals (Mateles and Tannenbaum, 1968; Waslien, 1975). Compared to other yeast, brewers yeast is higher in lysine but lower in tryptophan (Mateles and Tannenbaum, 1968). Yeast is a good source of the water soluble vitamins, except for vitamin C (Waslien, 1975; Mateles and Tannenbaum, 1968).

Much work has been reported evaluating the protein quality of yeast (Lipinskey, 1970; Waslien, 1975; Mateles and Tannenbaum, 1968; Ingledew et al, 1977; Kuzela et al, 1978). Most studies use the entire yeast cell. Little work has been done on yeast protein isolates or concentrates. Different Net Protein Ratio (NPU), Protein Efficiency Ratio (PER), Net Protein Utilization (NPU), True Digestibility (TD), and Biological Value (BV) have been reported for yeast protein (Mateles and Tannenbaum, 1968; Waslien, 1975; Vananuvat, 1977; Ingledew, 1977; Kuzela et al, 1978). This variability is partially due to different yeast grown on different media. Much of this difference may be due to different dietary formulation, test animals, and test periods (Bodwell, 1977, Vaghefi, 1974). In their review, Vaghefi et al (1974) explained how different dietary formulations, feeding levels, and feeding periods can mislead scientists. In Bodwell's book, Miller and Lachance (1977) discuss several methods of measuring nitrogen retention. They pointed out that precision is the common problem with all the methods. From previous studies, yeast was reported to have 77% to 92% digestibility, 0.7 to 1.7 PER value, 32 to 47 BV value, and 22.2 to 57 NPU. Waslien (1975) said that it is unwise to compare these data because different yeasts grown on different media were used in these tests. Besides, several of those studies did not present dietary formulas. Most of these studies showed fairly high TD and low PER,

BV values when compared with casein (Reed, 1981; Waslien, 1975, Mateles and Tannenbaum, 1968; Lipinsky et al, 1970; Sinsky, 1975). When protein synthesizing ability of skeletal muscle was used as a measurement of nutritional value, yeast protein isolate was found to be superior to disintegrated yeast, lypholized yeast and wheat gluten but still inferior to casein (Waslien, 1975). Kuzela et al (1978) reported that yeast protein concentrate had higher digestibility than dried yeast or casein. With 0.3% methionine supplementation, yeast protein concentrate has a BV of 80.9, even higher than that of casein (74.15). But its PER still was lower than that of casein. The work of Kuzela et al (1978) was consistent with previous studies using dried or fresh yeast (Waslien, 1975; Mateles and Tannenbaum, 1968; Vananuvat, 1977). The previous investigations showed that the addition of methionine doubled PER value. Biological Value was also increased in some cases. It is not surprising that protein concentrate has a higher digestibility than dried yeast. After the cell wall is ruptured, it can no longer serve as barrier to digestive action. The fact that yeast protein has high digestibility but low PER and BV which can be improved by fortification of methionine indicates that yeast protein has an inadequate amino acid balance. There are several factors influencing the nutritional value of yeast protein (including biomass). Mateles and Tannenbaum (1968) reported that dried yeast has higher nutritional value than fresh yeast. They suggest drying caused some changes in the cell membrane and improved digestibility. Burico (1978) claimed that the autolysis of yeast cells would improve the nutritional value of yeast protein. In his paper, Burico did not give any data to support his hypothesis. However, this hypothesis is a logical assumption because autolysis will result in degradation of RNA and protein as well as produce better flavor. RNA is believed to be one of the factors responsible for the adverse effect reported in feeding trials (Reed, 1981; Waslien, 1975; Mateles and Tannenbaum, 1968). Lysozyme

(autolysis) digests cell membrane which allows the cytoplasmic protein to leach out. As a result, the nutritional value of yeast protein is improved. Investigation showed that pelleting could improve the nutritional value of yeast meal (Vananuvat, 1977). Very likely, the improvement is a result of the combination of physical changes (such as rupture of cell wall or denaturing of the cell membrane) and chemical changes (such as inactivation of growth inhibitors). Heat treatment with alkali during extraction of yeast cytoplasmic protein resulted in hydrolysis of protein, depolymerization, racemization of amino acids, and formation of lysinoalanine. With the exception of protein hydrolysis, those reactions reduce amino acid content of the protein isolate as well as its nutritional value. In addition, free lysinoalanine was found to be hazardous to humans (Shetty and Kisella, 1980).

Many scientists have studied the safety of consuming yeast protein (Waslien, 1975; Mateles and Tannenbaum, 1968; Sinsky et al, 1975). Although there are reports describing yeast consumption by animals and human subjects without adverse effect, there are many other reports of gastrointestinal disturbance, abdominal pain, diarrhea, nausea, allergic reactions, liver necrosis, low growth rates in the second or third generation, sterility, liver and kidney lesions and death due to ingestion of yeast (Mateles and Tannenbaum, 1968; Lipinsky et al, 1970; Waslien, 1975; Sinskey et al, 1975; Vananuvat, 1977). There are unconfirmed theories for those adverse effects:

1. Diarrhea, gastrointestinal disturbance, vomiting and nausea might be due to unobserved contamination or simply because the subjects are unused to this new ingredient (Reed, 1981).
2. Sterility might be due to a shortage of vitamin E in the diet (Mateles and Tannenbaum, 1968; Sinskey et al, 1975).
3. Liver necrosis and kidney lesions might be due to an unbalanced diet (Mateles and Tannenbaum, 1968; Sinskey et al, 1975).

These three explanations are not very convincing because the same symptoms are reported by scientists working with different animal species, diets, and yeast levels.

Another potential hazard of consuming of yeast protein is the elevation of blood uric acid level. Uric acid, the end product of the purine portion of nucleic acid catabolism, is only slightly soluble at the pH of blood and there is risk that salt may be deposited in the renal tract and possibly other tissue if the diet contains excessive purine (Kamel and Kramer, 1978; Waslien et al, 1970). Waslien et al (1970) and Kamel et al (1978) found that the level of blood uric acid increased when men were fed yeast protein isolate or pizza containing yeast protein.

Yeast cell wall is composed mainly of glycan and is undesirable because it reduces the bioavailability of yeast protein and may contain antigenic, allergenic and other factors causing nausea and gastrointestinal disturbance De. Drot, 1977; Cheftel, 1977). Nucleic acids, if ingested at high levels, may result in development of gout or kidney stone formation, adverse gastro-intestinal reactions, adverse skin reaction (Kamel and Kramer, 1978; Waslien, 1970; Waslien, 1975; Mateles and Tannenbaum, 1968; Sinskey et al, 1975; Reed, 1981). For these reasons, it is desirable to separate the protein fraction of yeast from the cell wall and nucleic acids. Many studies concerning single cell protein (SCP) isolation have been reported in the past 30 years. Many of them are patents and are of limited value. Most published papers or patents described the use of mechanical force to rupture the cell wall (Shetty et al, 1979; Newell, 1975 a and b; Lee et al, 1979). Other used alkaline extraction (Huang and Rha, 1971 and 1978). Only a few references suggested acid hydrolysis (Tsang et al, 1979; Trevelyan, 1976) or treatment of the cell with exogeneous lysis enzymes (Reed and Peppler, 1973; Knorr et al, 1979). Mechanical force ruptured the cell wall allowing the cytoplasmic protein to

flow out. Other treatments impair little, if any, of the integrity of the cell wall (Tsang et al, 1979). Both enzyme and chemical treatements are used to disorganize the cell membrane and enable the cytoplasmic material to leach out. After rupturing the cell wall or disorganizing the cell membrane, the cytoplasmic protein will leach out then can be easily separated from cell wall by centrifugation. Tsang et al (1979) compared the yield of both fresh and dried yeast by autolysis, acid lysis, alkaline extraction, and mechanical force. He found that the mechanical force method (use of a homogenizer to rupture the cell wall) gave the highest yield of protein. Knorr et al (1979) reported that the addition of pancreatin or pronase in addition to autolytic enzymes could accelerate the release of protein from the cell. This method was claimed to be better than those using organic solvents or NaCl to hasten the autolysis process. Autolysis is a long process and has a high risk of contamination, however, this process can be used to separate protein from cell wall and nucleic acids simultaneously (Knorr et al, 1979).

Methods of reducing the nucleic acids content in protein isolate can be divided into two groups: treatments of disrupted cells or intact cells. In the first category, the yeast cell homogenate (cell wall has been ruptured by mechanical force) was 1) Incubated in moderate alkaline at moderate temperature to allow RNAase (lysomal enzyme) to digest RNA with or without addition of exogeneous RNAase (Newell et al, 1975 b; Lee et al, 1979; Vananuvat and Kinsella, 1975 a) or 2) Incubated in strong alkaline at elevated temperature to hydrolyze RNA (Newell et al, 1975 a). Vananuvat and Kinsella (1975 a) and Shetty and Kinsella (1979) reported that succinic anhydride could activate the RNAase inhibitor and reduce the activity of endogeneous proteolytic enzymes thus improving the yield of protein. In a patent, Robbison et al (1976) suggested heating the cell homogenate to denature protein and obtain a low nucleic acid content protein concentrate. By this method, the cell wall frac-

tion is not separated from the yeast protein.

In the second category, the intact yeast is suspended in NaCl, NaOH, HCl, perchloric acid or trichloroacetic acid solution to extract RNA. The nucleic acids then can be removed by centrifugation or washed out (Tsang et al, 1979). Another enzymatic method is to suspend yeast in water, buffer or NaCl solution and then treat with heat for very short period of time to inactivate the RNAase inhibitor and destroy the membrane of yeast cell vacuole to release lysozyme. The lysozyme then digests RNA into nucleotides. The nucleotides can be removed by centrifugation or washed away. The third method to reduce the RNA content is use of exogeneous and/or endogenous proteolytic enzymes to digest cytoplamic protein and disorganize the yeast cell membrane but leave the RNA intact. The digested protein then diffuses into the medium but the intact RNA remains inside the cell. The digested protein then can be separated from cell wall and nucleic acids by centrifugation. Thio compounds (Shetty and Kinsella, 1978) and proteolytic enzymes from different sources (Hough et al, 1970; Cortell, 1972) can be added to accelerate the digestion of protein and inhibit the RNAase thereby preventing RNA from being broken into nucleotides and diffusing through the cell membrane.

Functional properties are important in determining the potential uses of new protein for the development of new product (Kinsella, 1976). There are very few publications concerning the functional properties of protein prepared from yeast cell. Huang and Rha (1971) examined the coagulation temperature of a protein concentrate made by alkaline extraction from Torula yeast. They reported that under the coagulation temperature, a pH 9.2 dope containing 20% solid has best spinnability. Balmaceda and Rha (1973) reported the coagulation rate of a Torula yeast protein dope made by alkaline extraction. They found that the coagulation rate increased with temperature but decreased with concentration. Vananuvat and Kinsella (1975 b) found that the amino

acid composition of yeast protein isolate varied with the isolation method. They also found that some functional properties of yeast protein isolate varied with their isolation method (Vananuvat and Kinsella, 1975 c). Their findings suggest that the functional properties and optimum fabrication condition of yeast protein isolates might vary with their sources and preparation methods. Huang and Rha (1978) incorporated reagents such as formaldehyde which could induce intermolecular linkage into a pH 9 dope containing 20% solid and found that the reagent changed both spinnability and apparent viscosity. The addition of gums improved the spinnability without significantly changing in viscosity. Tsintsadze et al (1979) reported that protein preparation method and freeze/thaw affects the microstructure and mechanical properties of yeast protein curd but that heat treatment (80^{o}C 30 min) prior to precipitation has no effect on structure and water holding ability of the curd. Sato et al (1979) studied the functional properties of a yeast protein isolate made from Hansenula yeast. They reported a solubility profile and low foam stability which are similar to what was reported by Vananuvat and Kinsella (1975 c). In their paper, Sato et al (1979) also found that the foam volume is concentration independent while the foam stability is concentration dependent. The foam stability and volume can be improved by the addition of salt or sucrose.

Schmandke (1978) found that the viscosity of a protein dope is dependent on concentration and pH. He reported that dialdehyde starch treatment improved the spinnability of a dope containing 19% yeast protein. This dope was texturized into meat analogue with good similarity to meat.

Little work has been reported on food applications of yeast protein. Most of the work which has been reported is in the patent literature. The application of yeast protein can be classified into three categories: flavor enhancer (Burica et al, 1978; Modic, 1978), protein fortification (Derkansov,

1978; Volpe et al, 1981; Bostian, 1978) and meat (Modic et al, 1978; Kurkela et al, 1981; Gajger et al, 1980) or fishery (Mistsui Toatsu Chem. Co., 1980) substitute.

SECTION I: EFFECT OF YEAST PROTEIN CONCENTRATE ON BREAD MAKING.

INTRODUCTION

Bread is universally recognized as a nutritious and economical food. The enrichment of white flour to restore the content of vitamins and minerals to match or exceed the level present in the whole grain is a widely practiced and nutritionally sound procedure. The need to enrich flour with protein or specific amino acids is still debated (Vaghefi et al, 1974; Pomeranz, 1970). In their view, Vaghefi et al (1974) questioned the accuracy of interpretation of data generated from rat feeding tests and showed how contradictory conclusions can be drawn if the feeding periods are different.

Protein malnutrition is a serious problem facing people in underdeveloped countries and poor people in many regions whose diet consists mainly of starchy food or cereals. It has been shown that fortification with high protein materials or specific amino acids such as lysine and methione improves the P. E. R. value of breads (Vaghefi et al, 1974; Pomeranz, 1970; Tsen, 1980). Bread is a popular dietary staple in underdeveloped and developing countries (Tsen et al, 1974), so fortification of flour with high protein materials would be one of the potential ways to combat malnutrition.

Many protein-rich materials such as full fat soy flour (Tsen and Tang, 1971 a; Tsen et al, 1971 b; Tsen et al, 1975; Tsen and Hoover, 1973), defatted soy flour (Hyder et al, 1974), isolated soy protein (Mizrahi et al, 1967), wheat germ (Pomeranz et al, 1969; Tsen et al, 1971 b), corn germ (Pomeranz, 1970; Tsen, 1980), defatted corn germ (Tsen et al, 1974; Tsen, 1975), fish protein concentrate (Tsen et al, 1971 b; Pomeranz et al, 1969 b), nonfat dried milk (Tsen et al, 1971 b; Pomeranz, 1969 b), cotton seed flour (Pomeranz et al, 1969 b; Matthews et al, 1970; Tsen et al, 1971 b), extruded soy product (Tsen et al, 1975), horsebean flour and protein isolate (Patel et al, 1975), chick pea flour (Tsen et al, 1975), peanut flour (Tsen et al, 1974; Matthews et al, 1970), sunflower seed flour (Matthews et al, 1970), and yeast

protein (Pomeranz et al, 1969 b; Volpe et al, 1981) have been added to white flour to make high protein bread. In those papers, the effect of fish protein concentrate, non-fat dried milk, and chick bean flour on dough were not reported. Horsebean flour was reported to have positive effect on dough mixing stability (Patel et al, 1975). The rest of the foreign materials were reported to decrease the mixing time, increase the absorption and decrease mixing stability. All nonwheat proteinswere reported to impair loaf volume. Potency of the effect varied with the protein source. In the presence of 0.5% SSL (sodium stearyl lactate), the addition of 12% soy flour did not significantly affect the loaf volume (Tsen and Tang, 1971 a; Tsen et al, 1971 b) while the addition of isolated baker's yeast protein containing equivalent amounts of protein did impair loaf volume significantly (Volpe et al, 1981). Isolated soy protein when added to flour at a level containing the same total protein as 12% soy flour decreased the loaf volume more than 50% compared to control (Mizrahi et al, 1967) while 12% soy flour alone reduced loaf volume by 13% (Tsen and Tang, 1971 a; Tsen et al, 1971 b). Factors responsible for the different effects of various protein are probably complex. Flour strength, formulation, processing method, surfactant potency, or combinations of these may be responsible. Certain specific proteins may be deleterious to loaf volume. Hyder et al (1974) reported the existence of such specific proteins. Pomeranz et al (1970) and Sullivan et al (1937) suggested that protein may not be the only factor responsible for the adverse effects of added nonwheat protein rich material.

Various mechanisms have been proposed to explain the adverse effect of protein fortification. Patel et al (1975) suggested a dilution theory to explain the adverse effect of the addition of horsebean flour and horsebean protein isolate on bread quality. This theory was also used to explain the difference in baking performance of those two additives. As the author did

not present loaf volume data, we are not able to compare the percent of bread volume decreased with the degree of gluten dilution. Pomeranz et al (1970) and Sullivan et al (1937) suggested that lipids and glutathione in wheat germ impaired the gluten network and reduced loaf volume. Aido and Tsen (1973) believed that soy protein intereacted with gliadin primarily hydrophobically and with glutenin hydrophilically to form a gliadin-soy protein-glutenin complex thus altering the gluten network. Hyder et al (1974) demonstrated the interaction between soy protein and gluten. An effort to isolate and identify this specific protein complex was unsuccessful. Pomeranz et al (1969 a and b) improved loaf volume of dough fortified with soy flour and other nonwheat protein concentrate by introducing free flour lipids or glycolipids to dough. The authors suggested that flour lipids might be involved in the impairing mechanism. Chung et al (1981) believed that soy protein might compete with flour lipids for gluten and thus changed the dough properties and reduced loaf volume. Evans et al (1980) using SEM to examine dough containing yeast protein isolate found that yeast protein changed the gluten network. The addition of surfactants alleviated part of the adverse effect. In their paper the authors did not propose a mechanism for the interaction of yeast protein isolate and gluten.

Methods reported to reduce the adverse effect of protein fortification on loaf volume include:

1. Heat treatment: Heat treatment was reported to be effective in counteracting the effect of wheat germ (Sullivan et al, 1937; Pomeranz et al, 1970).

2. Change formulation: Soft dough method (add more water than normally required flour absorption) has been shown to improve the loaf volume. But the product's volume was still too small to be acceptable (Matthews et al, 1970; Tsen et al, 1971a).

3. Shortened mixing time: Matthews et al (1970) reduced mixing time and improved loaf volume but the volume was far below the acceptable range.

4. Increase yeast level and decrease fermentation time: Tsen and Tang (1971 b) produced fairly acceptable bread containing 12% soy flour by cutting the fermentation time and increasing the yeast level.

5. Extrusion-cooking: Tsen et al (1975) used an extruder to cook soy products and found that extrusion cooking can alleviate some but not all of the adverse effects of soy flour.

6. Increase oxidant level: Generally fortifying the dough with non-wheat protein increases the oxidant requirement. But Mizarhi et al (1967) and Tsen et al (1971 b) showed that without surfactant the addition of soy flour or isolated soy protein induced a "bromate negative requirement" effect. With surfactant, increasing the oxidant level to a certain extent improved the loaf volume (Pomeranz, 1969 b; Tsen, 1975; Tsen et al, 1974; Tsen and Tang, 1971 a).

7. Use of strong flour: Patel et al (1975), Tsen et al (1971 b) and Pomeranz et al (1969 b) demonstrated that stronger flour can handle more protein additives than weak flour. They also reported that at the same protein addition level, the stronger flour always produced larger loaf volumes.

8. Add surfactants: Many surfactants such as SSL (sodium stearoyl-2-lactylate, CSL (calcium stearoyl-2-lactylate), EMG (ethoxylated monoglyceride), and lecithin have been reported to be very effective in relieving the effects of added proteins (Chung et al, 1981; Aido and Tsen, 1973; Tsen, 1980; Patel et al, 1975; Pomeranz et al, 1970; and Tsen and Tang, 1971 a).

9. Add natural or synthetic glycolipids: Pomeranz et al (1969 a and b) and Tsen et al (1974) improved the loaf volume by the addition of glycolipids to dough containing soy flour or corn germ or other protein rich materials.

The effect of isolated baker's yeast protein on dough properties and

baking performance has been reported by Volpe (1981). The protein composition and other constitutients of protein concentrate might influence the dough properties and bread quality. Thus Volpe's findings might not be directly related to effects of brewer's yeast as reported in this study.

Brewer's yeast has high protein content and is high in lysine, the first limiting amino acid in cereal. Fortifying bread with brewer's yeast protein may be an effective way to combat protein malnutrition in some areas of the world. The purpose of this study was to determine the effect of brewer's yeast protein concentrate on bread-making.

MATERIALS AND METHODS

Materials

Wheat flour. Wheat flour was obtained from the Ross Company. Protein content was 12.1% with good volume potential and a medium long mixing time of 4.6 min. Baking absorption of the flour was 62%.

Yeast protein concentrates. Two protein concentrates (heat treated and untreated) were prepared by the Miller Brewing Company and shipped frozen to our laboratory. Both protein concentrates had a protein content of about 72% (N x 6.25, 5% moisture basis). (See part II of this thesis.)

Methods

Mixograms. Mixograms were made on 10 g mixograph by the method of Finney and Shogren (1972).

Baking procedure (Straight dough method). Formula water, mixing time and oxidant level were determined by preliminary test. Formula included:

Flour (14% mb)	100 g
Salt	1.5 g
Sucrose	6 g
Non-fat dried milk	4 g
Shortening	3 g
Yeast	2 g
$KBrO_3$	optimum

Doughs were punched after 105 and 155 min and panned after 180 min of fermentation. Proof time was 55 min and loaf was baked 24 min at 420°F. Loaves were weighed as they came from the oven and volumes were determined by rapeseed displacement.

Short-time baking process. Bread formula and mixing time are the same as those used in the straight dough method. In the short time process,

fermentation time was reduced 25%. Doughs were punched after 95 and 129 min and panned after 145 min of fermentation. Proof time was 30 min. Loaves were baked 24 min at 420°F and weighed as they came from the oven. Bread volume was determined by rapeseed displacement.

Gasograms. Gasograms were made on a slurry gasograph by the method of Rubenthaler et al (1980).

RESULTS AND DISCUSSION

Effect of Yeast Protein Concentrate on Mixing and Flour Absorption

Various amounts of two types of brewer's yeast protein concentrate (heated and unheated) were added to flour to study their effect on water absorption and mixing time. Results are shown in Fig. 1 and Table 1. It was found that the addition of yeast protein concentrate increased water absorption. The increase in water absorption was 1% per 1% protein concentrate. Mixograph data showed that heat treatment had no effect on absorption (Fig. 1 and Table 1). As shown in Table 1, the addition of yeast protein concentrate (YPC) decreased optimum mixing time of the dough. The decrease in mixing time was a function of the amount added (Table 1). The addition of heat treated YPC also decreased optimum mixing time. This effect leveled off when addition level exceeded 2% (Table 1). Mixograph studies showed that added YPC (both heated and unheated) had very little, if any effect on peak height. However, YPC did decrease the band width (Fig. 1). The decrease in tolerance to over-mixing and reduced band width may indicate a reduction of dough strength. This finding is in agreement with Evans et al (1980) who demonstrated that the gluten in dough was altered when baker's yeast protein concentrate was added at a 6% level.

Effect of Yeast Protein on Bread Quality

Graded amounts of yeast protein concentrate (heated and unheated) were added to flour to study their effect on bread quality. Results of baking tests are summarized in Table 2. The addition of YPC reduced loaf volume. At 2% addition level, the loaf volume decreased about 15% from the control. The loaf volume of dough containing 5% YPC was about 50% of that of control. When 10% YPC (flour weight basis) was added, the loaf volume was as small as that of an unleavened bread. Heated YPC had a greater loaf volume reducing

Table 1. Mixogram peak time and height of doughs containing graded amounts of YPC

Treatment	Peak time	Peak height
Control (n = 5)*	4.6 ± 0.1	4.9 ± 0.2
2% YPC w/o (n = 3)**	3.4 ± 0.1	4.8 ± 0
5% YPC w/o ***	3.1 ± 0.2	4.5 ± 0.1
10% YPC w/o	2.7 ± 0.1	4.5 ± 0.2
2% YPC W/H ***	3.7 ± 0.2	4.6 ± 0.1
5% YPC W/H	3.6 ± 0.1	4.8 ± 0.1
10% YPC W/H	3.6 ± 0.1	4.8 ± 0.1

*n indicates number of observations

**control group had 5 observations

experimental group had 3 observations

***w/o indicates unheated

W/H indicates heated

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Fig. 1. Mixograms of flour with and without added YPC.

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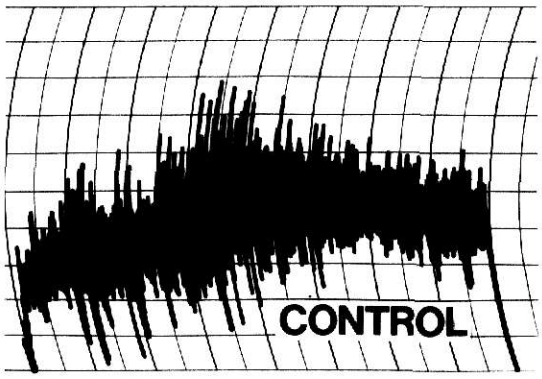
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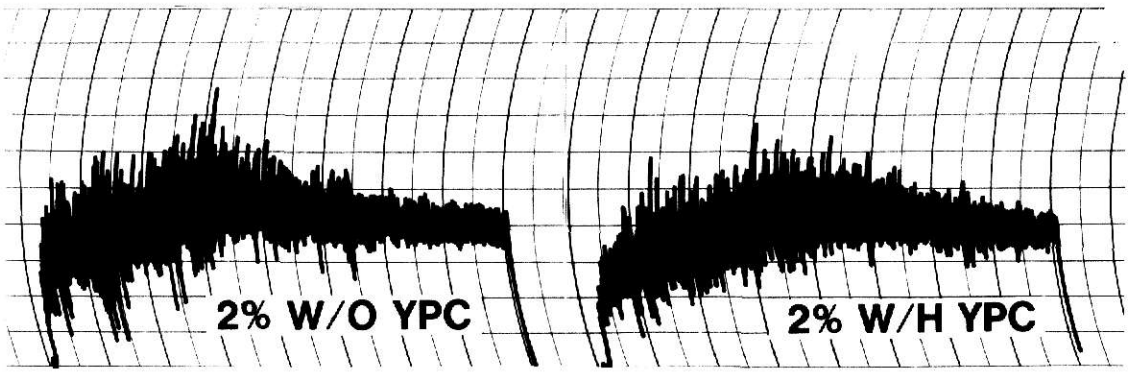
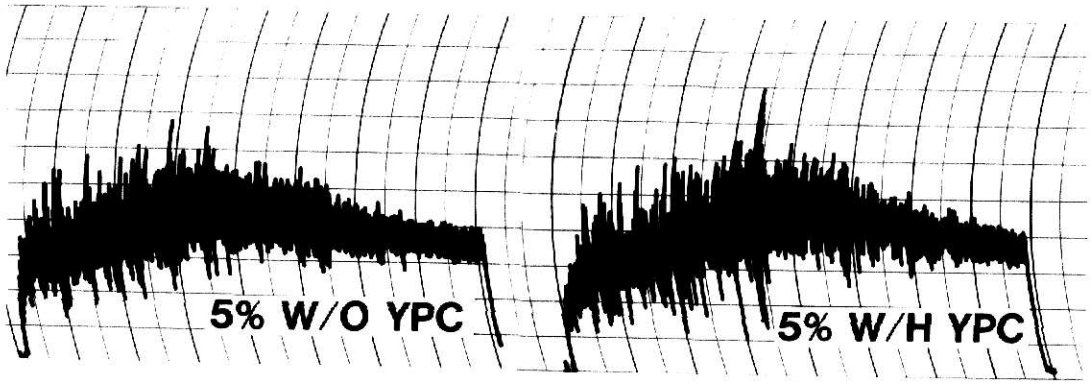
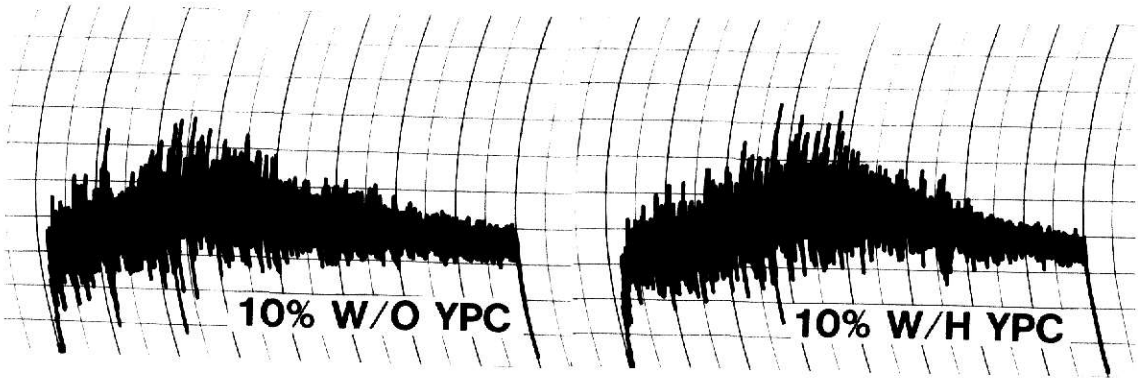

2% W/O YPC
2% W/H YPC


5% W/O YPC
5% W/H YPC


10% W/O YPC
10% W/H YPC

effect than did unheated YPC (Table 2). The loaf volume of dough containing 2% heated YPC was only 75% of control.

It was observed that all breads containing YPC (heated and unheated) had a strong brewery flavor even after cooling. Breads containing more than 5% YPC still had strong brewery flavor after storage in a sealed plastic bag for 1 day. The strong brewery flavor made those bread samples unacceptable.

Effect of Reducing Fermentation Time

According to Volpe (1981), the loaf volume lowering effect of yeast protein was not changed by additions of surfactants, oxidants, or salt. Those treatments have been reported to be effective in reducing the adverse effects of foreign protein on bread volume (Sullivan et al, 1937; Pomeranz et al, 1970; Chung et al, 1981; Adio and Tsen, 1973; Tsen, 1980; Patel and Johnson, 1975; Tsang and Tang, 1975). All doughs with added YPC had higher bowl height (the distance between the top of the rounded and fermented dough to the edge of the fermentation bowl) than did controls. Doughs containing YPC, except the 2% level, had smaller proof height. The dough containing 2% YPC was weaker at rounding. At 5% level, the dough lost its visco-elasticity and ruptured at the edge when punched. When 10% YPC was added, the dough lost its consistency and was barely able to form a dough. Gasograph data indicated that addition of YPC increased gas production (data shown in part II of this thesis). These findings suggest that doughs containing YPC produced more carbon dioxide but was unable to retain the gas. Two possibilities may account for the result. One is that the added YPC altered the gluten network and thereby reduced its ability to retain gas. The other is that YPC may stimulate both fermentation rate and the production of unfavorable materials which impaired the gas retention ability of the dough and resulted in smaller volume.

Tsen and Tang (1971) reported that a reduction of fermentation time

Table 2. Loaf volumes of bread with graded amounts of YPC (straight dough method)

Treatments (n = 6)***	Loaf volume (ml)
Control	971 ± 27
2% YPC (w/o)**	826 ± 18
5% YPC (w/o)	468 ± 15
10% YPC (w/o)	<425
2% YPC (W/H)	742 ± 5.7
5% YPC (W/H)	<425
10% YPC (W/H)	<425

*$LSD_{0.05}$ = 23.3 ml

**w/o indicates unheated

W/H indicates heated

***n indicates the number of observations for each treatment

improved the loaf volume of bread containing soy flour. With this in mind, fermentation time was reduced in an attempt to improve loaf volume. Results of this trial are summarized in Table 3. Reducing fermentation partially alleviated the adverse effect of YPC on bread volume. For dough containing 2% unheated YPC, the loaf volume was about 90% of the control. This is slightly better than the loaf volume of bread made by the straight dough method which was 85% of control. For dough containing 2% heated YPC, the loaf volume was also about 90% of the control. When compared to that made by straight dough method, the loaf volume was improved by 15%. This data indicates that reducing fermentation time and rest time eliminated the effect of heat treatment on the loaf volume reducing effect of YPC (Table 3). Heat treatment has been shown to enhance the loaf volume reducing effect of YPC (Table 2).

Table 3. Loaf volume of breads with graded amounts of YPC (short time dough method)

Treatment n = 6*	Loaf volume (ml)
Control	871.3 ± 25.6
2% YPC w/o**	785 ± 19.4
3% YPC w/o	750 ± 10
2% YPC W/H**	801 ± 25.6
3% YPC W/H	742 ± 7.6

*n indicates the number of observations for each treatment

**w/o indicates unheated

W/O indicates heated

***$LSD_{0.05}$ = 20.6 ml

CONCLUSIONS

The addition of YPC (heated and unheated) increased absorption but decreased mixing time, dough strength, and bread volume. Heat treatment was found to enhance the loaf volume reducing effect of YPC. This adverse effect of heat treatment could be eliminated by reducing the fermentation and rest time. Reducing fermentation time alleviated part of the adverse effect of YPC on bread volume, but this effect was limited. Bread volume was unacceptable with 3% YPC added. Breads containing more than 2% YPC had strong brewery flavor even after cooling and storage for 1 day and, therefore, were considered unacceptable.

SECTION II. EFFECTS OF YEAST PROTEIN CONCENTRATE AND DRIED WHOLE YEAST ON EXTRUDATES PROPERTIES

INTRODUCTION

Food extrusion refers to three types of process: low pressure cold forming, low pressure cooking, and high pressure cooking and puffing (Seib, 1976). The first, low pressure cold forming, is exemplified by pasta production (Moore, 1973). In this process, extrusion creates shaped pieces of premixed materials which are further processed by drying, baking, or deep-fat frying into products such as bread sticks or doughnut holes. The second process uses heat and low pressure to cook and form the product. In this process, dry materials are mixed, moistened (by adding water or steam) and then fed into extruder and cooked at temperatures below 100^{o}C. After exiting the extruder, the product can be cut and/or shaped, then toasted or puffed. This process is widely used in the production of breakfast cereals. The third process applied high heat (much above 100^{o}C) and high pressure to cook the feed materials. Much of the heat is generated by friction as the feed materials are moved through the barrel. High pressure is created by constriction of the discharge end of the barrel and/or by increasing the root diameter of the extruder screw from the feed to the metering zone. Upon exiting the extruder, the product expands because the sudden drop in pressure allows the super-heated moisture present in the extrudate to vaporize.

High Temperature Short Time (HTST) extrusion is an important and versatile type of extrusion cooking. There are several advantages of HTST extrusion cooking (Moore, 1973; Faubion, 1980; Stearns, 1974; Hawk, 1979):

1. Large volume production may be achieved on a continuous basis at relatively low cost.

2. Microorganisms are destroyed even in cases where the raw materials are highly contaminated. This is due to heat as well as the pressure and shear caused by the extruder screw.

3. The high temperature, pressure, and shear may destroy growth inhibitors and antipalatability factors in oilseeds or pulses and thereby improve the nutritional value of the product.

4. The high temperature short-time process seems to be less destructive to protein availability because of the very brief time period required to reach the peak temperature.

5. Enzymes, including those deterimental to food quality, are inactivated.

6. Extrusion cooking is a flexible system. By changing the operating condition or simply by changing the extruder die cap, a wide variety of product with various shapes and sizes can be made.

7. Extrusion requires low manpower and space.

On the other hand, HTST extrusion also has disadvantages. In his dissertation, Faubion (1980) listed the following:

1. Extrusion fades colors added to the premixes and creates brown color because of the browning reaction.

2. Extrusion decreases protein availability.

Taso et al (1978) reported that lysine can be lost under conditions similar to extrusion. Krukar (1980) reported that in addition to color problems, extrusion flashes-off almost all volatile flavors. For underdeveloped or developing countries, electricity is a relatively expensive energy source. So extrusion in those countries may not be an economical process.

The use of extrusion cooking in the food industry has greatly increased during the past few years. Primarily this has been due to an increased demand for convenience and snack-type foods (Lawton et al, 1972; Mercier et al, 1977) and the advantages extrusion offers over other production methods (Faubion, 1980). This technology has been applied in production of snack foods, infant formulas, pregelatinized starch, precooked flours and grits, cooking whole

grains and oilseeds, pet foods, gruels, breakfast cereal, quick-cooking noodles, precooked beverage powders, dry soup bases, and processed egg products (Bookwalter et al, 1971; Stearns, 1974; Moore, 1973; Lawton et al, 1972; Seib, 1976; Faubion, 1980; Kim et al, 1980).

The food extruder used today was designed for plastics extrusion and has been studied both from a practical and theoretical point of view. Papers concerning extrusion are concentrated on new product development and mechanical design, or modification of the extruder. Most extruder modifications have been made on the basis of practical experience, not theory (Lawton et al, 1972; Hauck, 1982). Basically, the food extruder consists of a close tolerance screw rotating inside a barrel. The barrel is constricted at the discharge end which creates a back pressure to facilitate mixing and to shape the product. Feeders may be a gravimetric, vibratory, dry force, or a feeder-conditioner combination. Screws come with varied pitch and depth of flight to give various degrees of shear and compression to the product. The compression of the material within an extruder may also be altered by increasing the shaft diameter of a constant pitch screw or by tapering the screw in a conical barrel. The inside of the extruder barrel may be either smooth or grooved. The grooves help in development of frictional heat and may be of different shapes or depth and straight or riffled. Straight grooves cause the development of more friction heat than the riffled grooves. The heat control system may be either steam or electrical and have a liquid or air-cooling system. The die which controls the shape of the product may be of any shape or size as long as continuous flow is maintained throughout the die. The size and shape of the die also has direct effect on the pressure within the extruder barrel (Seib, 1976; Hauck, 1982).

It has been long believed that the materials are plasticized and melted into a homogeneous melt in the metering zone of the extruder barrel. The

homogeneous melt is devoid of any structure above that of individual molecules of protein, amylose, and amylopectin. This melt when exiting the die encounters a sudden pressure drop and the super-heated water vaporizes to expand the extrudate (Faubion, 1980). However, this model failed to explain the effects of gluten, soy protein isolate, and flour lipids on extruded starch and thereby was questioned by Faubion (1980). As suggested by his findings, Faubion (1980) suggested that material was plasticized but not necessarily mixed into a homogeneous melt. When exiting the die, the starch granule hila become nucleation sites where super-heated water vaporizes due to the sudden pressure drop. The extrudate is made up of individual expanded starch granules which are connected by the leached-out material. Faubion's concept was derived from work by Dr. R. Carl Hoseney at Kansas State University. Hoseney (1983) studied the expansion of the popcorn kernel and postulated that its expansion is due to the expansion of each individual starch granule which is connected by the leached-out material.

Not many basic studies of extrusion are available. Most papers studied the effects of operating conditions or moisture content on extrusion cooking of various starches and cereal flour. Different optimum extrusion temperatures for expansion have been reported for starches and flours (Moore, 1973; Mercier, 1971; Mercier and Feillet, 1975; Mercier, 1979). Moisture has a negative effect on the expansion and strength of extruded starch (Stearns, 1974; Faubion, 1982a).

Mercier and Feillet (1975) reconstituted corn starch by blending various amounts of waxy (high amylopectin) and high amylose corn. They reported that except for material extruded at 225^{o}C, amylose was found to produce negative effect on expansion and water solubility of extruded starches. They also found that increasing extrusion temperature had a positive effect on the water solubility of extruded starch. Polysaccharide chains are not broken down due

to extrusion as indicated by their solubility in 80% alcohol (ethanol) (Mercier and Feillet, 1975). Cabrera-Lavedre (1978) reported that surfactants had negative effect on the expansion of extruded starch. He also found that between pH 4 and 9, pH had no effect on expansion. When the pH of pure wheat starch was reduced below 4, the expansion of extrudate was greatly reduced. Faubion (1982b) fractionated flour components and studied their effects on extrusion. He found that flour lipids and gluten decreased both the expansion and the strength of the extrudates. His findings suggest that additives can be used to modify the texture of extruded snack foods.

Proteins and polysaccharides have important carrier and protective functions for aromatic compounds which allow the flavor chemicals to withstand high temperature (Krukar, 1980). Yeast protein is a high quality protein which can be added to snack foods as a nutrition fortifier and possibly as a flavor carrier. In our study, a starch system was used as a model to study the effect of yeast protein concentrate (YPC) on extrusion.

MATERIALS AND METHODS

Materials

Yeast protein concentrate, yeast cell wall, and yeast cell homogenate. These materials were prepared by a brewing company as described in Fig. 1. They were shipped frozen to the Department of Grain Science and Industry at Kansas State University and lypholized on receipt. They were stored at 2°C before use.

Starch. Prime wheat starch was obtained from Midwest Solvent Co., Inc., Atchison, KS.

Methods

Moisture determination. Moisture was determined according to AACC Method 44-19.

Free lipids determination. Free lipids were determined according to AOAC Method 30-25.

Bound lipids determination. A 5 g sample (YPC) was suspended in 200 ml of water-saturated butanol. The suspension was allowed to stand 30 min with occasional stirring. The suspension was then centrifuged and the supernantant decanted. The precipitate was then extracted with an additional 200 ml of water-saturated butanol for 30 min. This process was repeated to a total of 3 extractions (Fig. 1). The combined supernatants were evaporated in a reduced pressure rotary evaporator at 30°C. The residue was dissolved in 40 ml petroleum ether and transferred to a tared beaker (the round flask was rinsed with additional 10 ml petroleum ether) then dried at 100°C for 30 min. Bound lipids content is defined as the amount of materials extracted as a percentage of the sample weight (as is).

Bound lipids extraction. Bound lipids were extracted by the same scheme used to determine bound lipids, except the sample-solvent ratio was reduced to 1/6. The defatted YPC was recovered by centrifugation (the precipitate

Fig. 1. Production process for yeast protein concentrate (YPC).

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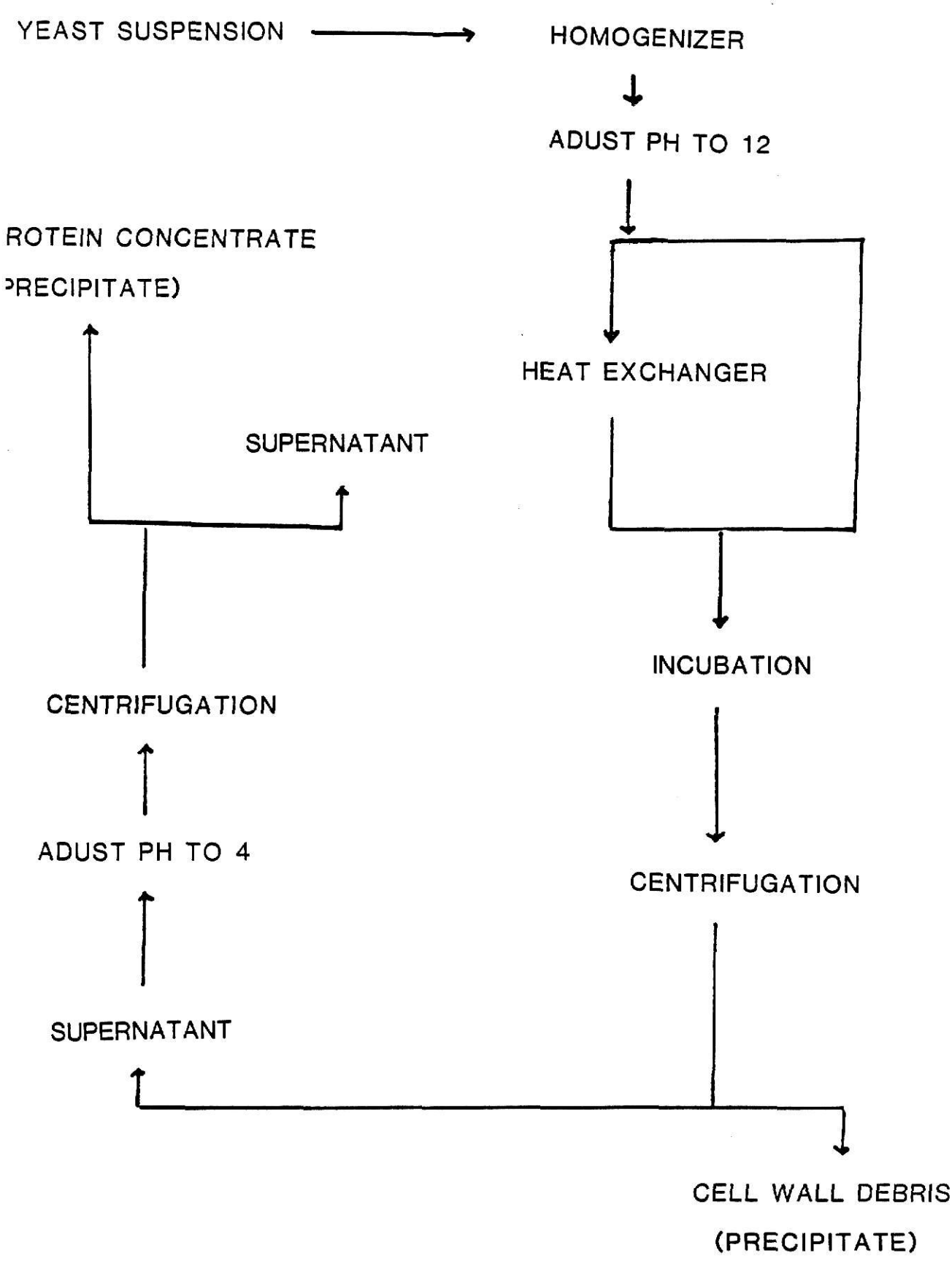
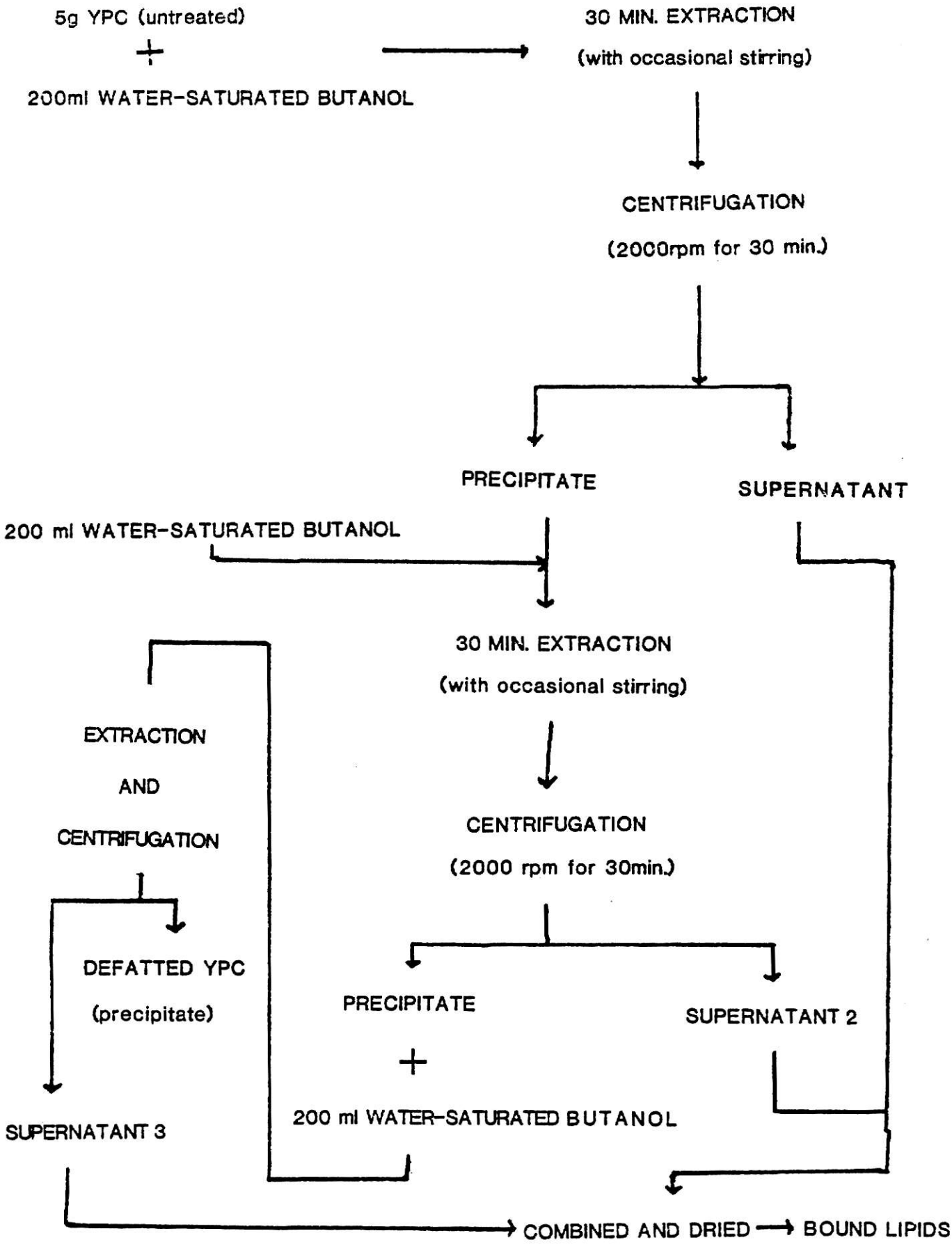

YEAST SUSPENSION
HOMOGENIZER
ADUST PH TO 12
HEAT EXCHANGER
INCUBATION
CENTRIFUGATION
CELL WALL DEBRIS
(PRECIPITATE)
SUPERNATANT
ADUST PH TO 4
CENTRIFUGATION
SUPERNATANT
ROTEIN CONCENTRATE
PRECIPITATE)

Fig. 2. Extraction scheme for bound yeast lipids.


5g YPC (untreated)
+
200ml WATER-SATURATED BUTANOL
30 MIN. EXTRACTION
(with occasional stirring)
CENTRIFUGATION
(2000rpm for 30 min.)
PRECIPITATE
SUPERNATANT
200 ml WATER-SATURATED BUTANOL
30 MIN. EXTRACTION
(with occasional stirring)
CENTRIFUGATION
(2000 rpm for 30min.)
EXTRACTION
AND
CENTRIFUGATION
DEFATTED YPC
(precipitate)
PRECIPITATE
+
200 ml WATER-SATURATED BUTANOL
SUPERNATANT 2
SUPERNATANT 3
COMBINED AND DRIED
BOUND LIPIDS

fraction) and dried at room temperature until no solvent odor remained. The bound lipids (the supernatant fraction) were recovered either by drying at room temperature under N_2 or by addition to starch and drying under vaccuum at room temperature overnight.

Preparation of YPC-starch, WY-starch and cell wall-starch mixtures. Appropriate amounts of dry starch (moisture content about 11%), wet starch (moisture content about 21%), and additives, if any, to give a 200 g sample of the desired moisture (19.5%) were blended. Mixtures were allowed to equilibrate overnight in a double sealed plastic bag. Moisture content of the mixtures was checked after preparation and prior to extrusion.

Starch-lipids mixture preparation (direct addition method). 100 g starch (moisture content 12.4%) was put in a Stein mill cup (Fred Stein Laboratories, Atchison, KS) followed by an appropriate amount of lipids and ground for 30 sec. The mixture then was added to the remainder of the dry starch (moisture 12.4%) and ground for 30 sec using a Stein mill. These mixtures were then blended with appropriate amount of wet starch to make the desired lipids-starch mixture at 19.5% moisture.

Starch-lipids mixture preparation (premix method). A premix was made by suspending starch in petroleum ether containing an appropriate amount of bound lipids. The suspension was dried under vacuum at room temperature overnight. The desired lipids-starch mixture was made by blending appropriate amount of wet and dry starch with the premix.

Preparation of reconstituted YPC. Reconstituted YPC-starch mixtures were made by blending an appropriate amount of starch-lipid mixture with desired amount of defatted YPC. The starch-lipids mixture was either made by direct addition or the premix method.

Extrusion process. All studies were carried out on a laboratory size, single screw extruder, model 2403 manufactured by C. W. Brabender Co., South

Hackensack, NJ. The extrusion process was carried out as described by Faubion (1980) except that wet corn grit (moisture content about 25%) was extruded before first test sample and a hand-operated feeder was used. The extrusion conditions were:

Screw speed	120 rpm
Heating zone 1	air cooled
Heating zone 2	110°C
Heating zone 3	175°C
Die diameter	6.35 mm
Compression ratio of screw	5:1

Feed rate was maintained to require 9 amps to drive the screw.

Texture analysis. Texture studies were done with an Instron Model 1130 Universal Texture Analyzer. Shear force was defined as the maximum force needed to cut through two-thirds of the extrudate. Shear force was measured by using a Warner-Brazler shear apparatus to shear across the rod at right angle to its axis. Breaking force was measured by using a round, vertically driven anvil 1.0 cm in diameter to break the sample in the middle. The sample was supported at both ends with a 9.25 cm free span. Deformation distance was defined as the distance the anvil traveled between touching the sample and breaking it. Because the chart and the anvil were driven at a fixed speed, the deformation distance is proportional to the deformation time. Deformation time is defined as the time between the anvil touching the extrudate and the time the sample broke. The operating conditions of the texture studies were: For shear force determination:

Blade speed	5 cm/min
Chart speed	10 cm/min
Load cell	50 kg
Calibration range	calibrated at range 5 using a 5 kg weight

Operation range	either range 5 or 10

For breaking force and deformation distance:

Anvil speed	2.5 cm/min
Chart speed	50 cm/min
Calibration range	calibrated at range 10 using a 1 kg weight
Operation range	operating at either range 5 or 10

Extrudate diameter determination. The expansion of the extruded product was determined by measuring its diameter with calipers. Ten pieces of extrudate (about 15 cm long) were selected from each run, five diameter measurements were taken at different points on each piece.

Extrudate density determination. Sample density was calculated by weighing the same extrudate sample used to determine expansion and length. Volumes were calculated using average diameter and length value. Density was calculated as g per cc.

Scanning electron microscopy. Sample preparation for scanning electron microscopy was described by Faubion (1980).

Standard deviation and Least Significant Difference (LSD). Mean, standard deviation, and LSD were calculated for each sample property tested and were carried out as described by Snedecor and Cochran (1964).

RESULTS AND DISCUSSION

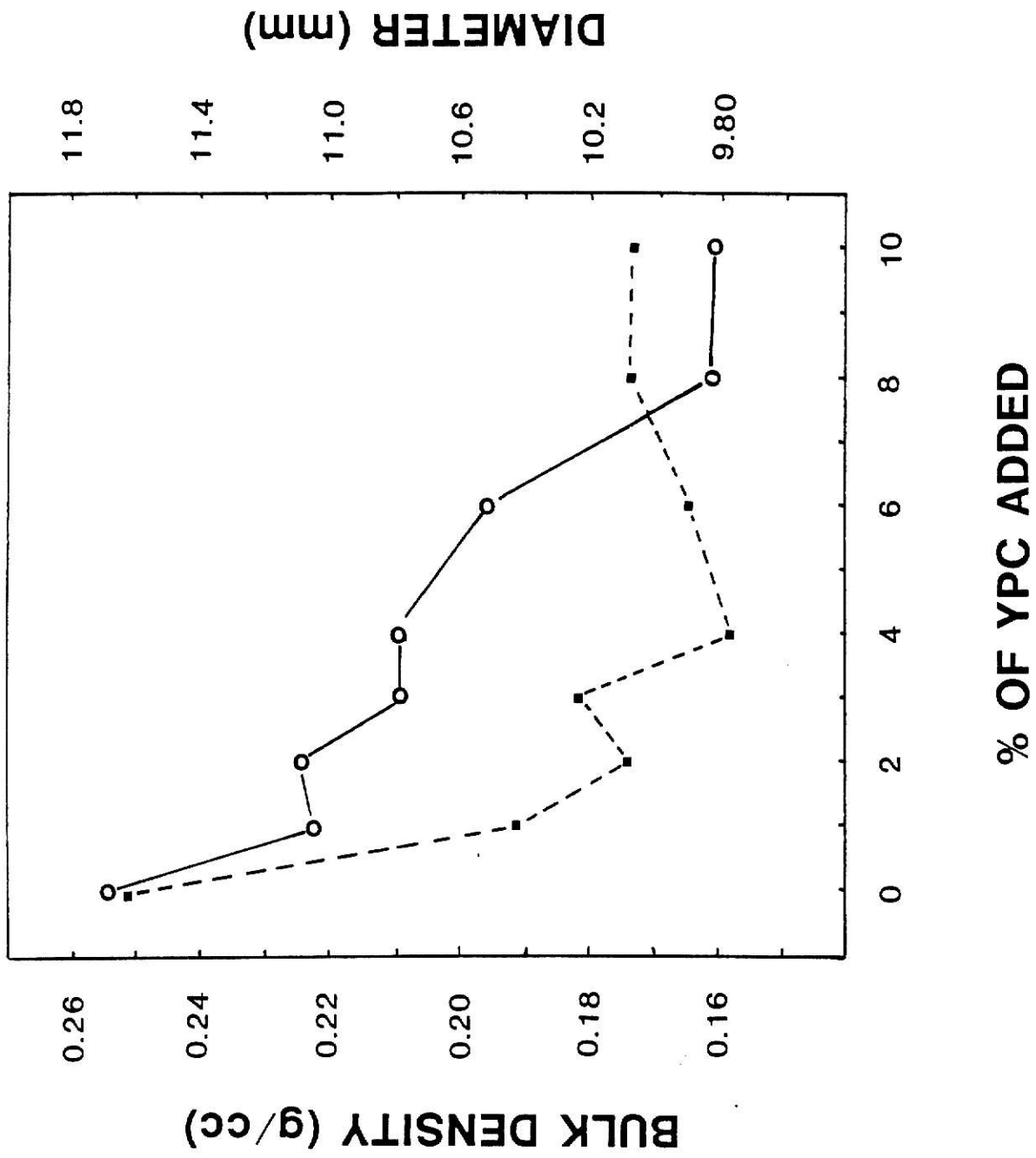
Effects of YPC on Extrudate Properties

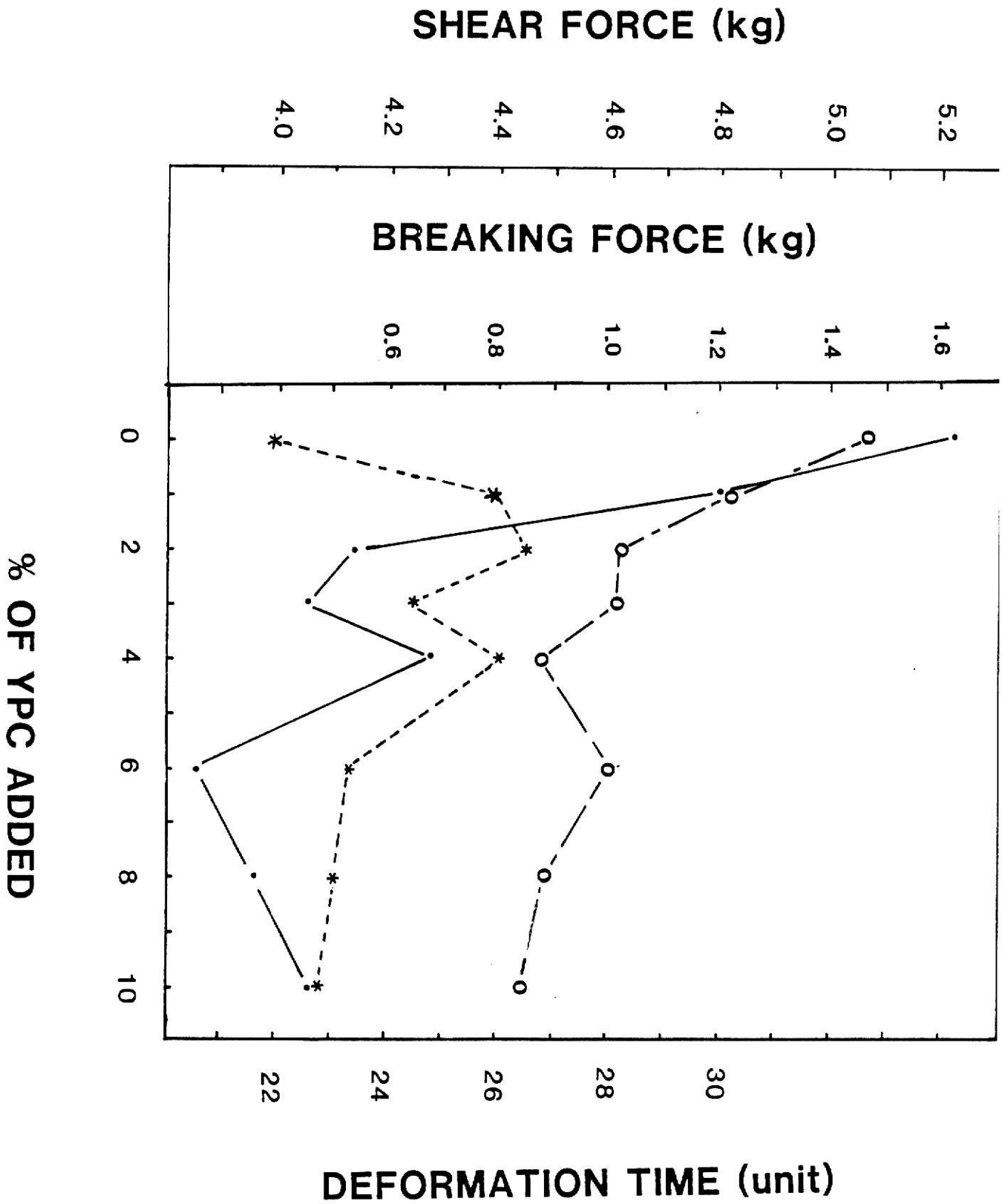
To determine the effect of yeast protein concentrate (YPC) on the extrusion parameters of extruded starch, various amounts of YPC were added to wheat starch and extruded. The effects of YPC addition were measured as changes in expansion, breaking force, shear force, and deformation time of extruded materials.

The addition of YPC decreased the expansion of extruded starch. Due to substantial variation within samples of the same extrudate, the expansion of extrudates containing adjacent levels of added YPC are frequently not significantly different. However, Fig. 3 showed a decreasing trend in expansion of extrudates with the amount of YPC added. The reduction in expansion was proportional to the addition level of YPC (Fig. 3). The added YPC decreased the bulk density of extruded starch (Fig. 3). The bulk density of extrudates containing YPC reaches minimum when addition level is 2% (W/W) and remains constant thereafter ($\alpha = 0.05$) (Fig. 3). The fact that a decrease in expansion was not accompanied by an increase in bulk density indicates that the production rate may be altered by the addition of YPC.

As shown in Fig. 2, the addition of YPC produced a negative effect on both breaking force and shear force of extrudates. This deleterious effect leveled-off when the addition level of YPC exceeded 2% ($\alpha = 0.05$) (Fig. 4). The effect of added YPC on deformation times of extrudates is shown in Fig. 4. At 95% confidence level, only the deformation times of extrudates containing 1%, 2%, and 4% YPC are significantly different from that of control (extruded wheat starch) (Fig. 4). The deformation times of extrudates containing 3%, 6%, and 10% YPC are neither significantly different from the control nor from those of extrudates containing 1%, 2%, and 4% YPC (Fig. 4).

Fig. 3. Expansion (◦——◦) and bulk density (•——•) of extrudates containing graded amounts of YPC.


DIAMETER (mm)
11.8
11.4
11.0
10.6
10.2
9.80
BULK DENSITY (g/cc)
0.26
0.24
0.22
0.20
0.18
0.16
0
2
4
6
8
10
% OF YPC ADDED


SHEAR FORCE (kg)
4.0
4.2
4.4
4.6
4.8
5.0
5.2
BREAKING FORCE (kg)
0.6
0.8
1.0
1.2
1.4
1.6
% OF YPC ADDED
0
2
4
6
8
10
22
24
26
28
30
DEFORMATION TIME (unit)

The decrease in breaking force and shear force may indicate that the added YPC reduced the hardness and strength of extrudates.

Cross sections (SEM) of extruded starch (control) are shown in Figure 5. Cross sections of extrudates containing 1%, 6%, and 10% YPC are given in Figure 6. These pictures are taken at same magnification. We can see that the added YPC reduced the expansion. The decrease in cell size appears to be proportional to the amount of YPC added (Figs. 5 and 6). The addition of YPC also altered the morphology of cell wall of extruded starch. The cell walls of extruded starch appear to be rougher than those of extrudates containing YPC (Figs. 5 and 6). The peripheral cells in the cross section of extruded starch and extrudates containing 1% YPC are relatively well expanded and elongated (Figs. 5 and 6). They are much larger than those in extrudates containing 6% and 10% YPC which appear to be very small and round (Figs. 5 and 6). Dense areas which are made up of small cells are seen in extrudates containing YPC. These areas are not found in control (Figs. 5 and 6). Some chunks are seen in extrudates containing YPC (arrow in Fig. 6c) which, under higher magnification, appear to be composed of cells that failed to expand (Fig. 7). The small round holes seen in Figure 7 (arrow) are suspected to be the hila reported by Hoseney (1983). Presented in Figure 8 are the surface of extrudates with or without added YPC. Similar to what reported by Stearns (1974) and Faubion (1980), domes, ripples, and pits are seen on the surface of extruded starch (Fig. 8a). Stearns (1974) and Faubion (1980) believed that domes indicates the existence of well expanded cells which set before cell collapse occurs while ripples are considered to be signs of cell collapse. This hypothesis seems to be consistent with our findings. From Figure 5a, we can see parts of the peripheral cells are well expanded (relatively large and round) while some are partially collapsed (arrows). For extrudates containing YPC, in addition to domes, ripples and

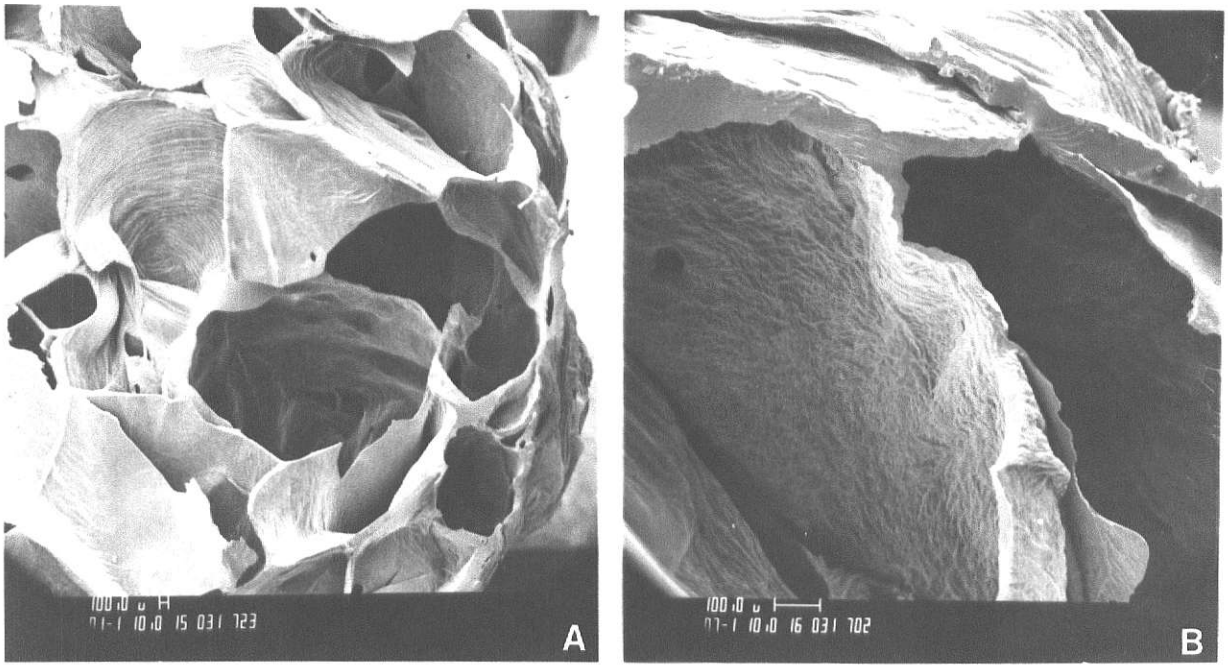
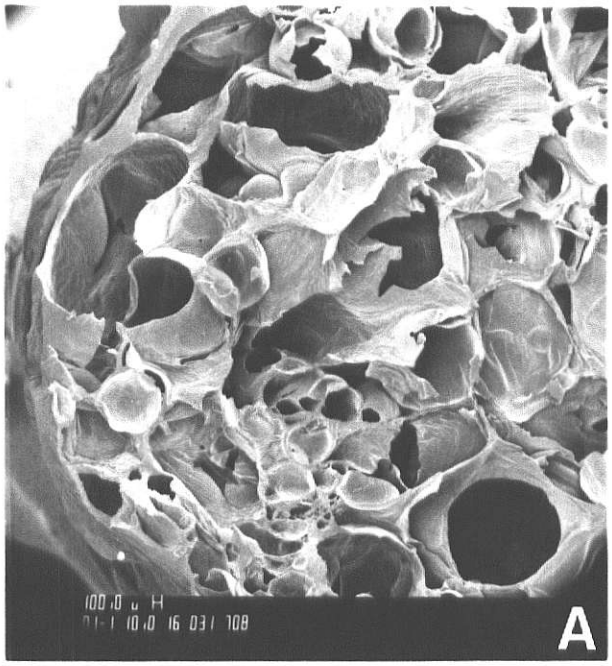
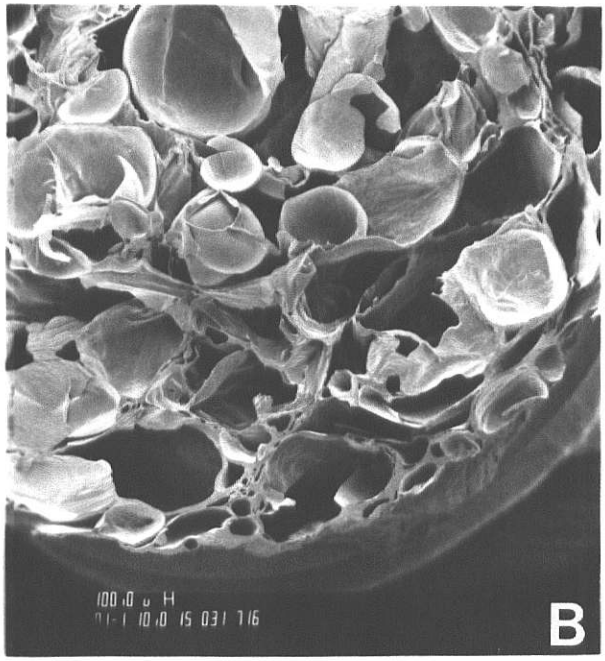

A
B

Fig. 6. Scanning electron micrographs of extrudates containing (A) 1%, (B) 6%, and (C) 10% YPC.


A


B

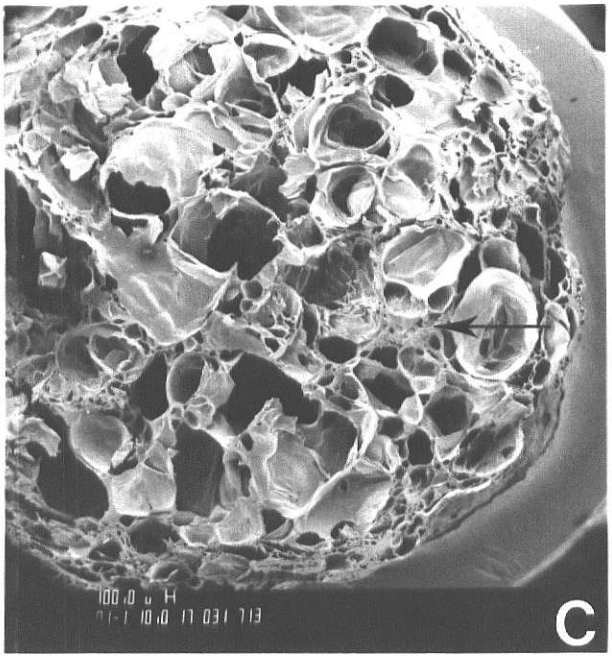

C

Fig. 7. Enlarged view of area pointed out by the arrow in figure 6(C).

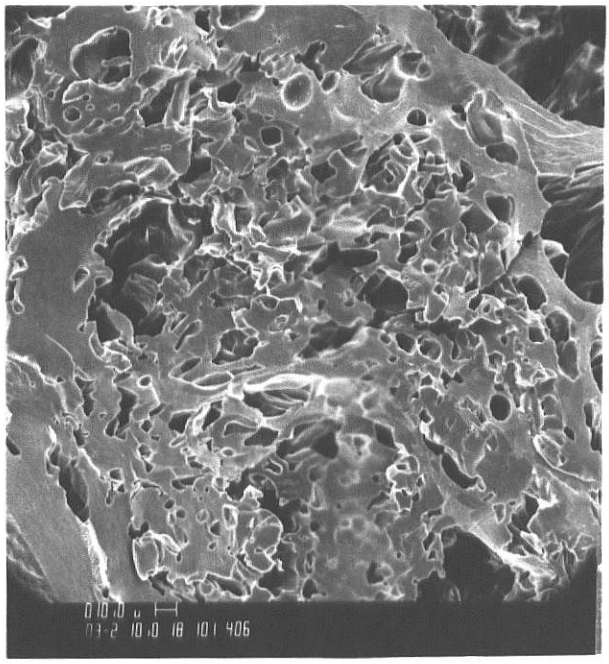
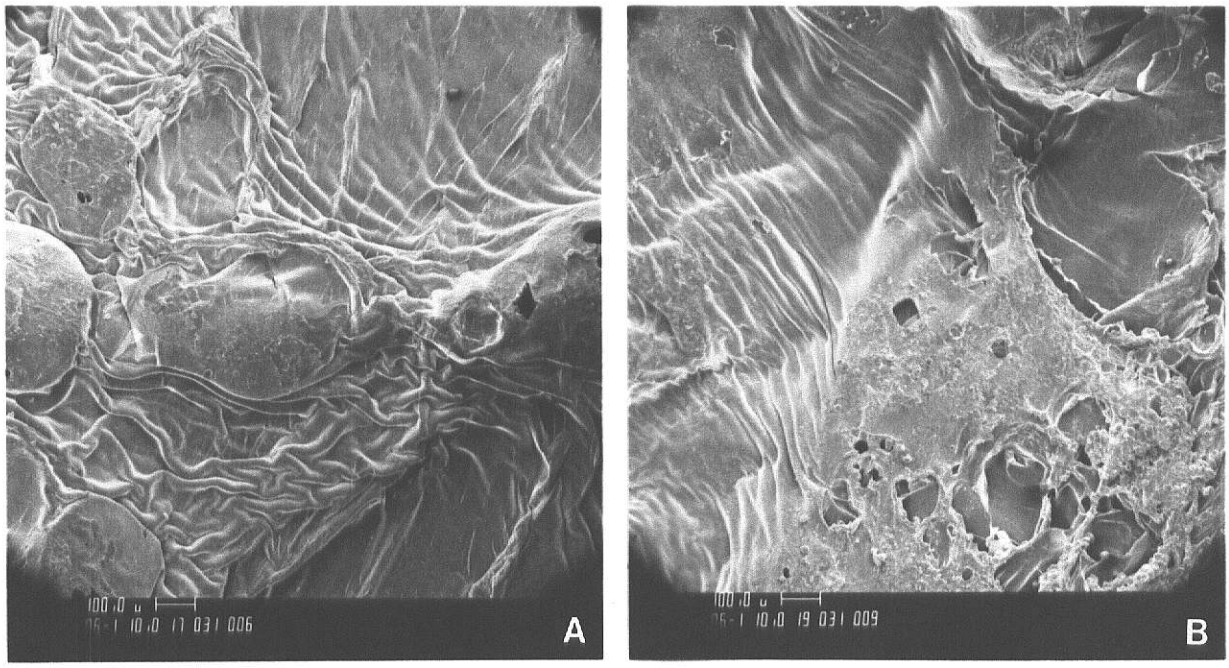

0 10.0 u
03-2 10.0 18 101 406

Fig. 8. Scanning electron micrograph of extrudate surface (A) control (starch) and (B) control plus 1% YPC.


A
B

pits regions that were severely torn are also seen (Fig. 8b). As the addition level increased, those regions become dominant. Domes are not seen in extrudates containing 10% YPC whose surface also appears to be rough and torn (Fig. 12b). Some small granules which may be starch are often seen in those rough regions (Fig. 8b). Similar to Faubion's findings (1982 b) we occasionally found some thread-like materials which bridge cell wall of poorly expanded cells of extrudates containing YPC (Fig. 9a). Higher magnification of these thread-like materials shows they are fiberous and that there may be starch granules embedded in them (Fig. 9b).

Faubion (1982 b) reported that flour lipids reduced the expansion, breaking force, and shear force of extruded wheat starch-gluten mixutre, and soy protein-starch mixture. Thus, we wanted to see if the lipids present in YPC were responsible for the deleterious effects of YPC on the strength and expansion of extruded starch. As determined by AOAC Method 30-25 using petroleum ether as solvent, YPC contained 0.21% free lipids while dried intact yeast contained 0.62% free lipids. That YPC contained lower free lipids content was expected since lipids may become bound to protein after the cell was ruptured by mechanical force or concentrated during protein isolation process. Water-saturated butanol was used to extract bound yeast lipids. It was found that YPC contained 6.3% bound lipids.

To determine the effect of bound yeast lipids, mixtures of starch-bound yeast lipids, starch-defatted YPC, and starch-reconstituted YPC were extruded. Results are summarized in Table 1.

Bound yeast lipids-starch mixtures were made both by premix and direct addition method. In terms of expansion and bulk density, there was no difference ($\alpha = 0.05$) between the extrudates made by these two methods (Table 1). Scanning electron microscopic pictures of the extrudates made by these two methods showed that their ultrastructure were quite similar (Fig. 10). The

Fig. 9. Scanning electron micrographs of thread-like structures seen in extrudates containing 1% YPC.

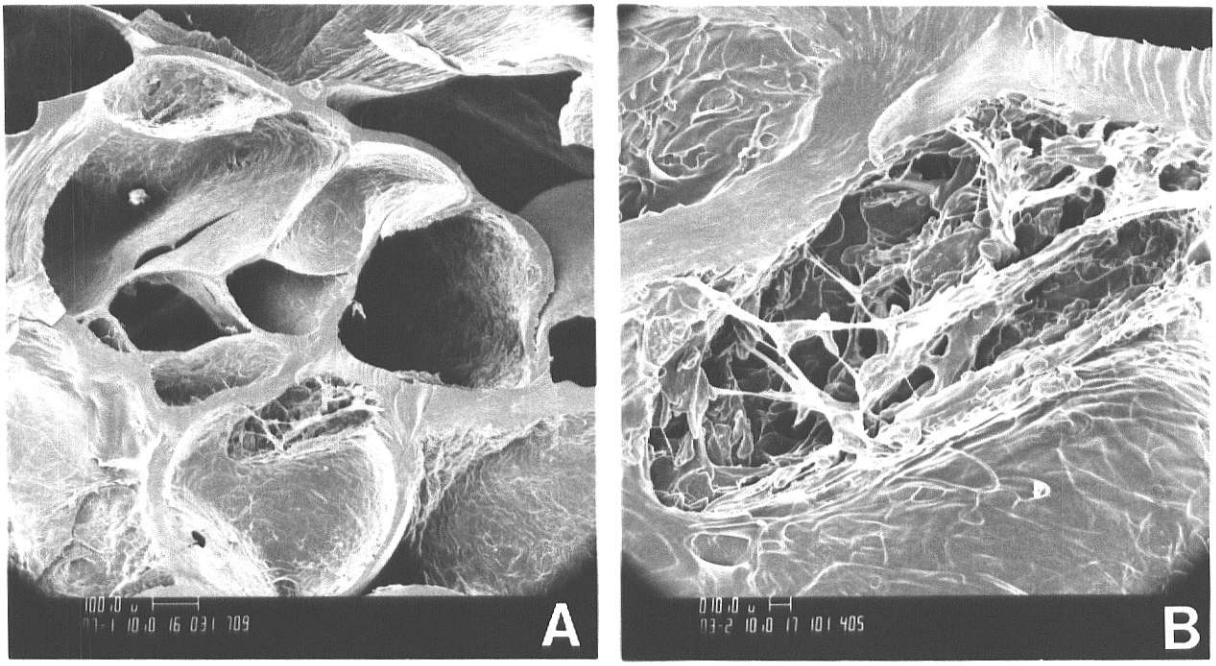

A
B

Table 1. Extrusion parameters of extruded mixtures of starch-lipids, starch-defatted YPC, and starch-reconstituted YPC

Extruded mixtures	Diameter[1] (mm)	Bulk density[2] (g/cc)	Breaking force[3] (kg)	Deformation time[4] (units)	Products rate (g/min)
lipids-starch[5] (direct addition)	10.75±1.06a*	0.261±0.048a	4.854±1.059a	50.3±10.5a	109.71± 9.26
lipids-starch[5] (premix method)	10.74±0.98a	0.254±0.039a	-	-	-
defatted YPC[6]	9.51±0.47b	0.194±0.011b	0.627±0.107b	39.6± 6.5b	96.5 ± 9.26
reconstituted YPC[7] (direct addition)	9.52±0.68b	0.332±0.038c	0.811±0.987b	33.2± 5.0c	83.0 ± 3.18
reconstituted YPC[7] (premix method)	9.67±0.72b	0.300±0.019d	-	-	-
10% YPC	9.82±0.64b	0.174±0.012b	0.848±0.152b	22.62± 4.6d	-
control[8]	11.66±0.96c	0.251±0.022a	1.471±0.306c	21.9 ± 5.4d	-

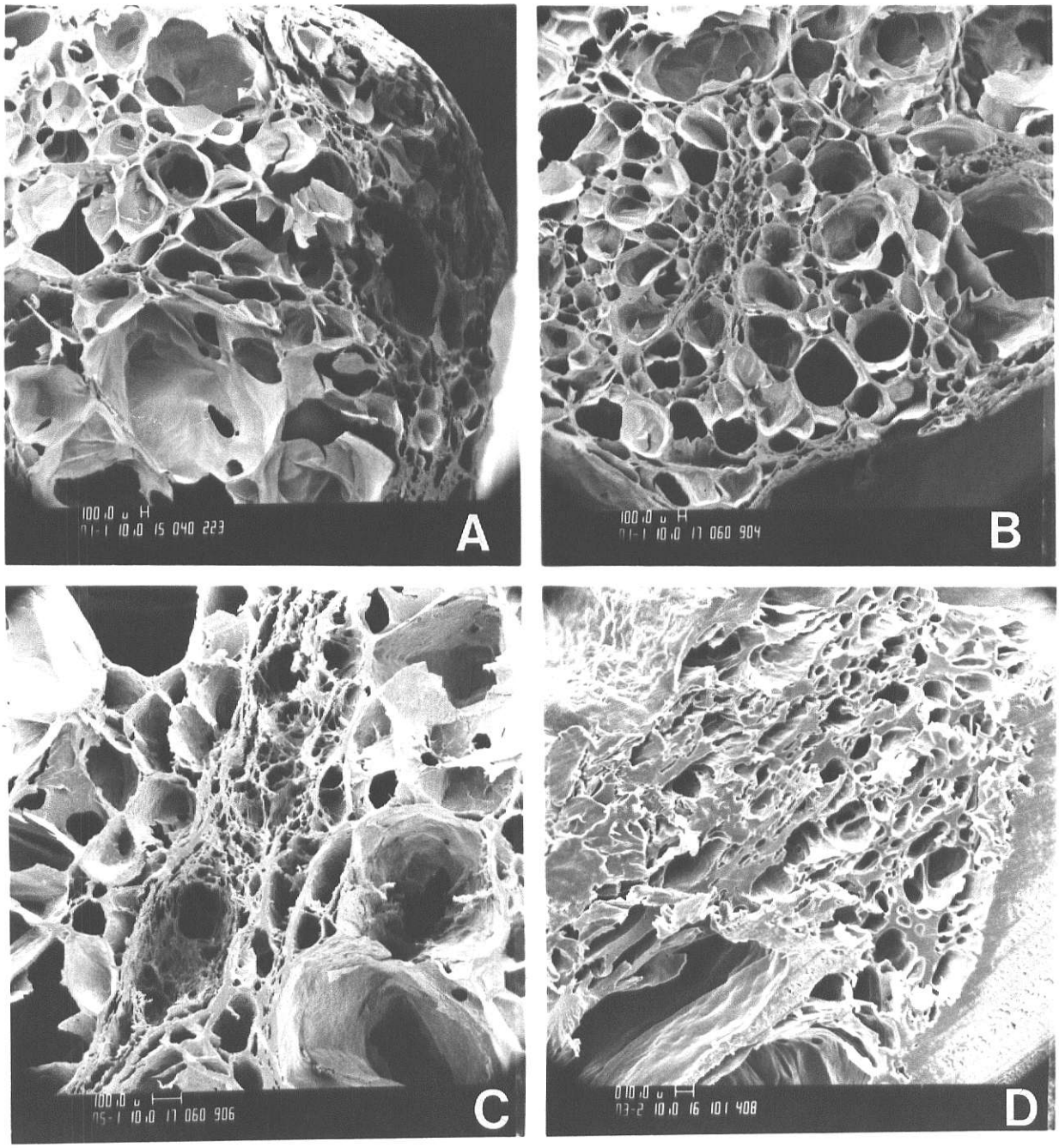
1. n = 50 $LSD_{.05}$ = 0.32
2. n = 10 $LSD_{.05}$ = 0.029
3. n = 17 $LSD_{.05}$ = 0.485
4. n = 17 $LSD_{.05}$ = 5.0
5. The amount of lipids added to starch was equivalent to 10% YPC.
6. The amount of defatted YPC added was equivalent to 10% YPC.
7. The amount of reconstituted YPC was equivalent to 10% YPC.
8. Control is extruded wheat starch.

* Values reported are mean and standard deviation
Values that have same letter footnote are not significantly different at = 0.05.

distribution of cell size is not even. There are areas of large cells and small cells interspread in the extrudate. For the extruded bound lipids-starch mixture (made by direct addition method), bound yeast lipids reduced the expansion of the extruded starch without affecting the bulk density (Table 1). This might be the result of production rate change. As shown in Table 1, the production rate of extrusion-cooking was altered by the addition of lipids or protein. The data showed that lipids can increase the production rate and defatting the YPC decreases the production rate (Table 1). The added bound yeast lipids increased both breaking force and deformation time of extruded starch (Table 1). Our findings are contrary to those reported by Cabrera (1978) and Faubion (1980) for other lipid systems. Cabrera reported that emulsifiers such as monoglycerides decreased expansion and production rate for extruded starch. Faubion (1980) reported that flour lipids decreased the expansion and strength (as measured as breaking and shear force) of extruded starch-gluten and starch-soy protein mixtures. Until the components of the bound yeast lipids and their individual effects on extrusion parameters are determined, it will be difficult to conclude whether the effect of "bound yeast lipids" is contributed by the lipids or other materials present in water-saturated butanol extract. Our data indicates that the resistance to mechanical force of extruded starch was increased due to the addition of bound yeast lipids.

Cross section views of extrudates containing bound yeast lipids (the lipids-starch mixtures are made by direct addition or premix method) are shown in Figure 10a and b. The added bound yeast lipids decreased the average cell size (Fig. 10a and b). As with extrudates containing YPC, dense areas and chunks were also seen (arrows in Fig. 10a and b). The dense areas appear to be composed of small cells (Fig. 10c). Chunks appear to be composed of cells that failed to expand (Fig. 10d) which are very similar to what is seen

Fig. 10. Scanning electron micrographs of extrudates containing bound yeast lipids;(A) starch-lipid mixture prepared by pre-mix method, (B) starch-lipid mixture prepared by direct addition method, (C) enlarged view of dense area indicated by the arrow in Figure 10(B), and (D) enlarged view of structures seen in Figure 10(A).


A
B
C
D

in extrudate containing 10% YPC (Fig. 7). Similar to what was found in extrudates containing YPC, thread-like materials bridging the cell wall of cells in dense areas (Fig. 11a) were also seen. At higher magnification, granules which may be starch granules embedded the the thread-like materials were found (Fig. 11b). Some small cells in the dense area whose cell wall surface were rough and torn on which granules that may be starch granules were found (Fig. 11c and d).

Defatting the YPC did not significantly influence the expansion, bulk density, and breaking force of extruded starch-YPC mixture (Table 1). Yet the removal of bound lipids resulted in a substantial increase in deformation time (Table 1). Faubion (1980) speculated that the breaking force is related to strength and resistence to mechanical force of the extruded materials. Deformation time may be a good measure of the ultimate stress which an extrudate can resist before breaking. Deformation time may be affected by the size and orientation of the expanded cells. However, as shown in Figure 6c and Figure 12a, the ultrastructure of extrudates containing defatted YPC or intact YPC were very similar. No major change in cell size and distribution were observed. Nevertheless, change in the appearance of surface of extrudates due to defatting the YPC were seen (Fig. 12b and c). The surface of extrudate containing 10% intact YPC appear to be rougher than that of extruded defatted YPC-starch mixture (Fig. 12b and c).

Reconstitution of YPC (by both direct addition and premix method) did not restore the original effect of YPC on extrusion (Table 1). When compared with extrudate containing 10% intact YPC, the extruded reconstituted YPC-starch mixture had higher bulk density and deformation time. This suggests that the water-saturated butanol treatment changed the functional properties of YPC. Figure 13a, b, and d showed the ultrastructure of extrudate containing 10% reconstituted YPC. They showed different parts of a cross section of the

Fig. 11. Enlarged view of (A and B) thread-like structures and (C and D) small cells seen in dense areas indicated by the arrow in Figure 10A and B.

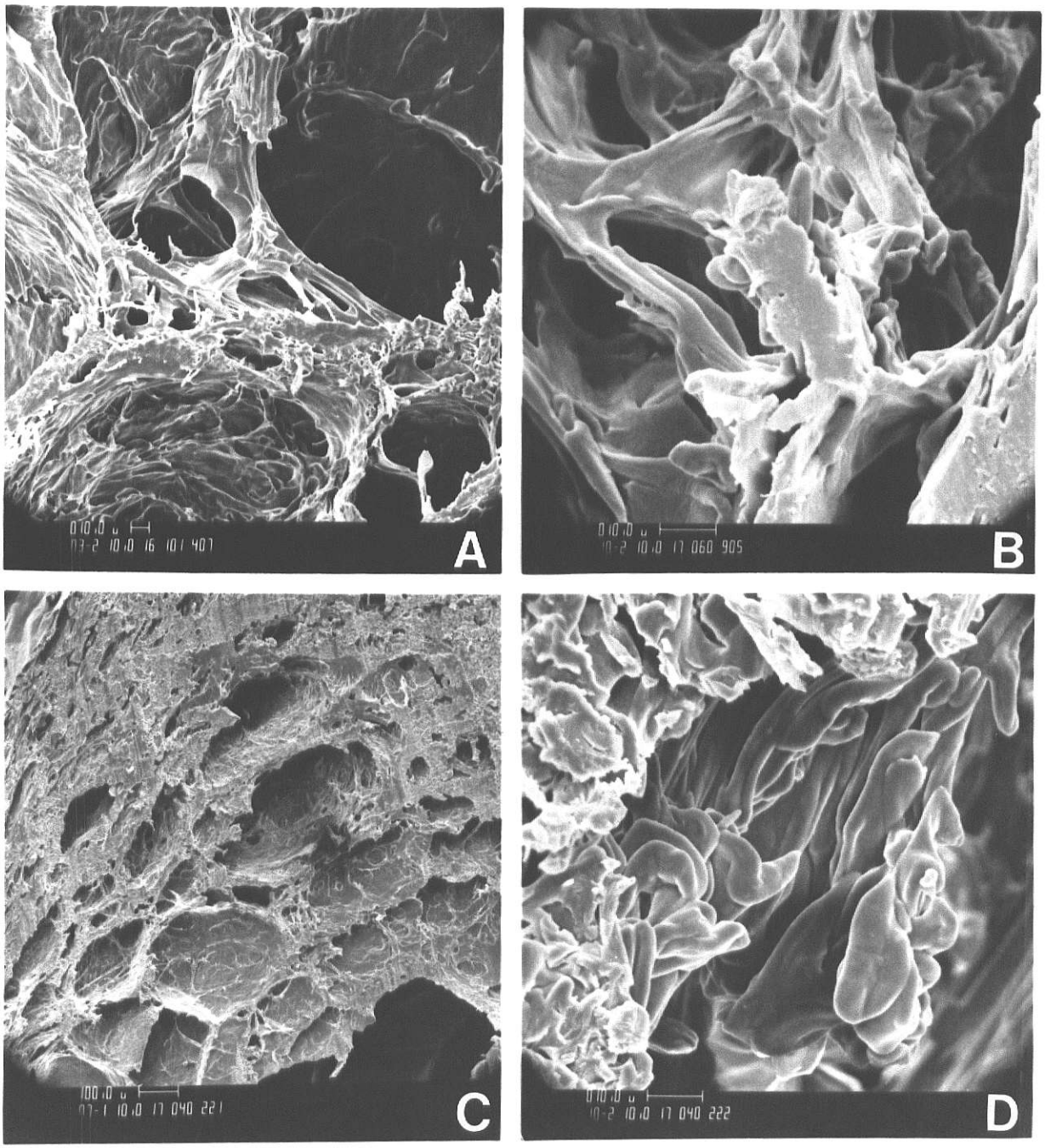
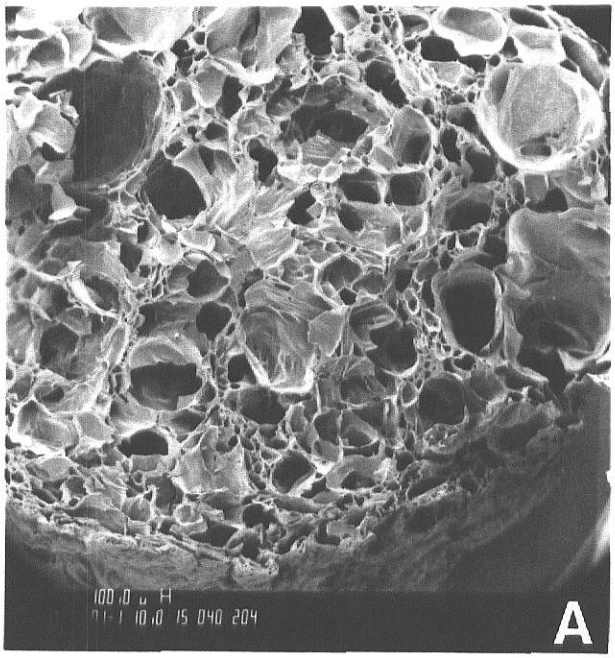
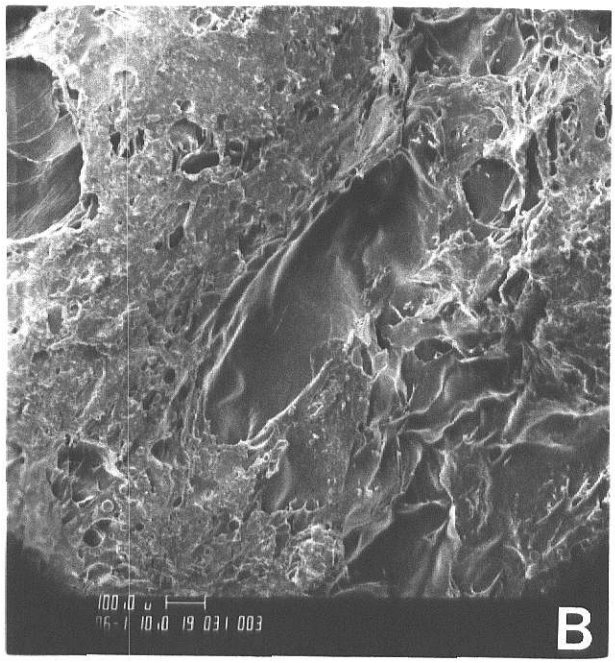

A
B
C
D

Fig. 12. Scanning electron micrographs of extruded (A and C) defatted YPC-starch and (B) intact YPC-starch mixture.


A


B

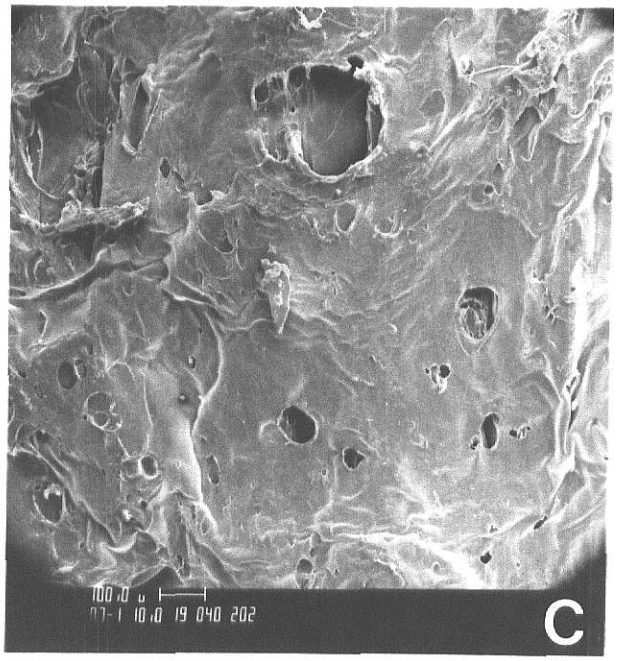
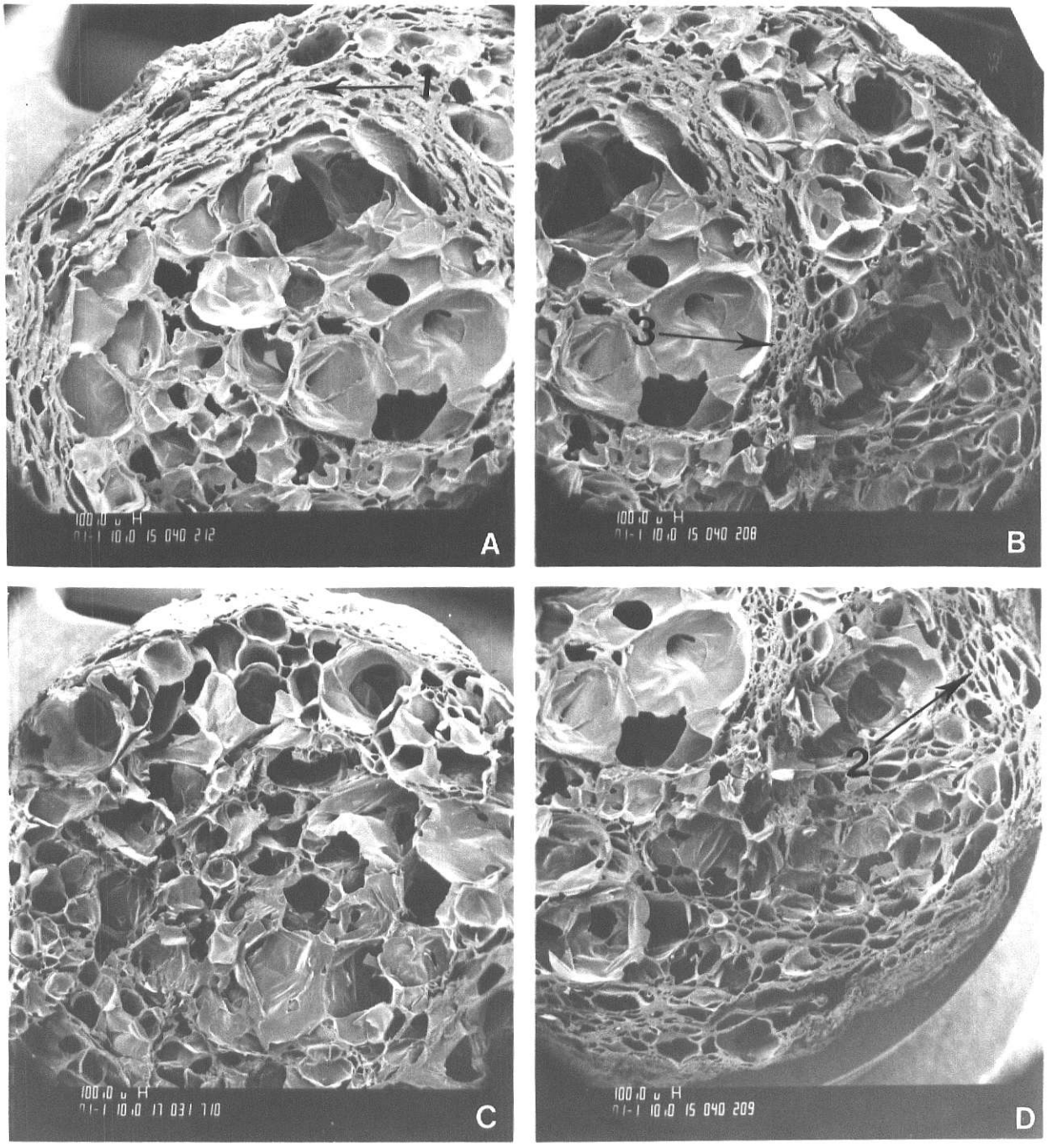
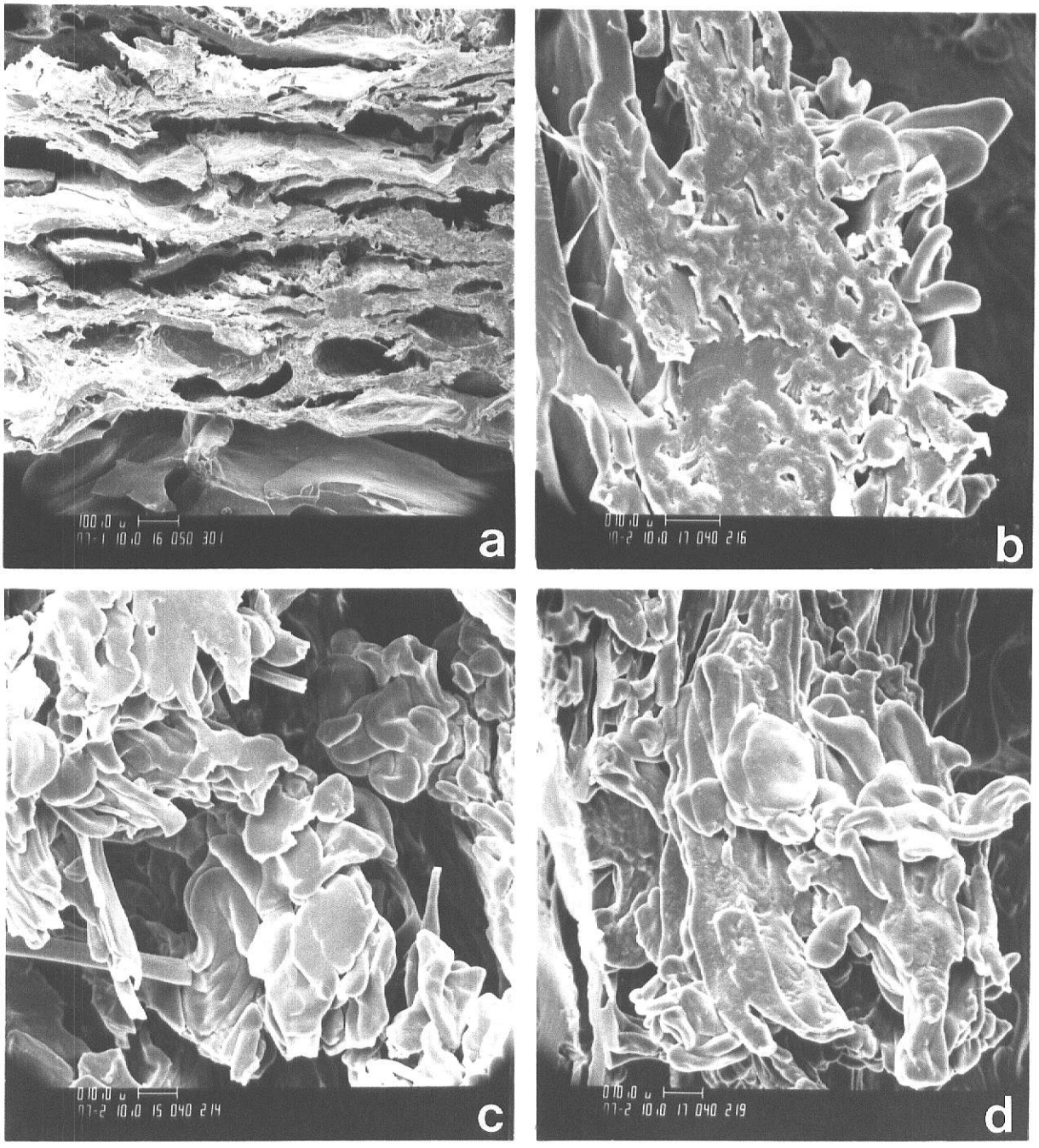

C

Fig. 13. Scanning electron micrographs of extruded reconstituted YPC-starch mixture (A, B, and D) and extruded YPC-starch mixture (C).


1
A
3
B
C
2
D

same speciman. Figure 13c showed the cross section of extrudate containing 10% intact YPC. The ultrastructure of extruded YPC-starch mixture is obviously altered by fractionation and recombination of YPC. Bands which consist of either a multiple layer structure (marked as region 1 in Fig. 13) or small and thick walled cells (region 3) are often seen in extruded reconstituted YPC-starch mixture (Fig. 13). Areas of small and elongated cells are also often seen (marked as region 2 in Fig. 13). These three (multiple layer structures, small thick-walled cells and areas of small and elongated cells) are not seen in extrudate containing intact YPC. At higher magnifications, the multiple layer structure appears to be layers with "melted" or "partially melted" starch granules adhering to them (Fig. 14a and b). Thread-like materials bridging the cell wall were found in regions 3 and 2 (Fig. 14) which appear to be very similar to those seen in extruded starch-bound lipids mixture (Fig. 10b, c, and 11a). Granules, which may be starch, embedded the thread-like materials. As with extruded starch-bound lipids mixture, granules which may be starch were found on the rough and torn surface of cell wall of the cells in region 2 and 3 (Fig. 14c and d).

Fig. 14. Scanning electron micrographs of extruded starch-reconstituted YPC mixture; (A, B) area of layered structure labeled 1 in Fig. 13 (A) and (C, D) enlarged view of areas marked 2 in Fig. 13D and areas marked 3 in Fig. 13B.


a
b
c
d

Effects of Dried Whole Yeast on Extrusion

To determine the effect of whole yeast on extrusion parameters, graded amounts of lypholized whole yeast were mixed with starch and extruded. The effects of intact dried yeast were examined and expressed in terms of expansion, bulk density, breaking force, deformation time, and shear force.

The addition of low levels of whole yeast ($\leq$ 6% W/W) reduced the expansion of the extruded starch. The reduction in expansion was inversely proportional to the amount of intact dried yeast added (Fig. 15). When addition was increased to between 8% to 12%, the added whole yeast increased the expansion of extruded starch. When addition level exceeded 12%, the added yeast again decreased the expansion. When the addition level was 15%, the expansion of extruded starch-whole yeast mixture decreased to a value very close to that of control (Fig. 15).

The addition of dried whole yeast reduced the bulk density of extruded starch (Fig. 15). The bulk density of extruded starch-whole yeast mixture reached a minimum limit when addition level reached 2% and further additions had no additional effect (Fig. 15).

The increase (or decrease) in expansion was not accompanied by a decrease (or increase) in bulk density indicating that the production rate was changed due to the addition of whole yeast (Fig. 15).

The addition of whole yeast (up to 12%) substantially increased the load required to cut through the extrudates (Fig. 16). The increase in shear strength was inversely proportional to the amount of dried whole yeast added. When the addition level was increased to 15%, the shear strength of extruded starch-whole yeast mixture decreased to a value that was very close to the control (Fig. 16). In this study, shear force was defined as the maximum force recorded before the blade cut two-thirds through the test sample.

Fig. 15. Expansion (x———x) and bulk density (•———•) of extrudates containing graded amounts of dried whole yeast.

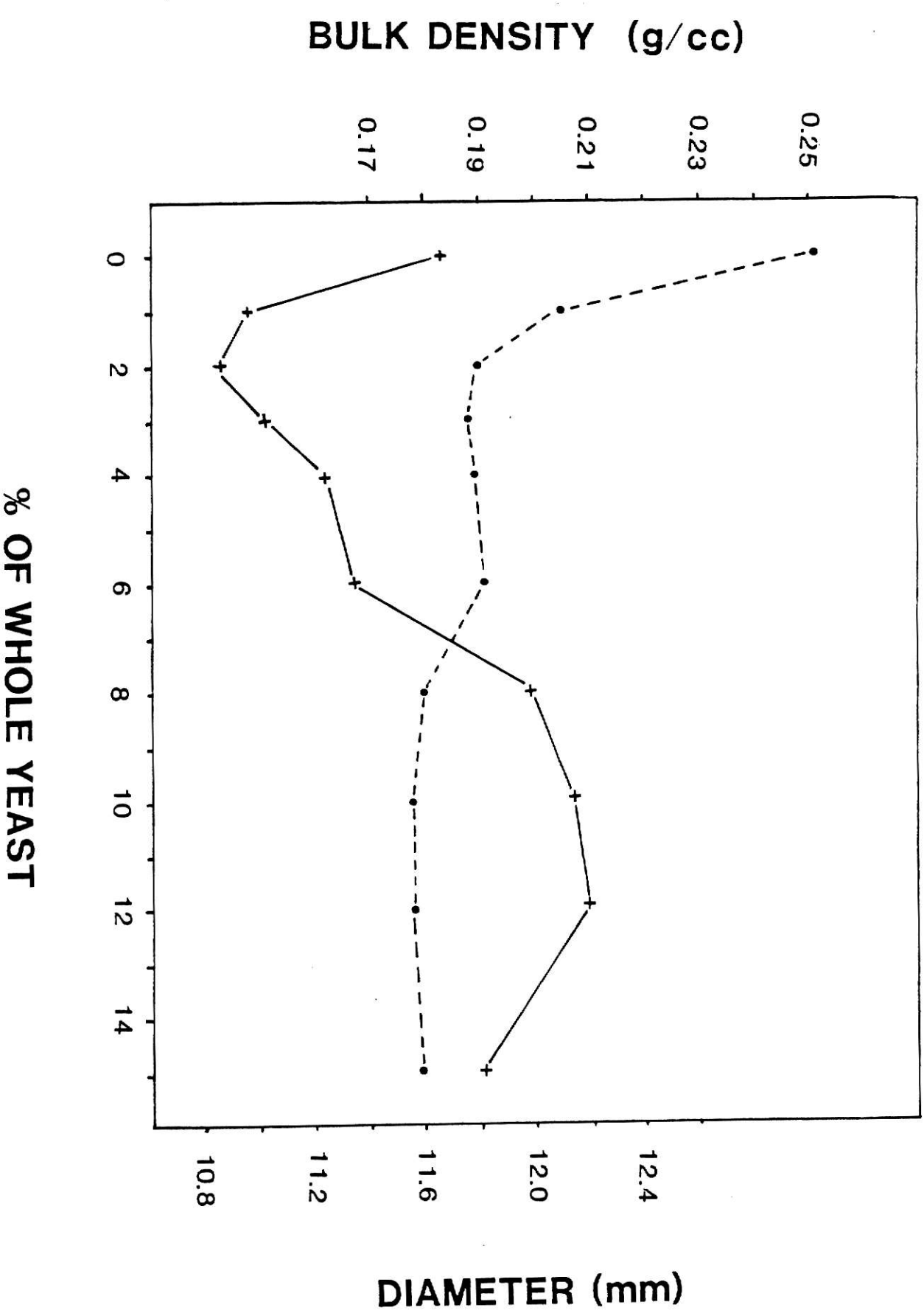
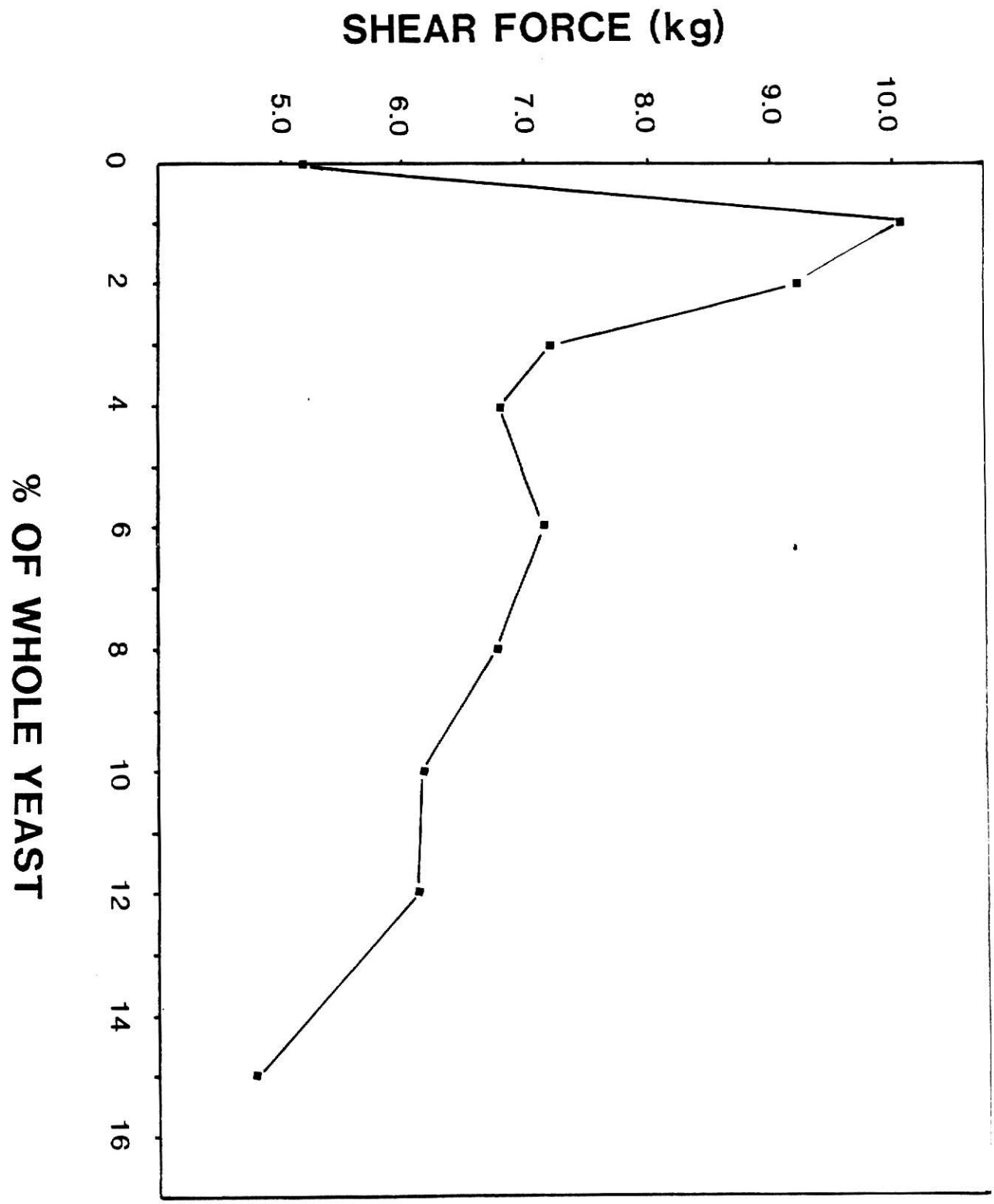

BULK DENSITY (g/cc)
0.25
0.23
0.21
0.19
0.17
0
2
4
6
8
10
12
14
% OF WHOLE YEAST
10.8
11.2
11.6
12.0
12.4
DIAMETER (mm)

Fig. 16. Force required to cut through extrudates containing dried whole yeast.


SHEAR FORCE (kg)
5.0
6.0
7.0
8.0
9.0
10.0
0
2
4
6
8
10
12
14
16
% OF WHOLE YEAST

Occasionally extrudates containing 1% or 2% whole yeast did not break while being cut, instead they were compressed and formed a dense material in the blade guide. This problem was reflected by the unusually high shear force for these samples which was beyond our operational range. As these unusually high values do not indicate the true shear force of the extrudates as defined, for this study, they were excluded from the statistical analysis.

Low addition levels (up to 6%) of dried whole yeast slightly decreased the breaking strength of extruded starch. The breaking strength of extruded starch-whole yeast mixtures reached minimum when addition level decreased to 6%. As addition level was in the range of 6% to 8%, the effect of added yeast reversed. But when addition level exceeded 8%, the breaking force of extruded starch-whole yeast mixtures decreased with the amount of whole yeast added (Fig. 17).

Figure 18 showed the effect of added whole yeast on the deformation time of extruded starch. No deformation times of extrudates containing whole yeast were significantly different from that of control ($\alpha = .05$).

Our data suggests that expansion of extruded starch can be improved without affecting the texture (as determined by shear force, breaking force, and deformation time) by adding 10% to 12% whole yeast.

Low levels of addition of whole yeast changed the ultrastructure of extruded starch (Fig. 5 and 19a). The cells appeared to be shallower and smaller than control (Fig. 5a and 19a). As the addition level increased, both cell depth and size increased (Fig. 19a, b, and c). At 12% addition, the extrudates resemble the extruded starch in cell size and distribution (Fig. 5a and 19c). At all addition levels, whole yeast did not affect the morphology of the cell wall (Fig. 5 and 19).

The effects of dried whole yeast on starch extrusion are quite different from those of yeast protein concentrate (YPC). Scanning electron microscopic

Fig. 17. Force required to break extrudates containing dried whole yeast.

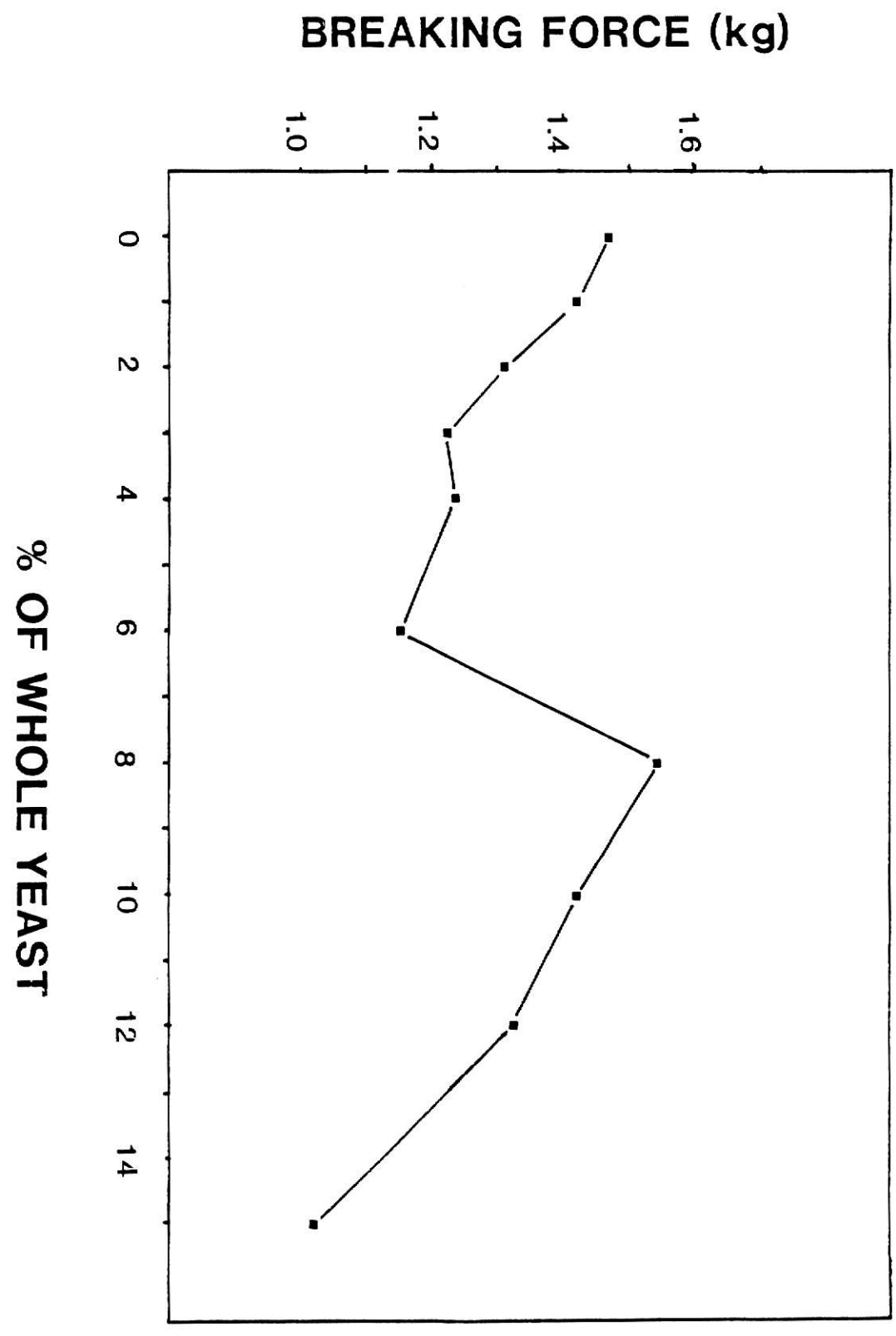

BREAKING FORCE (kg)
1.0
1.2
1.4
1.6
0
2
4
6
8
10
12
14
% OF WHOLE YEAST

Fig. 18. Deformation times for extrudates containing dried whole yeast.

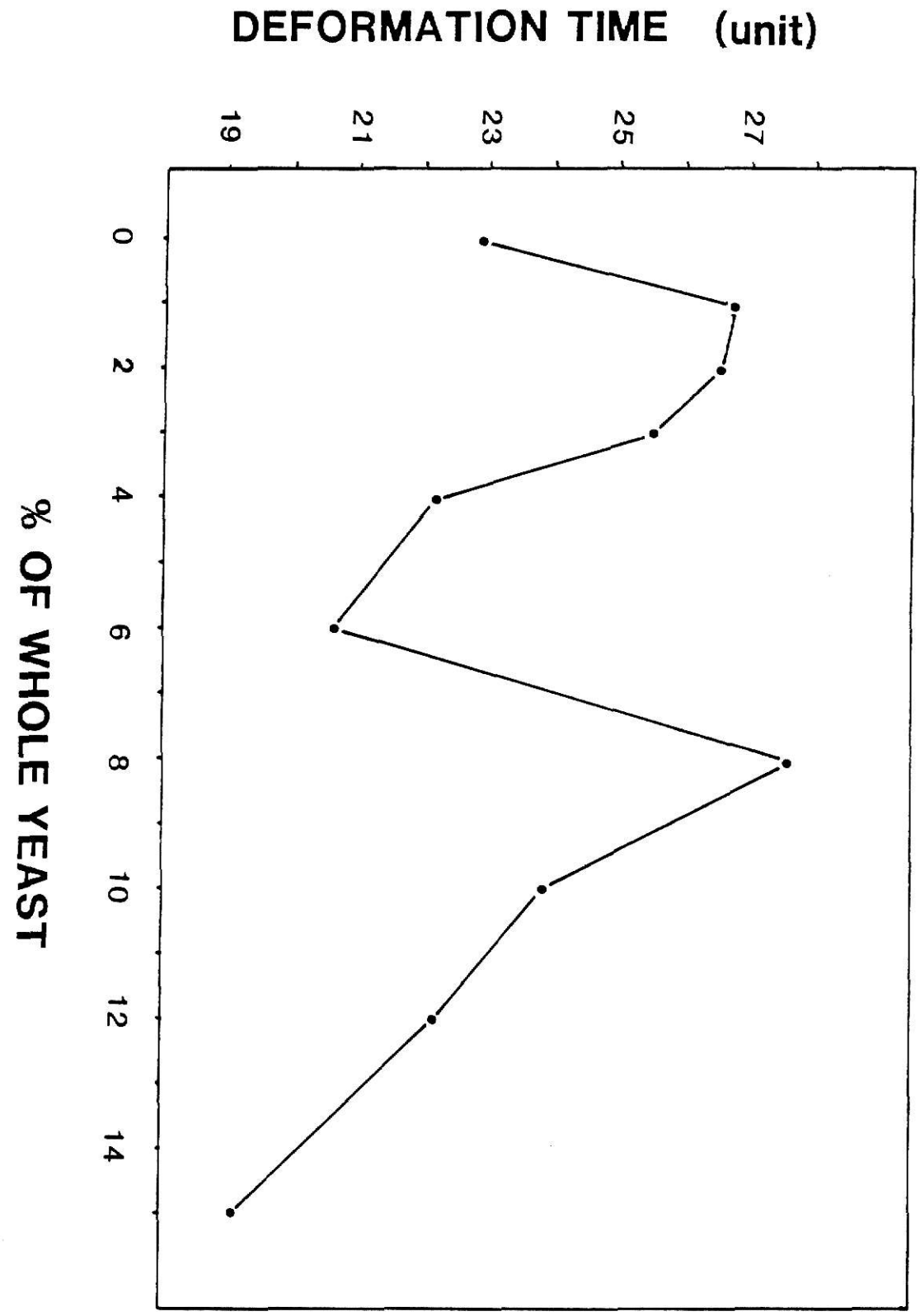
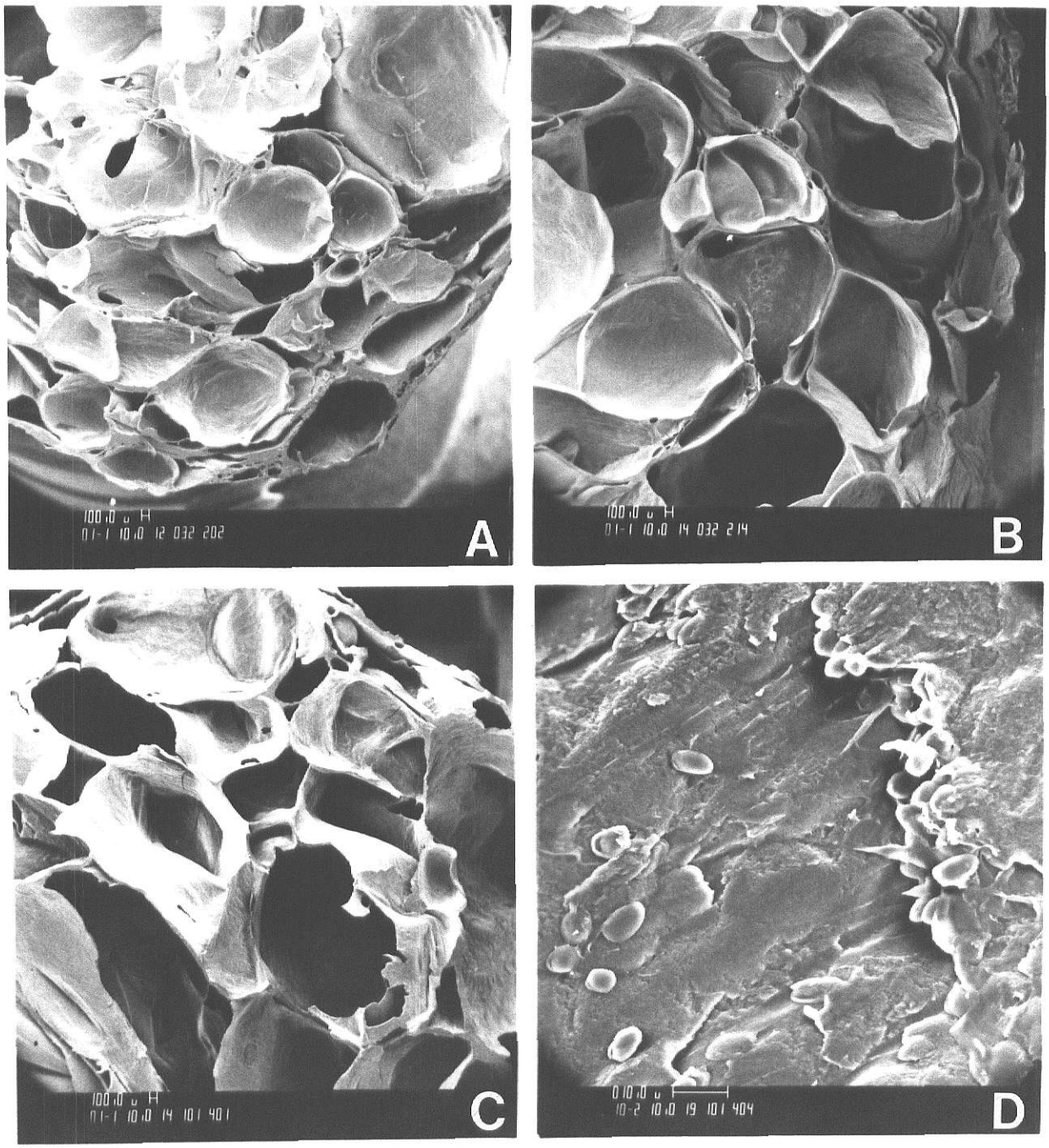

DEFORMATION TIME (unit)
19
21
23
25
27
0
2
4
6
8
10
12
14
% OF WHOLE YEAST

Fig. 19. Scanning electron micrographs of extruded starch-dried whole yeast mixture (A) 1% WY, (B) 6% WY, (C) and (D) 12% WY.


A
B
C
D

examination of extrudates containing whole yeast showed some granular structure which may be yeast cells embedded in the extrudate cell wall (Fig. 19d). Possibly, yeast cell wall encapsulates the cytoplasmic material (protein and lipids) and prevents them from interacting with starch. To test this hypothesis, yeast cells were ruptured by homogenization and the homogenized suspension centrifuged. The precipitated fraction was labeled cell wall material. Lypholyzed homogenized yeast (10%) was added to starch and extruded. As shown in Table 2, the addition of homogenized yeast resulted in a decrease in expansion, breaking force, and bulk density. In all respect, the extruded homogenized yeast-starch mixture resembled extrudates containing 6% YPC. Data summarized in Table 2 supports the hypothesis that the yeast cell wall encapsulated the cytoplasmic material and this was responsible for the difference in the effects between YPC and intact yeast on extrusion (according to data from the brewing company that prepared the YPC and intact yeast, 6% YPC is about equivalant to 10% homogenized yeast). If that hypothesis is correct, then the effect of intact yeast cells on extrusion should be duplicated by that of yeast cell walls. One per cent and 2% cell wall debris were added to starch and extruded. Results are summarized in Table 2. The addition levels were chosen based on data from the brewing company. It was found that extruded cell wall-starch mixture did not duplicate the effect of whole yeast (Table 2). The extruded cell wall-starch mixture had less expansion but had a much greater deformation time and slightly higher shear force (Table 2). Two factors might be responsible for these results. One is that this cell wall materials have been contaminated with cytoplasmic material during isolation. The other possibility might be an effect of pH. The pH of extruded, homogenized yeast-starch and cell wall-starch mixtures was determined by the method of Stearns (1974). The pH of the extrudates containing 1% and 2% cell wall, and 10% homogenized yeast were 9.20, 9.35, and 9.50, respectively. To

Table 2. Effect of cell wall on extrusion

Treatment	Diameter (mm) n=50	Bulk density (g/cc) n=10	Breaking force (kg) n=17	Shear force (kg)	Deformation time (unit) n=17
Control	11.66±0.96	0.251±0.837	1.740±0.306	5.222±0.837	21.9± 5.4
10% whole yeast	12.14±0.79	0.178±0.009	1.420±0.353	6.166±0.947	22.8± 4.9
6% YPC	10.53±0.69	0.167±0.017	1.009±0.195	3.855±0.618	23.4± 5.2
10% yeast cell homogenate	10.71±0.65	0.163±0.012	1.043±0.230	-	24.1± 4.6
1% cell wall	10.74±0.84	0.209±0.014	1.498±0.189	8.820±0.681	42.4±11.9
2% cell wall	10.30±0.67	0.167±0.017	1.298±0.160	7.350±1.344	50.1±11.7

determine if pH was the factor affecting expansion and texture of extrudates containing cell wall and homogenized yeast, cell wall materials and homogenized yeast were resuspended. HCl (4N) was added to bring the pH to 7.0. Neutralized cell wall materials and homogenized yeast were lypholized, added to wheat starch, and extruded. Results are summarized in Table 3. As shown in Table 3, neutralization improved the expansion of extruded starch-cell wall mixture and starch-homogenized yeast-starch mixture. The diameter of extrudate containing 1% neutralized cell wall is very close to that of 10% intact yeast cell. The diameter of extrudates containing 10% neutralized yeast cell homogenate is smaller than that of extruded starch-neutralized cell wall mixture but is larger than those of 6% YPC and 10% pH 11 yeast cell homogenate. Data suggest that pH adjustment affected production rate as reflected by an increase in diameter without decreasing the bulk density (Table 3). The pH adjustment altered the strength of extrudates containing yeast cell wall but did not influence the texture of extruded starch-yeast cell homogenate (Table 3). The deformation time and breaking force of extrudates containing neutralized yeast cell wall is higher than that of 10% dried whole yeast cell (Table 2 and 3).

Apparently the added neutral yeast cell wall did not duplicate the effect of intact dried yeast. This could be due to that after cell walls are ruptured, the cell membrane and glucan of cell wall (the interior side of the cell wall) become available to interact with starch and, therefore, alter the texture of the extrudate.

Table 3. Effect of pH adjustment on extrusion cooking of cell wall-starch and yeast cell homogenate-starch mixtures

Treatments	Diameter (mm)* n=50	Bulk density** (g/cc) n=10	Breaking force*** (kg) n=17	Deformation time**** n=17
pH = 7 cell wall	12.23±0.84	0.219±0.019	2.061±0.224	63.8± 9.7
pH > 9 cell wall	10.74±0.84	0.209±0.017	1.498±0.189	42.4±11.9
pH = 7 homogenate	11.29±0.71	0.212±0.007	0.914±0.196	20.0± 3.9
pH > 9 homogenate	10.71±0.65	0.163±0.012	1.043±0.230	24.1± 4.6

*$LSD_{.05}$ = 0.44 (expansion)
**$LSD_{.05}$ = 0.014 (bulk density)
***$LSD_{.05}$ = 0.146 (breaking force)
****$LSD_{.05}$ = 5.6 (deformation time)

SECTION III. ISOLATION OF FERMENTATION STIMULANT(S) FROM A YEAST PROTEIN CONCENTRATE

CONCLUSIONS

Results of this study demonstrate that fractionation and recombination of the major components of brewer's yeast and brewer's yeast protein concentrate are effective methods for investigating the effect of starch extruded in combination with them. Several conclusions can be drawn from the data. First, yeast protein concentrate reduced expansion and strength of extrudate. Second, bound yeast lipids reduced expansion but increased textural strength of extruded starch. Third, due to our inability to defat YPC without altering yeast protein, the effect of that protein alone on extrusion remained unclear. Fourth, the effect of dried whole yeast on extrusion depends on its addition level. Data suggest that it is possible to modify either expansion or textual strength without affecting the other by adding appropriate amounts of dried whole yeast or appropriate combinations of either YPC and intact yeast or YPC and cell wall. Fifth, the effect of cell wall and homogenized yeast on extrusion varies with pH. A final conclusion concerns the relationship between expansion, internal structure, and texture of the extrudates. Formerly, it was believed that a poorly expanded product would be dense and hard while a highly expanded product would be delicate and fragile. Faubion (1980) reported the reverse. Data in this study indicates that the textural strength is not related to expansion. Rather, expansion is related to internal structure. Generally, the expanded products have larger but fewer cells than the poorly expanded ones. In addition to the difference in cell size and number, areas of small cells are only seen in poorly expanded products.

INTRODUCTION

Yeast, the only living ingredient added to dough, has three basic functions in breadmaking: (a) leavening action, the result of carbon dioxide production; (b) flavor development, the result of formation of alcohols, acids, esters, and other flavor precusors; and (c) modification of doughs rheology properties (Magoffin and Hoseney, 1974; Pyler, 1973; Seeley, 1962; and Hoseney et al, 1979).

Papers reporting the effect of fermentation on dough rheology and bread flavor are available. However, in this review we shall concentrate on the factors affecting gas production.

Effects of several factors on yeast fermentation have been reported (Pyler, 1973; Magoffin and Hoseney, 1974; Reed and Peppler, 1973; Pomper, 1969; Seely, 1962). Yeast can tolerate extremes of pH for short time periods. However, prolonged exposure to low pH will decrease yeast activity. In a dough system, the pH is rapidly reduced to about pH of 5.0. This is near optimum range for fermentation. The rapid drop in pH is because of the dissolution in the aqueous phase of carbon dioxide initially produced by yeast. Temperature has a potent effect on the rate of fermentation. Literature values indicated that glucose fermentation is twice as fast at 35^{o}C as at 25^{o}C. Under both conditions, yeast was reported to achieve its maximum activity at 38^{o}C. However, the high level of activity did not last long at that temperature. As temperature increases, the reaction rate increases; however, the potential for inactivation also increases. At higher temperatures, the inactivation effect becomes more significant. Thus at 35^{o}C yeast has lower activity than it has at 38^{o}C, but the activity persists longer. Yeast is a very osmotolerant organism. High concentrations of sugar and salt affect the fermentation rate but are not lethal. The fermentation rate in dough is

lowered when salt concentration exceeds 2% or sugar concentration exceeds 5% (Pyler, 1973) to 10% (Pomper, 1969). Pomper (1969) reported that sucrose had a positive effect on yeast fermentation at level less than 6%.

For maximum yeast activity, carbon, nitrogen, potassium, magnesium, sulfur, and phosphorous are required. In addition, vitamins such as thiamine, pyridoxine and nicotinic acid have been reported as necessary for maximum activity (Alkins et al, 1945; Schultz, 1937 and 1938). Yeast can utilize most common monosaccharides (except galactose) and such disaccharides as sucrose and maltose. It also utilizes the oligosaccharides of the glucofructosan series. However, yeast ferments different sugars at different rates. Glucose and fructose are fermented rapidly while maltose is not fermented when monosaccharides and sucrose are present. When readily fermentable sugars are exhausted, the yeast will adapt to the fermentation of maltose. Pomper (1969) and Pyler (1973) have discussed the mechanism of maltose adaption. Antimicrobial agents, pesticides, and herbicides have been reported to retard fermentation (Pyler, 1973). However, certain antimicrobial agents such as monolaurine retarded fermentation only when mechanical force was applied to mix the flour-water mass into dough (Finney et al, 1982). Monolaurine was found to stimulate fermentation when excess water was added and mechanical force was not applied (Finney et al, 1982). Sulfite and certain metal ions, such as Cd, Cu, Hg, Os, and Pd are fermentation inhibitors (Reed and Peppler, 1973). In other papers, Cu was reported to act as fermentation activator (Chen, 1959) or to have no effect on fermentation (Ling and Hoseney, 1977). Some naturally occurring substances, such as protamine hydrochloride and purothionin isolated from wheat flour, have an inhibitory effect on fermentation. However, the adverse effects of purothionin and protamine hydrochloride can be destroyed by heat, protease action, low concentration of salt, and certain water soluble materials from flour (Reed and Peppler, 1973).

Factors that can stimulate fermentation can be classified into three groups: vitamins, minerals, and unknown factors.

Vitamins such as thiamine, pyridoxin, nicotinic acid, biotin, pantothenate and inositol were long believed to be necessary for optimum yeast growth and maximum activity (Reed and Peppler, 1973; Pyler, 1974; Schultz, 1937 and 1939). However, Schultz et al (1937a) reported that thiamine may inhibit certain types of yeast while stimulating others. Reed and Peppler (1973) reported that thiamine is not needed for optimum yeast growth but it is a potent stimulant for the fermentation of dough. Ling and Hoseney (1977) found that inositol was not effective in stimulating gassing power. These may seem contradictory, however, the amount and type of vitamins required for optimum yeast growth or fermentation varies greatly with the type (strain) of yeast, yeast source (including feed composition, growth condition and state, harvest method, storage condition after harvest), and the composition of medium used in the test (Reed and Peppler, 1973). Also current yeast producers add vitamins to the culture medium during yeast production. Those added vitamins may accumulate in the yeast (Ling and Hoseney, 1977).

Certain amino acids such as β-alanine were reported to be able to accelerate fermentation (Schultz, 1937). But later work showed that they had no effect on fermentation (Reed and Peppler, 1973). Minerals that were reported to be fermentation activators include: K, Na, Mg, Zn, Cu, Co, I, Mo, and Mn (Reed and Peppler, 1973; Pyler, 1973; Chen, 1959). The requirement for these minerals varied with the type, source of yeast, and medium used in the test. Although the functions of vitamins and minerals are not completely known yet, they are certainly involved in metabolism. They are found to be co-enzymes or enzyme co-factors. Instead of considering them as fermentation activators, it is preferable to call them "essential yeast nutrients".

The last category of reported fermentation activators are unknown

materials. This class includes malt flour, yeast extract, autolyzed yeast, autoclaved yeast, cotton plant extract (Euler and Swartz, 1924; Schultz et al 1937b); Lee and Geddes, 1959; Hoseney et al, 1969; Atkin et al, 1945; Reed and Peppler, 1973; Pyler, 1974). It is well known that in dough, milk solids will cause an acceleration in gas production. Lee and Geddes (1959) attributed the improving effect of milk solids on gas production to its buffering effect. Pyler (1973) attributed the improvement effect of milk solids to the fact that yeast is stimulated by the milk solids. Pyler (1974) cited no data to support his theory.

Hoseney et al (1969) found that the dialyzable fraction of flour water solubles stimulated yeast activity. They also found that the effect of this fraction could be replaced by appropriate amount of yeast or NH_4^+. It seems likely that this improving effect of flour water solubles on gas production was a result of nitrogen supplementation rather than yeast stimulation by the dialyzable fraction of wheat flour water solubles. Euler and Swartz (1924) reported that malt embryo extract, rye extract, and yeast extract could accelerate yeast growth and fermentation. The authors postulated a growth factor, called "Z" factor, that was not able to be replaced by vitamins. Their data proved that the stimulating effect was neither due to enzymatic action nor mineral supplementation.

It is a well known fact that the addition of malt improves bread volume. Pyler (1973) suggested that the improvement might be a result of amylase action but that other unknown mechanisms might also be involved. Magoffin and Hoseney (1974) doubted that the effect of added malt on fermentation rate was contributed by amylase action. Euler and Swartz (1924) reported that a malt extract could accelerate fermentation rate and this effect was not a function of enzymatic action. They did not describe their extract preparation method, thus their findings can not be associated with malt flour

action. However, their findings strongly suggest that the effect of malt flour was not due to enzymatic action or mineral supplementation. The vitamins present in the malt are unlikely to have had much effect. Thus, there may be an unidentified growth factor(s) which can accelerate yeast growth and fermentation.

Euler and Swartz (1924) first reported that yeast extracts could stimulate yeast growth and fermentation. Heat treatment alone did not change the effect, but heating under oxidative or alkaline conditions did. The effect was totally lost when the extract was ashed. Schultz et al (1937b) found that yeast extract, autoclaved yeast, and vitamin B_1 precusors were fermentation accelerators. They thought vitamin B_1 was responsible. Shultz's conclusions are not convincing. The fact that some vitamin B_1 structural analogs, products of thiamine degradation during heat treatment, have some effect does not mean that the effect of autoclaved yeast and yeast extract are contributed by vitamin B_1. However, Shultz's work does show that the effect is not changed by heat treatment. This implies that the improvement effect is not a function of enzymatic action. Neither Euler and Swartz (1924) nor Shultz (1937b) described their extraction and preparation methods so it is difficult to compare their work.

A water soluble extract of autolyzed yeast was found to accelerate fermentation rate without affecting yeast cell population or cell size (Lee and Geddes, 1959). The authors also reported that the active constituent was dialyzable but fractionating the extract always resulted in partial loss of activity.

To date no one has successfully isolated any yeast fermentation activators from yeast extract (or autolyzed yeast). A growth factor for Lactobacillus hichi was isolated from yeast extract by Japanese scientists. It was shown to be two peptides containing glutamic acid (Higashi et al, 1978).

Because this growth factor was heat labile, it could not be responsible for the stimulating effect of yeast extract on fermentation.

Our previous study (part I of this thesis) showed that the addition of YPC to dough resulted in higher bowl and pan height but reduced loaf volume. High bowl and pan height are signs of high fermentation rate. Gasograph data confirmed this supposition. In this study, our goal was to isolate and identify the factor(s) responsible for the increase of gassing power in yeast.

MATERIALS AND METHODS

Materials

Flour. Wheat flour was obtained from the Ross Company. Protein content was 12.1% with good volume potential and a medium long mixing time of 4.6 min. Baking absorption of the flour was 62%.

Non-fat dried milk. Land O'Lakes Company 33.4% protein (N X 6.25 as is basis.

Soy protein concentrate. Ralston Purina 85% protein (N X 6.25, as is basis).

Pronase (protease type XIV). Sigma Chemical Company.

Reagents. Reagents are reagent grade.

Cellophane film (dialysis film). Union Carbide Company. Cut-off range approximately 10^4 Daltons.

Methods

Gasograph. Gasograph gas production was determined on a slurry as described by Rubenthaler et al (1980). The slurry contained 15 ml H_2O, 10 g flour (14% mb), 0.15 g NaCl, 0.6 g sucrose, and 0.2 g compressed yeast. Gas production during 4 hr fermentation was recorded and expressed as gasograph units (GU); 1 GU = 2.38 ml of CO_2.

RESULTS AND DISCUSSION

Effect of Nitrogen Present in YPC

Previous work (part I of this thesis), showed that the addition of YPC to dough resulted in higher bowl and pan heights but lower loaf volume. The high bowl and pan heights were taken as evidence of high fermentation rate. Preliminary work showed that the addition of 5% YPC (base on flour weight) increased gas production by 25%. It was suspected that this increase might be the result of the nitrogen present in YPC. Thus, nonfat dried milk, soy protein concentrate, and 5% YPC were added to 10 g of flour (14% mb) and a gasograph test conducted to determine their effect on fermentation (Table 1). Nonfat dried milk improved yeast gassing power but the effect was limited. Soy protein concentrate had no effect on gas production and 5% YPC increased gas production by 25%. The effect of added protein on fermentation varied greatly with protein source strongly suggesting but not conclusively proving that the improvement effect of YPC on gassing power is not a function of added nitrogen. Ling and Hoseney (1977) used a synthetic mixture which with adequate amount of sugar was able to maintain a gassing rate equal to that of flour plus ammonia. The formulation per 10 ml total volume of this synthetic mixture was:

$(NH_4)_2HPO_4$	2×10^{-2} g
$MgSO_4\ 7H_2O$	1.4×10^{-2} g
KCl	6.0×10^{-3} g
Thiamine HCl	3.0×10^{-5} g
Pyridoxine HCl	3.0×10^{-5} g

To determine if the improvement effect of YPC was due to its nitrogen content, 4 ml of the synthetic mixture was used to replace 4 ml of the 15 ml of water in Rubenthaler's formula (1980). A gasograph test was used to

Table 1. Effect of YPC, NFDM and soy protein concentrate on fermentation

Gas production after 4 hr fermentation (GU)***	Protein added n=2**			
	None	NFDM	Soy protein	YPC
Gas production*** after 4 hr fermentation	51.7±1.5*	55.5±0.7	52.7±1.5	64.4±0.5

*Standard deviation

**n = 2 n = number of observations within each treatment

***Gas unit 1 G.U. = 2.38 ml CO_2 (under $30^{o}C$, 1 atm)

determine the effect of YPC on a system optimally fortified with nitrogen. The addition of 2%, 5%, and 10% YPC increased gas production by 11%, 20.5%, and 25.8%, respectively (Table 2). This result proved that the improvement effect of YPC was not due to its nitrogen content.

Effect of Fermentable Sugar Present in YPC

The improvement effect of YPC occurred during the third and fourth hours of fermentation (Table 2). The improvement was suspected to be due to yeast growth or to the fermentable sugar present in YPC. A yeast plate count showed that the yeast population did not change significantly during the 4 hr fermentation. This finding is consistent with that reported by Lee and Geddes (1959). They reported that a water soluble fraction of autolyzed yeast accelerated yeast fermentation without affecting cell population. To study the effect of fermentable sugar present in YPC, 5% YPC was replaced with a calculated amount of sucrose. The calculation was based on the assumption that YPC contained 25% fermentable sugar (according to our analysis YPC contained 72% protein (N x 6.25; as is basis), 6% moisture, and 4.2% ash. If fermentable sugar present in YPC was responsible for gassing power improvement, then its substitute (sucrose) should also have a significant effect. The gasograph data showed that the additional sucrose had no effect on fermentation (Table 3). This proved that the effect of YPC was not contributed by any fermentable sugar it might contain.

Effect of Minerals Present in YPC

Chen (1959) and Reed et al (1973) reported that some mineral ions (Fe, Zn, Cu, and Mn) could stimulate yeast fermentation. Possibly the flour used in this test was low in some of the essential metal ions. To test the above hypothesis, YPC was hydrolyzed with HCl under vaccum at 100^{o}C for 24 hr. The HCl was removed by reduced pressure evaporation. A gasograph test

Table 2. Improvement effect of YPC on yeast gassing power (N-supplemented)

Treatments (n=2)	Gas production during fermentation (GU)			
	1st hr	2nd hr	3rd hr	4th hr
2% YPC	8.3±0.4	25 ±0	46.3±0.4	66.2±0.1
5% YPC	8.5±0	23.9±0.2	47.2±0.3	71.6±0.5
10% YPC	8.6±0.1	24.3±1.1	47.5±0.7	74.8±0.4
Control	8.3±0.4	25 ±0.2	43 ±1.4	59.5±0.4

1. LSD .05 = .09

Table 3. Effect of sucrose replacement

Treatments*	Gas production during fermentation (GU)**			
	1st hr	2nd hr	3rd hr	4th hr
Control	7.5±0.4***	23.9±0.2	41.5±0	58.4±0.6
5% YPC	8.4±0.2	26.4±0.2	50.8±0.4	73.0±0
Substitute	7.4±0.3	22.4±3.1	42.3±4.3	58.5±3.1

*n = 2 Number of observations within each treatment

**Gas unit 1 G.U. = 2.38 ml CO_2 (under $30^{o}C$, 1 atm)

***Standard deviation

was used to determine the effect of hydrolyzed YPC (Table 4). The hydrolyzed YPC had a limited effect on fermentation, compared with intact YPC. The small improvement could be due to the metal ions and/or certain acid resistant vitamins. This data proved that the metal ions present in YPC were not a major factor in the acceleration of fermentation.

Effect of Free Vitamins Present in YPC

The vitamin requirements of yeast for maximum activity have been reported (Pyler, 1974; Chen, 1959). To eliminate the possibility that flour used in this series of tests was low in certain specific vitamins which then were supplied by the addition of YPC, the following trial was conducted. YPC was dialyzed against distilled water. About 10% of the YPC was removed by dialysis. Gasograph data showed that the dialysate had only a limited effect on gassing power. The dialyzed YPC possessed more than 80% of the original activity. Reconstitution of YPC by combining appropriate amounts of dialysate and dialyzed YPC (DYPC) restored the original activity. Thus the dialysate contributes some but not a major improvement (Table 5). This conclusion was confirmed when the addition level of dialysate was raised to 5% (base on 14% mb flour weight). The high level did not produce any additional effect (Table 5). The data showed that the major effect of YPC was associated with nondialyzable fraction.

Effect of Enzymes Present in YPC

Previous research has shown that certain enzymes, such as amylase, accelerate fermentation (Pyler, 1973). To determine what portion, if any, of the effect of YPC was contributed by enzymatic action, YPC was boiled for 20 min. As shown in Table 6, heat treatment did not alter the effect of DYPC on fermentation. This finding suggests that the effect of YPC was not due to enzymatic action.

Table 4. Effect of hydrolyzed YPC on yeast gassing power (N-supplemented)

Treatment (n=2)	Gas production during fermentation (GU)			
	1st hr	2nd hr	3rd hr	4th hr
Control	5.4±0.3	19.2±0.3	37.5±0.3	54.6±0.3
5% YPC	5.5±0.1	20.5±0.1	42.7±0.1	64.8±1.3
5% Hydrolyzed YPC	5.6±0	16.7±0.1	34.4±0	57.9±0.4

1. LSD .05 = 0.7

Table 5. Effect of dialysis on YPC activity

Treatment	Gas production after 4 hr fermentation
Control	57.5±0.4
Dialysate (0.5%)	60.7±0.4
Dialyzed YPC (DYPC) (4.5%)	66.2±0.2
Reconstituted YPC (0.5% dialysate + 4.5% DYPC)	68.4±0.4
YPC	68.3±0.3
Control	59.5±1.3
Dialysate (5%)	63.2±2.1
DYPC (5%)	70.3±0.6

Table 6. Effect of heat treatment on YPC activity

Treatment	Gas production after 4 hr fermentation
Control	56.5±0.2
DYPC (4.5%)	65.3±1.4
Boiled DYPC (4.5%)	65.6±0.1
YPC (5%)	66.8±0.7
Boiled DYPC (4.5%) + 0.5% Dialysate	68.3±0.3
Control	57.5±0.4

Effect of Protein Present in YPC

Because the activity of YPC was associated with materials that were not dialyzable and the high protein content of the YPC (72%), it was suspected that the effect of YPC was contributed by either protein or by a substance bound to protein. If this hypothesis was true, we should be able to precipitate (isolate) the yeast protein and retain the activity. YPC (20 g) was suspended in 500 ml of distilled water and the pH adjusted to 12. Ammonium sulfate (200 g) was then added to precipitate the protein. Both the precipitate and the solubles were dialyzed for 72 hr to remove ammonium salts (Fig. 1). After dialysis, the dialyzed salt solubles (DSS) constituted about 1% of the untreated YPC, while the dialyzed salted-out protein (DSOP) accounted for 74% of the intact YPC. The remaining 25% of the YPC was lost in the dialysate. The effect of DSOP and DSS on fermentation is shown in Table 6. DSOP retained 70% of the original activity while the DSS was essentially ineffective. Reconstitution of DYPC by combining appropriate amounts of DSS and DSOP did not restore the effect of DYPC (Table 7). The fact that the DSOP possessed most but not all of the original activity indicates that the major effect of YPC is associated with yeast protein and that some dialyzable material is also important.

Effect of Pronase Digestion

If the effect of YPC is due to protein, then the YPC's activity should be destroyed by hydrolyzing the protein. To test that hypothesis 10 g of YPC was suspended in 200 ml of distilled water and the pH adjusted to 7.0. Pronase was added and the YPC was digested for 8 hr at 35^{o}C with occasional stirring. Paper chromatography indicated that the protein was partially digested (Fig. 2). Pronase digestion did not alter the activity of YPC (Table 8). This result did not disprove the existence of a peptide fermenta-

Fig. 1. YPC solubility separation scheme.

FIGURE 1

SOLUBILIZATION--
Y.P.C. PRECIPITATION SCHEME

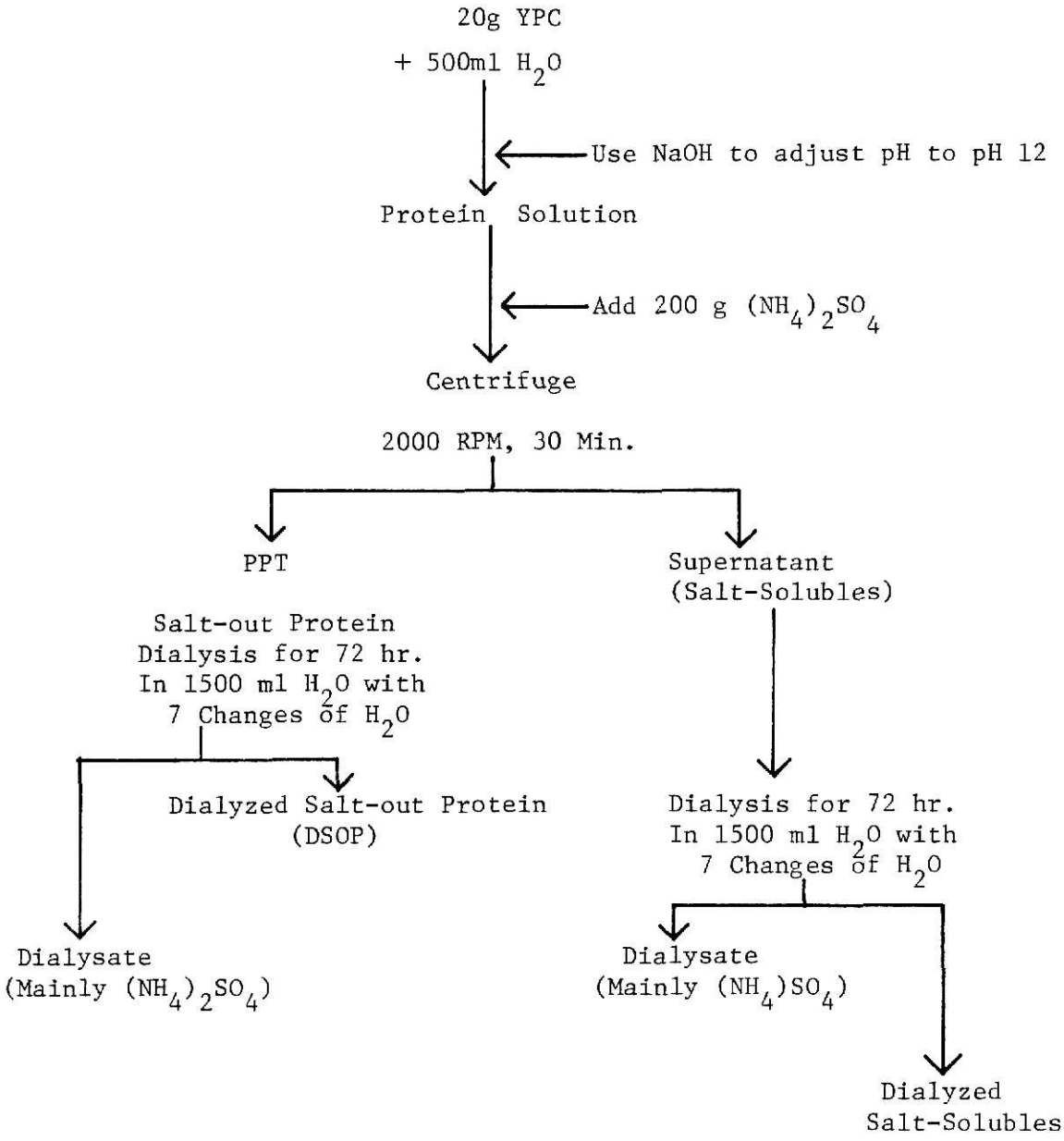


Table 7. Effect of dialyzed salt-out yeast protein and salt-solubles on yeast gassing power

Treatment* n=2	Gas production after 3 hr fermentation	Gas production after 4 hr fermentation
Control	38.2±0.2	54.5±0.7
5% YPC W/H	43.1±0.3	65.8±0.4
DYPC W/H (4.5%)	42.4±0.2	64.3±0.2
Dialyzed** salt-out protein	41.0±0.5	62.4±0.4
Dialyzed** salt-soluble	38.4±0.2	55.7±0.5
DS-OP*** + Salt soluble	40.4±0.1	62.4±0.1

*n = Number of observations within each treatment.

**Salt-out protein = material which precipitated when pH 12 YPC solution was half saturated with $(NH_4)_2SO_4$.

***Wt. of dialyzed salt-out protein + dialyzed salt solubles.

= 75% of YPC

$$\frac{\text{Wt. of D.S.O.P.}}{\text{Wt. of salt solubles}} = \frac{74}{1}$$

Fig. 2. One-dimensional descending paper chromatogram of (Y) intact YPC, pronase digested YPC (E), soluble (S) and insoluble (U) fraction of pronase digested YPC, "through" fraction (N) and "retained" fraction (P) of soluble fraction of pronase digested YPC on an ion-exchange (Amberlite IR-210) column. Developing solvent: n-Butanol:Acetic acid:Water = 4:1:1 (V/V).


P N U S E Y

Table 8. Effect of pronase digestion on YPC activity

Treatment* n = 2	Gas production after 4 hr fermentation (GU)
Control	53 ±0.3
5% YPC	67.3±0.2
5% Pronase digested YPC (EYPC)	66.5±0.2
Control	50 ±1.4
Solubles of EYPC (3%)	66.8 -
Unsolubles of EYPC (2%)	66.1±0.8
5% YPC	67.5±0.3
3% Solubles + 2% unsolubles of EYPC	69.2±2.4
Control	56.4±0.6
2.3% Solubles of neutralized YPC	64.6±0.9
2.7% Unsolubles of neutralized YPC	62.5±0.6
2.3% Solubles and 2.7% unsolubles (reconstitute)	66.1±0
5% YPC	68.0±0.7
Control	57.7
5% YPC	69.0±0.7
2.3% Solubles of neutralized YPC + pronase	71.0±0
Control	56.6±0.3
5% YPC	65.3±0.4
2.7% Unsolubles of neutralized YPC + pronase	67.7±0.7

*n = Number of observations within each treatment.

tion stimulant as it might not have been digested by pronase. The pronase digested YPC was fractionated into soluble and insoluble portions by centrifugation (2000 g; 30 min). Both the soluble and insoluble portions were as effective as intact YPC at the addition level of 60% and 40% (by weight) of the intact YPC (Table 8). Pronase digestion increased the potency of YPC. To further study the effect of pronase digestion, 10 g untreated YPC was suspended in 200 ml of distilled water and pH adjusted to 7.0. This neutralized intact YPC was fractionated into soluble and insoluble fractions by centrifugation (2000 g; 30 min). By this method, 45% of the YPC was solublized. Gasograph data showed that both the soluble portion and the insoluble portion only retained part of the original activity (Table 8). When these two fractions (soluble and insoluble) were treated with pronase, they both became at least as effective as intact YPC or perhaps slightly more active (Table 8). These results suggest that enzyme digestion renders the active component more available to yeast. The fact that both the soluble and insoluble portions of the neutralized intact YPC have the same potential effect on fermentation indicates that the active component is present in more than one protein or that there are multiple factors.

Isolation of the Active Component from YPC

To isolate the active component, the soluble portion of pronase digested YPC was subjected to an ion exchange column (3.5 cm 40 cm packed with Amberlite IR-120). The column was eluted with distilled water (flow rate was 6 drops/min) until three times the void volume was collected. This fraction was designated as "through". The column was then eluted with 1N NH_4OH until the eluent became alkaline. This fraction was designated as "retained". Both the retained and through fractions were concentrated under reduced pressure and lypholyzed. Material recovery from the ion exchange column varied

greatly between tests but the weight ratio of the retained and through fractions remained approximately the same. Recovery from the ion exchange column was 33% and 53% for duplicate samples. The weight ratio of retained and through fraction was about 1:1. Gasograph data showed that the retained fraction had about 98% of the original activity while the through fraction had little effect (Table 9). It is not known why the recovery was low and not consistent. However, it is clear that the effect of YPC was contributed by materials bound on Amberlite IR-120. This finding again strongly suggested that the effect of YPC was due to protein.

To determine the effect of oxidation on the active component, the retained portion (RSEDP) was dissolved in distilled water (0.2% protein solution W/V) and compressed air bubbled through the protein solution. This system was incubated at $55^{o}C$ for 16 hr. As shown in Table 9, oxidation did not alter the activity of RSEDP.

If the effect of YPC was contributed by some specific amino acid or material bound to protein, then it might be possible to hydrolyze the protein and retain the activity. The previous study showed that pronase did not completely digest YPC and acid hydrolysis destroyed the activity (Fig. 2 and Table 4). Therefore alkaline hydrolysis, as described by Knox et al (1970), was tried. After hydrolysis, carbon dioxide was introduced into the hydrolysate to precipitate Ba^{+2}. The precipitate was removed by centrifugation (2000 g; 30 min). This process was repeated until no precipitate was produced. The hydrolysate was lypholyzed. Gasograph data showed that alkaline hydrolysis destroyed the activity of YPC (Table 9). This result indicates that the effect of YPC was either contributed by a specific peptide or some entity that is both acid and alkaline labile.

The RSEDP was then subjected to gel filtration (3.2 cm 38 cm, Sephadex G-25 superfine) in attempt to isolate the active component. The column was

Table 9. Effect on components of solubles of pronase digested YPC on yeast gassing power

Treatment* n = 2	Gas production after 4 hr fermentation (GU)
Control	50.5±0.1
Retained (0.6%)	62.3±0.3
Through (0.6%)	53.6±0.1
Retained and through	63.6±0.1
5% YPC	63.0 -
Control	56.6±0.3
Oxidized retained	64.7±0.7
5% YPC	65.3±0.4
Control	62.1±0.6
YPC	74.8±1.1
Acid treated retained	73.0±0.8
Alkaline hydrolyzed retained	64.0 -
Control	56.6±0.3
YPC	65.3±0.4
Alkaline hydrolyzed retained	59 -

*n = Number of observations within each treatment.

eluted with distilled water at 12 drops per min. The resulting chromatogram showed 6 peaks (as measured by the absorption of elutent at 280 nm). None of these peaks retained the original activity (Fig. 3 and Table 10). However, recombining material from the peaks restored the original activity. Extensive use of the Sephadex column reduced the recovery rate from 90% to 50%. Recovery could be improved by repacking the column. It is not known why the recovery rate declined after extensive use of the column. In this study, the column was repacked after five runs. We did not quantitate each fraction because of the small quantities. The amount of RSEDP that was equivalent to 5% YPC was loaded on the column and material giving those peaks in Figure 3 was collected and lypholyzed in the reaction bottle used in the gasograph test.

As shown in Table 10, the activity was limited to first three fractions. The other four fractions appear to be ineffective. To verify the above hypothesis, the seven fractions were grouped into pooled fractions (Fig. 3) and a gasograph was used to determine their effect on fermentation. Table 11 shows the effect of fraction I and II on fermentation. The yield of fraction 1 and II was 37 mg and 5 mg, respectively. The reconstitution of RSEDP by combining 37 mg of fraction I and 5 mg fraction II restored 85% of the original activity. The improvement over control was plotted versus the amount of fractions I and II. It was found that the effect of fraction II leveled-off at levels exceeding 5 mg while the effect of fraction I was concentration dependent (Table 11). When the addition level of fraction I exceeded that of reconstituted RSEDP, fraction I still only had 56% of the original activity (29% less than that of reconstituted RSEDP). This result indicates an interaction between fraction I and II.

Fig. 3. Elution profiles of RSEDP (see text page 111) on Sephadex G-25.

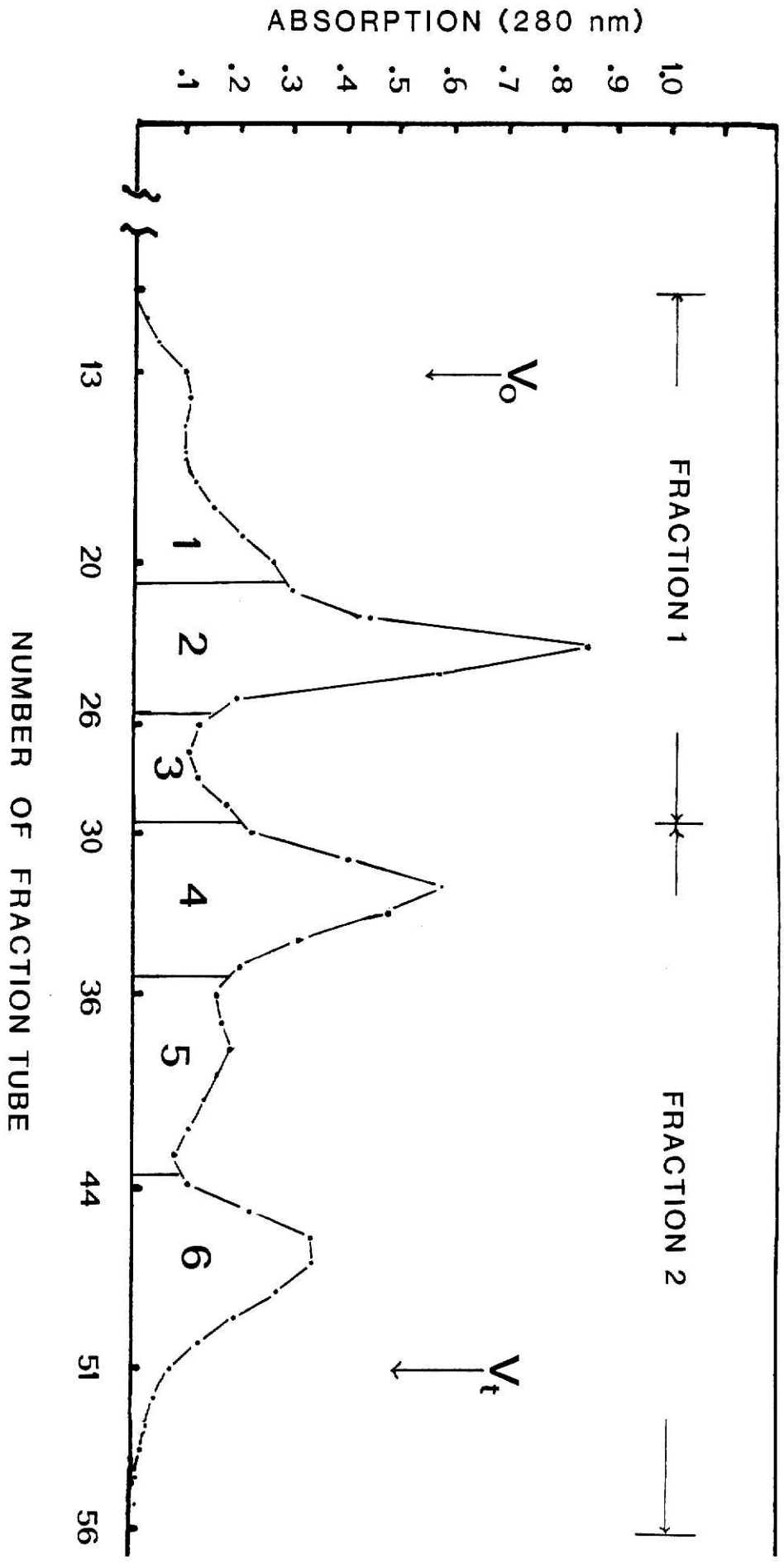

ABSORPTION (280 nm)
.1
.2
.3
.4
.5
.6
.7
.8
.9
1.0
FRACTION 1
FRACTION 2
V_o
V_t
1
2
3
4
5
6
13
20
26
30
36
44
51
56
NUMBER OF FRACTION TUBE

Table 10. Effect of fractions of RSEDP on a Sephadex G-25 column on yeast gassing power

Treatments	Gas production after 4 hr fermentation
Control	58.4
YPC	70.8
Reconstituted	68.1
Fraction 1	59.6
Fraction 2	60.6
Fraction 3	60.4
Fraction 4	58.3
Fraction 5	57.6
Fraction 6	58.4

Table 11. Effect of fraction I, fraction II and reconstituted RSEDP on yeast gassing power

Treatments	Gas production after 4 hr fermentation (GU)
Control	53.9
YPC	65.1
Reconstituted RSEDP*	63.3
5 mg Fraction II	56.3
10 mg Fraction II	55.4
15 mg Fraction II	56.7
10 mg Fraction I	54.8
15 mg Fraction I	56.9
30 mg Fraction I	58.0
50 mg Fraction I	60.2

*Reconstituted RSEDP is the combination of 37 mg of Fraction I and 5 mg of Fraction II.

CONCLUSIONS

The effect of YPC on fermentation is not contributed by nitrogen, metal ions, vitamins, or enzymes present in the YPC. The fact that protein retained most of the activity strongly suggests that the effect of YPC is associated with protein. The evidence became more convincing when it is found that the active constituents of YPC are retained on an Amberlite IR-120 column. Further study with Sephadex column showed that the effect is regulated by at least two factors.

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EFFECTS OF YEAST PROTEIN CONCENTRATE ON BREADMAKING

EFFECTS OF YEAST PROTEIN CONCENTRATE AND DRIED WHOLE YEAST ON EXTRUDATE PROPERTIES

ISOLATION OF FERMENTATION STIMULANTS FROM YEAST PROTEIN CONCENTRATE

by

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ABSTRACT

Results of this study indicate that a protein concentrate from brewer's yeast (YPC) decreased mixing time, dough strength, and bread volume but increased water absorption. Heat treatment of YPC increased its loaf volume reducing effect. Reducing fermentation time alliviated part of the adverse effect of YPC on bread volume but improvement was limited. A strong brewery flavor and small loaf volume rendered breads containing more than 2% YPC unacceptable.

YPC decreased expansion, bulk density, shear force, and breaking force of extruded starch. Bound lipids extracted from YPC reduced expansion but increased textural strength of extruded starch. The effect of dried whole yeast on extrusion depended on the level of addition. Effects of yeast cell wall and homogenized yeast on extrusion varied with pH. Textural strength was not related to internal structure. Internal structure was related to expansion of extruded materials.

Addition of YPC accelerates fermentation in dough. This effect was not due to vitamins, minerals, fermentable sugar, or nitrogen. The effect was not contributed by enzymatic action but was associated with protein. The active components of YPC could be retained on an ion exchange column. Further fractionation of the active components showed that the effect was regulated by at least two factors.