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GLUCOSE OXIDASE PRETREATMENT TO MINIMIZE NONENZYMATIC BROWNING IN POTATO CHIPS AND FRENCH FRIES

by

ZHIRONG JIANG

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

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OF Master of Science

IN

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DATED June 25

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled **GLUCOSE OXIDASE PRETREATMENT TO MINIMIZE NONENZYMATIC BROWNING IN POTATO CHIPS AND FRENCH FRIES** submitted by **ZHIRONG JIANG** in partial fulfilment of the requirements for the degree of **Master of Science in Food Processing.**

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Supervisor

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Date.... June 15, 1987

Dedicated to

My Beloved Parents

ABSTRACT

The reaction properties of two commercial preparations of glucose oxidase, DeeO 1500 in powder form and Fercozyme CBB-750 in liquid form, were studied using a polarographic method. The experimental activities of both preparations were much higher than declared. The Michaelis constant was 0.27 M and 0.26 M glucose for powder and liquid enzymes, respectively. Temperature had no appreciable effect on the reaction rate in the range studied (25 to 40°C). Both enzyme preparations had a relatively broad effective range of pH, from 4 to 7, with optimal pH's around 5.0 and 5.5 for powder and liquid enzymes, respectively. The liquid enzyme had a superior thermal stability to the powder enzyme.

The two glucose oxidase preparations were applied to minimize the undesirable nonenzymatic browning in potato chips and French fries. Apparent improvement of lightness and uniformity of color of potato chips was achieved, with potatoes marginally suitable for chipping (glucose content ≅ 0.3%), by dipping potato slices in 0.05% (w/v) powder enzyme or 0.10% (v/v) liquid enzyme at ≤40°C for 30 min or longer. In the French fry process, the steam blanching (7 min under atmospheric pressure) followed by water leaching (≤35°C for up to 15 min) of potato strips retained more flavor in French fries than the recommended hot water blanching (85°C for 15 min). Dipping of steam-blanched potato strips in 0.2% (v/v) liquid enzyme or 0.1% (w/v) powder enzyme at ≤35°C for up to 15 min improved, to a limited extent, the lightness

and uniformity of the color of French fries when compared to the control (dipping in tap water).

The oil content of potato chips was increased by the dipping treatment, either in tap water or in enzyme solution. Steam blanching followed by dipping in water or enzyme solution produced French fries of lower oil content than when hot water blanching was used. The glucose oxidase treatment imparted no off-flavor to potato chips or French fries.

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1. INTRODUCTION

The color of some potato products, such as potato chips and French fries, is of primary importance in determining the acceptance of the products. The discoloration of these potato products could be caused both enzymatically and nonenzymatically. Enzymatic browning, which occurs in peeled or cut potatoes when exposed to air, results from the activity of polyphenol oxidase on some phenols to form dark polymers (Lerner and Fitzpatrick, 1950; Mondy *et al.*, 1960). Nonenzymatic browning, also known as the Maillard reaction, is "a group of chemical reactions involving the amino and carbonyl functions present in foodstuffs and leading to browning and flavor production" (Mauron, 1981). The discoloration in fried potato products can be attributed mainly to nonenzymatic browning.

As reducing sugars are the limiting reactants in nonenzymatic browning, potatoes of appropriate variety, maturity and post-harvest handling with low reducing sugar contents, are therefore chosen for making fried products. In some cases, potatoes of high sugar content from low temperature storage could be reconditioned by holding the tubers at room temperature in order to produce acceptable products. In normal practice, sodium bisulfite, alone or with phosphoric or citric acid, is employed to treat potato slices or strips to prevent undesirable discoloration during frying (Smith, 1975b). Sulfiting agents, such as sodium bisulfite, are capable of inhibiting both enzymatic and

nonenzymatic browning.

Despite the long history of wide applications of sulfites in foods, the safety of sulfiting agents in foods has recently been questioned on the basis of their role in the initiation of asthmatic reactions in certain sensitive individuals (Taylor *et al.*, 1986). A series of actions has been taken by the U.S. Food and Drug Administration (FDA) to limit the use of sulfites in foods. In 1986, the FDA lifted the GRAS (Generally Recognized As Safe) status of sulfites used in fresh vegetables and fruits (except potatoes) intended for consumption in raw state, particularly in salad bars. Another FDA regulation came into effect in 1987, requesting the declaration of sulfites when the residual sulfite content, as total SO_2, exceeds 10 ppm. The final action on the GRAS status of sulfiting agents for use on potato products has been scheduled for June 1987 (Semling, 1987).

The safety problem of sulfites and the regulations regarding their use created a practical necessity for new approaches to prevent undesirable browning in foods. Glucose oxidase enzyme could be an agent to replace sulfites. By oxidizing glucose, one of the reactants in the Maillard reaction, to the unreactive gluconic acid, the enzyme is able to block nonenzymatic browning. The glucose oxidase/catalase system has been used successfully to prevent the browning of dried egg powder (Baldwin *et al.*, 1953; Scott, 1953).

Although it was stated as early as 1953 that the application of glucose oxidase would "no doubt be made to other products such as dehydrated mashed potatoes" (Scott, 1953), the enzyme has never been reported to have been applied to such products. The recent limitation on the use of sulfite in foods has presented major problems to the production of some color-sensitive potato products, such as potato granules, potato chips and French fries. Therefore, it is logical that the effectiveness of glucose oxidase in preventing nonenzymatic browning in these products should now be investigated. In this project, the focus was on the prevention or minimization of browning in potato chips and French fries with glucose oxidase. A parallel study was also performed on potato granules by other researchers in this laboratory.

The objectives of the present research were:

1. to study the chemical and kinetic properties of the commercial glucose oxidase preparations;

2. to establish the optimum conditions for glucose oxidase treatment of potatoes;

3. to determine the effect of glucose oxidase treatment on the color and general acceptability of potato chips and French fries.

2. LITERATURE REVIEW

2.1 Potato Production, Consumption and Processing

The white potato (*Solanum tuberosum*, L.) is one of the few crops capable of nourishing a significant portion of the world population. Despite the tremendous increase in quantities and varieties of food sources, the annual per-capita consumption of the potato in the United States and Canada has remained quite constant (Table 2.1). However, the consumption pattern of potatoes has been changing. In the United States, the consumption of fresh potatoes decreased from 1960 to 1980, while the consumption of processed potatoes almost tripled in the same period. Potato chips and French fries are the two major potato products, accounting for almost two-thirds of the processed potatoes (Figure 2.1).

2.2 Potato Chips

The production of potato chips in the United States has increased slowly but constantly (Figure 2.1). Potato chips (crisps) are the most important processed potato products in the United Kingdom (Smith and Davis, 1977). Because of convenience in serving and variety in flavoring, potato chips are expected to remain popular.

Table 2.1 Annual per-capita consumption of potatoes in U.S.A. and in Canada.

U. S. A.[1]		Canada[2]	
Year	Consumption (kg)	Year	Consumption (kg)
1945	55.39	1978	72.54
1950	48.12	1979	78.77
1955	49.49	1980	70.87
1960	49.03	1981	64.79
1965	49.03	1982	66.66
1970	53.57	1983	72.08
1975	54.93	1984	60.69
1980	53.57	1985	67.68

1: U.S.D.A., Agricultural Statistcs, 1972; 1985

2: Statistics Canada, 1979-1985

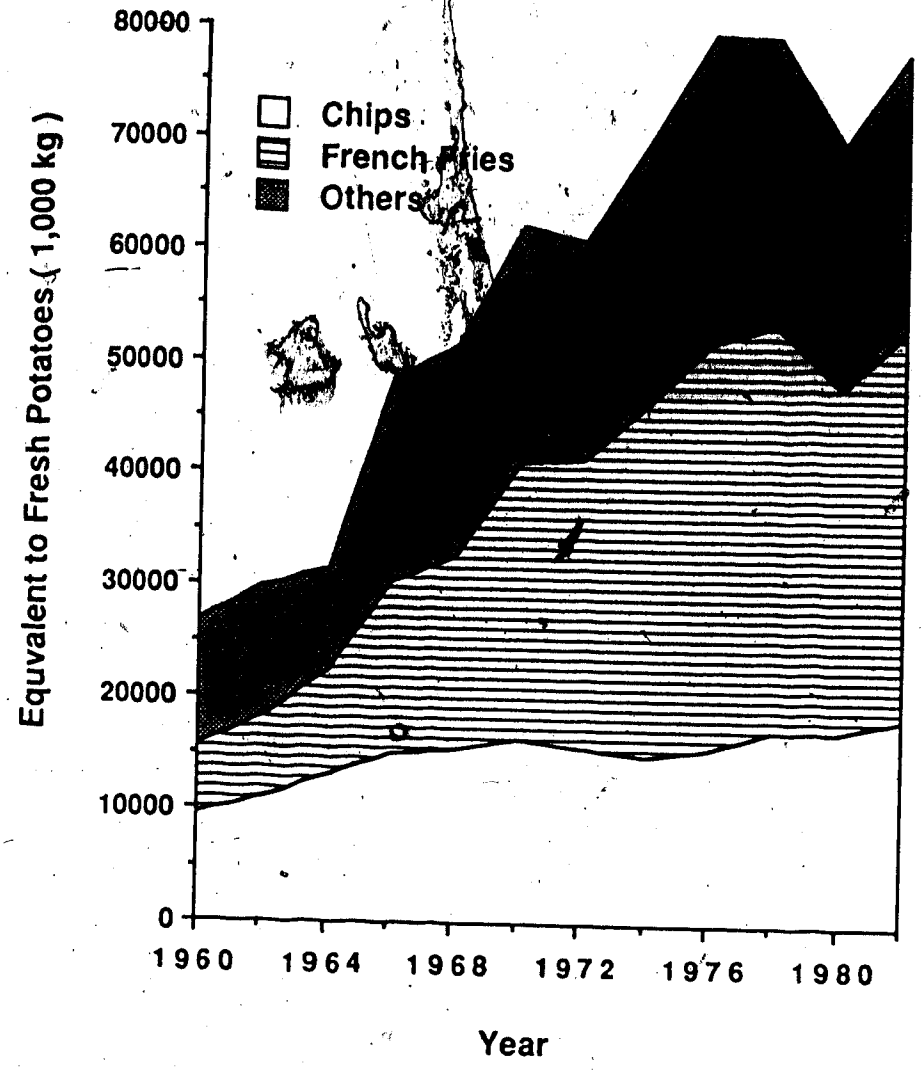


Figure 2.1 U.S.A. production of potato chips (including shoestrings), frozen French fries, and others (including dehydrated potatoes, other frozen products, canned potatoes and potatoes for starch and flour).

2.2.1 Processing procedures

The most commonly practised processing procedures for potato chips were described by Smith (1975b). After washing and peeling, potato tubers are sliced to a thickness of 1/15 to 1/30 in (1.70 to 0.85 mm). The slices are then washed in cold water to remove surface starch to prevent adhesion of the slices during frying. Treatment to improve the color of potato chips can be carried out at this stage. The slices are then partially dried and fried in oils at 350-375°F (177-191°C) until bubbling stops. The chips are now ready for salting and flavoring, inspection and packaging. A flow chart for potato chip processing is presented in Figure 2.2.

2.2.2 Quality of potato chips

The quality of potato chips can be evaluated from their physical appearance and their flavor. The most important factor in determining the quality of potato chips is the color of the product. The desired product has a uniform, light-yellow color.

2.3 French Fries

French fries, in a partially-fried frozen form, have become the most popular processed potato products in North America. In the United States, the production of French fries in 1981 was almost six times that of 1960 (USDA, 1966-1985). About half of the processed potatoes since 1972 was in the form of frozen French fries.

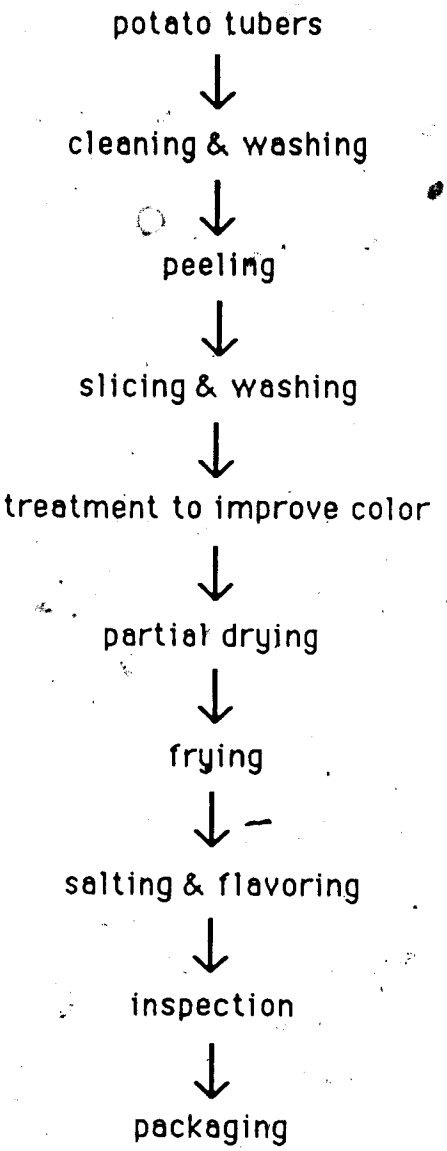


Figure 2.2 The processing procedures of potato chips.

2.3.1 Processing procedures of French fries

French fries can be produced either by finish frying potato strips or by partial frying the strips for a short time before freezing, producing the so-called par-fried frozen French fries. The general processing procedures for frozen French fries are presented in Figure 2.3. After washing and peeling, the potato tubers are sliced to generate strips, usually 1/4 or 3/8 in^2 in cross section. The strips are washed in cold water to remove surface starch. Blanching, using various methods, is then performed, followed by treatment, if any, to improve the quality of the final product. The strips are then partially dried and partially fried in oils at about 180°C for a short period of time, usually 30-90 sec. The partially fried potato strips are quickly frozen at -30°C and kept frozen until serving (Weaver *et al.*, 1975). The frozen French fries can be prepared for serving by finish frying in hot oil, by conventional oven heating, by microwave heating (Weaver *et al.*, 1975; Bushway *et al.*, 1984), or by infrared heating (Mohr *et al.*, 1960). In some restaurants, the potato strips, blanched or unblanched, are fried until they are ready for serving (Martino, 1969).

Novel methods of producing extruded French fries from freshly cooked and mashed potatoes (Weaver *et al.*, 1974) or from dehydrated potato granules (Jadhav *et al.*, 1976) have also been described.

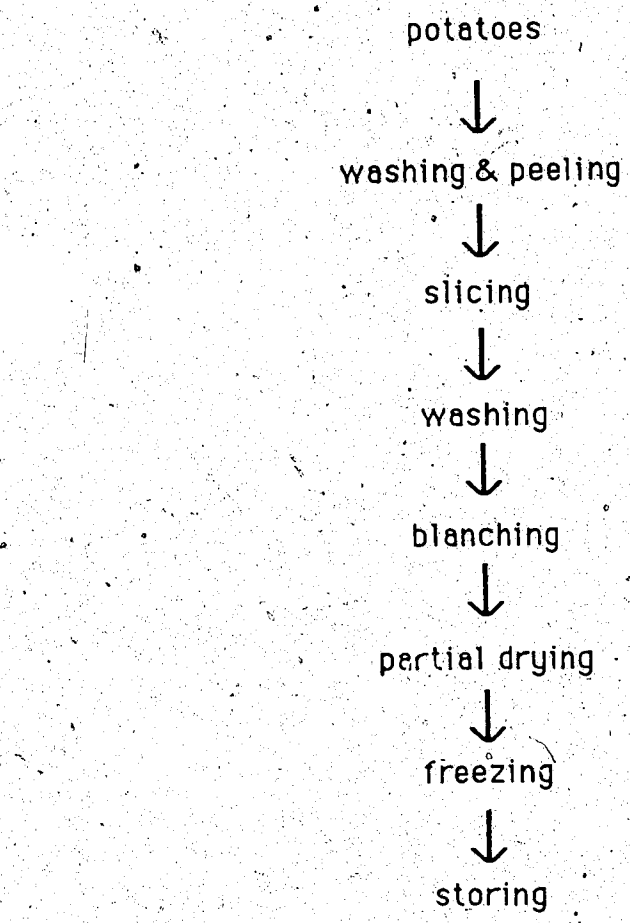


Figure 2.3 Common processing procedures for frozen French fries.

2.3.2 Blanching methods for French fries

Potato strips are usually blanched before frying. The advantages of blanching are: (1) reduction of fat adsorption through gelatinization of the surface layer of starch; (2) shortened frying time since potato strips are partially cooked by blanching; (3) inactivation of certain enzymes so that undesirable flavor and color are avoided during storage and subsequent handling; (4) improvement of the texture and flavor of the final product; and (5) removal of certain solutes, in water blanching, to prevent excess browning (Weaver *et al.*, 1975; Brown and Morales, 1970).

Several blanching methods are practised by the French fry industry, including hot water, hot air, steam and oil blanching. The most commonly practised method is hot water blanching. The temperature and time may vary from 66-88°C and 3-15 min, respectively (Vahlsing, 1965; Van Patten and Freck, 1973), with the optimum conditions recommended at 80°C and 15 min (Brown and Morales, 1970). Two or even more blanchers are commonly operated in series for greater flexibility and more effective control of color of the products (Weaver *et al.*, 1975). The first blancher can be used for hot water leaching, while the second may contain a dilute glucose solution to produce the desired golden brown color during frying. In some processing plants, more stages are used. The strips are blanched at 82°C (180°F) for 8 min, cooled in water at 45-50°C for 6 min, blanched at the same temperature for 8 min, and dipped in a dilute glucose

solution at 180°F for a short time (≈10 sec) before frying (Iwabuchi, 1986). However, the hot water blanching process can also result in the loss of desirable flavor constituents from potato strips, and serious impairment of texture and flavor is often encountered. In addition, the method has been reported to not always be effective in avoiding darkening (Weaver and Nonaka, 1976).

A technique, referred to as dry blanching, is also employed by the industry. It uses hot air to heat the potato strips to achieve blanching effect, and the product is reported to be more crisp and rigid (Weaver *et al.*, 1975).

Steam blanching is preferred in some processing procedures (Strong, 1968; Wilde 1972). Potato strips are blanched at atmospheric pressure for 2-10 min, preferably about 7 min. This process, with no solutes removed from the potato strips, avoids the possible loss of flavor components encountered in hot water blanching.

Recently, a new process using hot oil to blanch starchy vegetables, such as potatoes, has been reported (Fan and Arce, 1985). The potato cuts are dipped in a hot edible vegetable oil at 50-60°C for a short time before frying. The claimed advantages include less oil absorption, less potato solids loss, and improved flavor and texture when compared with that of water-blanched products.

2.3.3 Quality of French fries

The quality of frozen French fries can be evaluated according to the following scheme (Weaver *et al.*, 1975):

Factors	Maximum Score
Color	30
Uniformity of size and symmetry	20
Defects	20
Texture	30

Among the listed factors, the color of the French fries is the most sensitive and important parameter. The desired gold-brown colored French fries can be made from potatoes of proper variety, maturity and post-harvest handling. However, the discoloration of French fries is the most troublesome problem that often confronts processors.

2.4 Browning Problems in Potato Products

2.4.1 Types and occurrence of browning

There are three distinct types of discoloration that are associated with the browning of potato products (Smith and Davis, 1977): (1) enzymatic discoloration; (2) after-cooking darkening; and (3) nonenzymatic browning, also known as the Maillard reaction. The enzymatic browning which occurs in peeled or cut potatoes when exposed to air results from the activity of polyphenol oxidase on some phenols to form dark polymers (Lerner and Fitzpatrick, 1950; Mondy *et*

al., 1960). The cause of after-cooking darkening of cooked potatoes is generally believed to be the reaction of certain ortho-diphenols, such as chlorogenic acid, with the ferrous ions of potato tubers, forming a colorless complex, which is oxidized to yield a deeply-colored ferric compound (Hughes *et al.*, 1962; Hughes and Swain, 1962). More recently, ascorbic acid-Fe^{3+} complex was suggested as another cause of after-cooking darkening of potatoes (Muneta and Kaisaki, 1985). However, the above two types of browning are not important in potato chips and French fries. The discoloration of potato chips and French fries is attributed mainly to the nonenzymatic browning reaction.

2.4.2 Nonenzymatic browning

Nonenzymatic browning, also known as the Maillard reaction, is "a group of chemical reactions involving the amino and carbonyl functions present in foodstuffs and leading to browning and flavor production" (Mauron, 1981). The first coherent scheme of the Maillard reaction was put forward by Hodge (1953) in a comprehensive review. The Maillard reaction has also been the subject of several other reviews (Reynolds, 1963, 1965; Mauron, 1981; Kawamura, 1983; Danehy, 1986).

The complicated Maillard reaction can be divided into three stages: early, advanced and final reactions (Hodge, 1953; Mauron, 1981). The early stage involves a simple condensation process between the carbonyl group of reducing

sugars and the free amino group of the amino acids or proteins, followed by the Amadori rearrangement, a step which isomerizes an aldose derivative to a keto derivative. The advanced Maillard reaction, starting from the Amadori compounds, generates flavor components which are responsible for the roasted, bready and nutty flavor of heated foods. The final Maillard reaction produces the brown melanoidin pigments formed by polymerization of many highly reactive compounds generated during the advanced stage. More recently, Namiki and Hayashi (1983) reported a new pathway which involves the cleavage of sugar molecules to generate highly active two-carbon fragments prior to the Amadori rearrangement.

Among the parameters that affect the rate of the Maillard reaction, temperature and water content are by far the most important. The Q_{10} value of the browning reaction in dehydrated vegetables was reported to be 5-8.4 (Legault *et al.*, 1947), which was exceptionally high. The browning rate is near zero in the anhydrous state. It reaches a maximum at about 30% water content, and then decreases gradually as the moisture increases. The reaction stops at about 90% water content (Wolfrom and Rooney, 1953).

2.4.3 Browning of potato chips and French fries

The browning of potato chips and French fries had been associated with sugar content of potato tubers no later than 1930. Sweetman (1930) reported the correlation between the

brown color of potato chips and the high sugar content of tubers. He concluded that the formation of brown color was due to the caramelization of sugars at high temperature. Later, Thornton (1940) pointed out that the dark brown color of potato chips was caused by high reducing sugar, not by sucrose or total sugar content of potato tubers. Several years later, however, the browning of chips and other potato products was attributed, at least in part, to the Maillard reaction between reducing sugars and amino acids (Legault *et al.*, 1947). Fitzpatrick and coworkers (Fitzpatrick *et al.*, 1965; Fitzpatrick and Porter, 1966) were able to substantiate the above theory by tracing the changes in reducing sugars and amino acids during chip frying. The ratio of the consumption of free amino acid nitrogen to reducing sugars was 9:1 in low reducing sugar and 1:1.35 in high reducing sugar potato chips. The browning of French fries was also believed to be caused by the Maillard reaction between reducing sugars and amino acids.

2.4.4 Potato composition and browning

Although the discoloration of potato chips and French fries can be mainly attributed to the Maillard reaction, the relationships between the color of the products and the chemical components, such as sucrose, glucose and fructose, amino acids and dry matter, are very complicated. Wunsch and Schaller (1972) reported that the discoloration was essentially dependent on four groups of components: total

amino acids, glucose, fructose, and tyrosine and proline. Yada *et al.* (1985) found that approximately 60% of the variability seen in chip color could be accounted for by examining the fructose, glucose and sucrose content of potato tubers.

Habib and Brown (1956, 1957) obtained the correlation coefficients for both reducing sugars and amino acids with potato chip color. Both coefficients were significant at the 1% level. They also showed that there was a positive correlation between nonreducing sugar (sucrose) content and the lightness of potato chip color. However, Shallenberger *et al.* (1959) demonstrated that chip color was a function of both reducing sugar and sucrose contents of tubers. They postulated that sucrose must first be hydrolyzed, during frying, to participate in the nonenzymatic discoloration of chips.

Although both reducing sugars and amino acids participate in the Maillard reaction, amino acids and other nonprotein nitrogenous compounds do not correlate well with chip color (Miller, 1972). The limiting factor of the browning reaction was proposed to be the reducing sugar content (Marquez and Anon, 1986). Miller (1972) reported that glucose was the major determinant of chip color, while fructose appeared to be unimportant in browning. Sucrose had a minor, but significant role in color development, probably as a result of hydrolysis during frying. Miller *et al.* (1975) were able to substantiate those results.

2.4.5 Factors affecting the color of the products

The color of potato chips and French fries is determined by the chemical composition of potato tubers which, in turn, is affected by many factors during potato cultivation and post-harvest handling.

The cultivation conditions related to the color of potato chips and French fries include variety and maturity of potatoes, irrigation and soil moisture, and application of fertilizers and some other chemicals. Some well known varieties that usually produce chips of good to excellent color are Monona, Kennebec, Norchip, Russet Rural, Superior and Katahdin (Smith, 1975b). Several new good chipping varieties released in recent years are Rosa (Plaisted *et al.*, 1981), Simcoe (Johnson and Rowberry, 1982), Yankee Chipper (Reeves *et al.*, 1984a) and Islander (Reeves *et al.*, 1984b). Varieties which produce light-colored potato chips were found to contain the lowest amount of reducing sugars (Shallenberger, 1955).

Maturity of potato tubers when harvested is another well-known factor affecting the color of potato products. The more mature the potatoes, the easier they can be handled, stored and reconditioned to produce light-colored chips (Smith, 1975b). Shallenberger (1955) found the the more mature tubers produced light-colored chips not only on the day of harvest, but also after storage at different temperatures. The reducing sugar content was found, in general, to be higher in immature tubers than in more mature

ones.

Several other cultural conditions during the growing season may also affect the color of potato products. Kushman *et al.* (1959) reported that the darker color of chips made from potatoes grown under high soil moisture content was always apparent after storage at 60°F (15.6°C), and sometimes also appeared immediately after harvest. A heavy application of nitrogen during growing was often regarded as a factor that makes stored potatoes more likely to produce dark chips (Smith, 1975a). However, Kunkel and Holstad (1972), after an extensive 11-year study, reported that potato chip color was almost unaffected by nutritional balance or total amount of fertilizers used. Whenever the soil temperature at a depth of 4 inches goes below 4.4°C (40°F) for several days or nights, the potatoes subsequently harvested will produce dark chips (Smith, 1959b).

The effect of transit and storage conditions of tubers on the color of potato chips can not be overemphasized. The accumulation of sugars during low temperature storage of potatoes was reported as long ago as 1882 (Smith, 1975a). Early work by Sweetman (1930) showed that chips made from tubers stored at 32-37°F (0-2.8°C) were darker than those made from tubers stored at 40-55°F (4.4-12.8°C). Habib and Brown (1957) found that glucose content of potatoes was almost doubled after storage at 40°F (4.4°C) for 4 weeks, while the fructose and sucrose contents did not change significantly. Ewing *et al.* (1981) reported that glucose and

fructose content showed a similar increase in potatoes exposed to 1°C for 4 days or longer, except that the glucose level was almost always much higher, roughly twice that of fructose.

The sugars accumulated during low temperature storage of potatoes disappear when the tubers are held at temperatures of 18°C or higher for 1-3 weeks (Weaver *et al.*, 1975). This process, referred to as conditioning or reconditioning, has been practised extensively in the potato processing industry to produce properly colored chips and French fries from potatoes stored at low temperature.

However, not all potatoes respond to cold storage and warm reconditioning to the same extent. Potato varieties that accumulate less sugars in cold storage and respond more readily in warm conditioning should be selected for processing of potato chips and French fries. Also, more mature potatoes accumulate less sugars during storage and respond to reconditioning more readily (Shallenberger, 1955; Burton, 1965).

The change of potato sugar content has been associated with the activities of certain enzymes, such as invertase, phosphohexose isomerase and aldolase. The accumulation of reducing sugars was found to occur with concomitant formation of the enzyme invertase (Pressey and Shaw, 1966). During the initial period of cold treatment, when reducing sugars increased rapidly, invertase formation proceeded until the level of enzyme exceeded that of a proteinaceous

invertase inhibitor, resulting in a basal invertase activity. On transfer of cold stored potatoes to warmer temperatures, sugar content and invertase level decreased sharply, and a large excess of inhibitor developed.

Pressey (1969) pointed out that invertase participated in reducing sugar formation, but other factors were responsible for the regulation of starch-sugar conversion in potatoes during storage. A possible sequence of events in cold stored potatoes was proposed by Tishel and Mazelis (1966). The low temperature appeared to induce in the tubers (1) a temporary decrease in aldolase activity, (2) an increase in sucrose, (3) increased invertase activity, (4) accumulation of reducing sugars, and (5) a lowering of the activity of phosphohexose isomerase.

2.5 Various Methods of Improving the Color of Potato Chips and French Fries

It may often be almost impossible to make potato chips and French fries of acceptable color without some treatments of raw potatoes or potato cuts. The following are some of the treatments that have been used or tried.

2.5.1 Reconditioning of potatoes stored at low temperature

A common practice to improve the color of potato chips and French fries from low-temperature stored potatoes is the reconditioning process. Potatoes are removed from low temperature storage and held at room temperature (18°C or

higher) for 1-3 weeks before processing. However, as stated previously, some varieties do not respond readily to conditioning and, even in potato varieties that normally respond well to reconditioning, disorders such as sugar end can sometimes occur (Weaver *et al.*, 1975). In addition, reconditioning is expensive as it involves extra handling costs, ties up large quantities of potatoes, and requires large storage space and facilities for the treatment (Weaver and Nonaka, 1976). Nonetheless, the technique is widely used in industry.

2.5.2 Leaching techniques

Various leaching techniques have been tested to prevent the discoloration in potato chips and French fries by removing the solutes from the potato cuts.

Light-colored potato chips could be made from cold stored potatoes, without reconditioning, by dipping the slices in hot water at 153-163°F (67.2-72.8°C) for 6-7 minutes before frying (Townsley, 1952). Dexter and Salunkhe (1952a) reported that immersion in water at 70°C for 1.5 minutes, followed by soaking in cold water for 15 minutes, produced chips of excellent color.

Hot water blanching is a powerful leaching process for French fry production. The leaching of reactants by hot water blanching is so extensive that glucose has to be added back to produce the proper light golden color typical of French fries. However, the drastic water treatments often

remove all flavor, in addition to their undesired effects of extracting nutrients and giving rise to waste water disposal problems (Weaver *et al.*, 1974).

Some surface treatments of potato cuts to accelerate the leaching process have been reported. Weaver and Hautala (1972) patented a process for making French fries, wherein potato strips were dipped in a liquid refrigerant, such as liquid nitrogen or dichlorodifluoromethane, for a short period (about 7 seconds), followed by leaching in hot water at 50-60°C for 1-5 minutes. Another process, patented by Weaver and Nonaka (1976), described the production of fried potato products of improved texture, flavor and color from raw stock that exhibits excessive browning tendencies. Typically, potato cuts were prefried in edible oil for a short period, then leached with water. The treatment was reported to be applicable to both French fries and potato chips.

2.5.3 Microwave heating and other processing methods

The Maillard reaction is usually accelerated at a later stage in frying of potato products, when moisture content is relatively low and the temperature of potato cuts increases, as less water is evaporated (Sijbring and Van de Velde, 1969). To avoid excessive discoloration of chips from potatoes with high reducing sugar content, the slices can be partially fried and then subjected to other methods of drying, such as hot air drying at 250°F (121.1°C) (Smith,

1967a), infrared heating (Smith, 1975b), vacuum frying (Sijbring and Van de Velde, 1969), and microwave heating (Smith, 1965; Fitzpatrick and Porter, 1968). The preparation of French fries suitable for microwave heating has also been reported (Bushway *et al.*, 1984).

2.5.4 Chemical treatments of potatoes and potato cuts

Various aqueous solutions, such as ether, alcohol, salt and acid, were tested by Dexter and Salunkhe (1952b) to selectively remove reducing sugars from potato slices. Treatment with phosphoric or hydrochloric acid at pH 1.9 for 1.5 minutes, followed by washing in water for 3.5 minutes, produced chips of excellent color. Patton (1948) patented a process of dipping slices for various lengths of time in hot aqueous solutions of alkaline earth salts ($CaCl_2$, $MgCl_2$, calcium sulfamate) of concentrations from 0.005-0.1 M before frying. Simon *et al.* (1955) reported that retardation of nonenzymatic browning in dehydrated white potatoes by spraying with calcium chloride.

Sodium bisulfite solution has been used to treat potato slices from raw stocks of high reducing sugars (Smith, 1975b). Attractive, light-colored chips could be produced by dipping the slices for 1 minute either in 0.25% sodium bisulfite at 180-200°F (82.2-93.3°C), or in a solution of sodium citrate, sodium bisulfite and phosphoric acid held at 160-180°F (71.1-82.2°C). Xander (1952) claimed that uniformly colored and flavored chips could be produced year

round, regardless of the variety used or the reducing sugar content, if slices were immersed in an SO_2 bath and then washed with water to remove the residual SO_2 and reducing sugars.

Whole potato tubers were also treated with SO_2 gas (Smith, 1959a). The treated potatoes resulted in chips of lighter color for tubers stored as long as three months at 4.4°C (40°F) than untreated tubers.

2.6 Sulfites in Foods: Uses and Safety Problems

2.6.1 Applications of sulfites in foods

Sulfiting agents, in various forms, alone or with phosphoric acid, sodium citrate or citric acid, have been employed in industry to treat potatoes and potato cuts to prevent nonenzymatic discoloration during frying (Smith, 1975b). As a group of versatile food additives, sulfiting agents have found wide applications in various foods for different technical purposes, such as inhibition of nonenzymatic and enzymatic browning, inhibition and control of microorganisms, and as reducing agents and antioxidants. They have been widely used to prevent enzymatic browning in peeled or sliced vegetables and fruits such as prepeeled potatoes, sliced potatoes, cut apples and cut lettuce (Taylor *et al.*, 1986). The mechanism of sulfite action is not clear, but sulfites appear to both inactivate enzyme phenolase and interrupt the subsequent reactions leading to

colored products (Haisman, 1974). The sulfites play crucial roles in the inhibition and control of microorganisms in several food processes, such as in preserving soft fruits, in jam making, in sausage production, and in wine making (Roberts and McWeeny, 1972). As an antioxidant, sulfur dioxide has been shown to be effective in preventing loss of ascorbic acid during processing and storage of products such as dehydrated cabbage, fruit cordials and grape juice. It has also been shown to be a reasonably effective antioxidant for protection of lipids in lipid-protein-water emulsion, of essential oils and carotenoids in citrus juices (Roberts and McWeeny, 1972). Sulfites are also widely used as dough conditioners in the baking industry for biscuits, crackers, cookies and frozen pizza doughs, where they act as reducing agents (Taylor *et al.*, 1986).

Sulfites find wide use as inhibitors of nonenzymatic browning. They have been used for this purpose to control discoloration of dehydrated potatoes, vegetables and fruits, white grape juice and other fruit juices and drinks (Taylor *et al.*, 1986). Detailed mechanisms by which the sulfites exert their anti-browning action in foods are still largely unknown. It was proposed by McWeeny *et al.* (1974) that sulfites exhibited their anti-browning action by their ability to react with various carbonyl intermediates formed during the nonenzymatic browning reaction. The theory is illustrated in Figure 2.4.

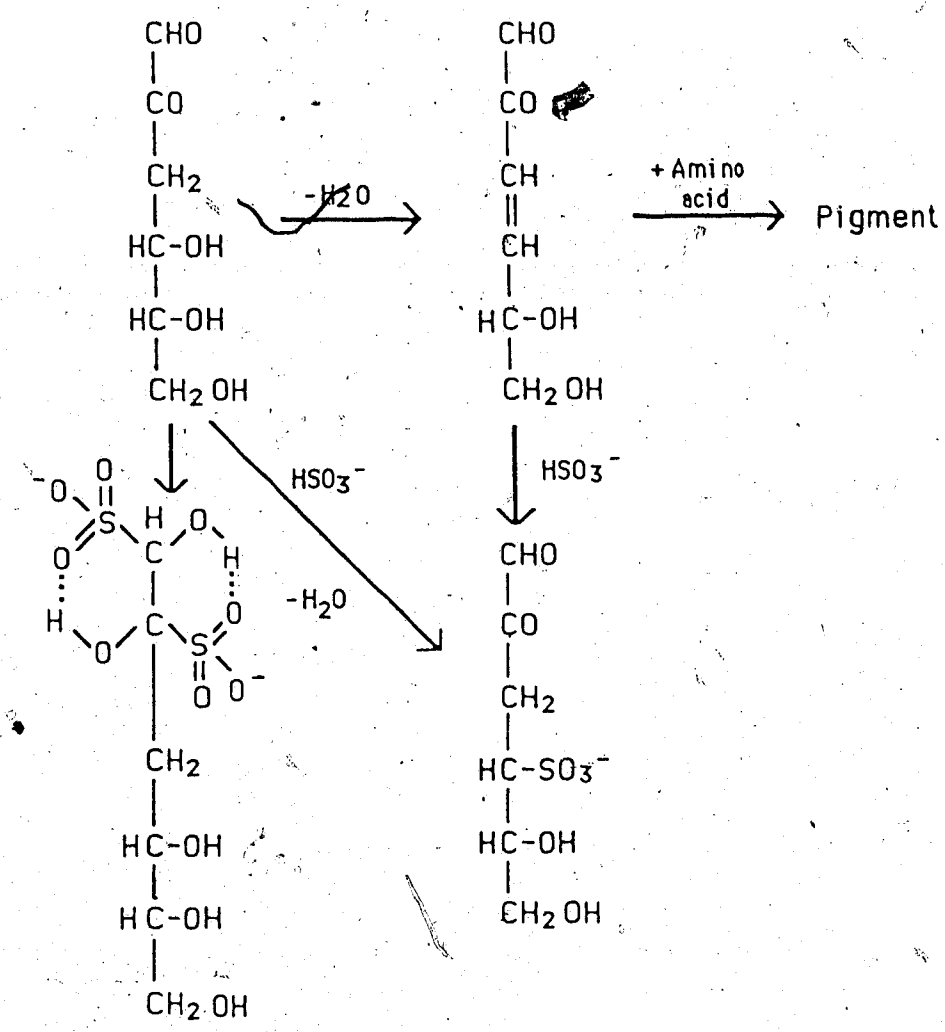


Figure 2.4 Reactions of sulfites with carbonyl intermediates (adapted from McWeeny *et al.*, 1974).

2.6.2 Safety problems of sulfites in foods

Recently, the safety of sulfites in foods has been questioned based mainly on their alleged role in the initiation of asthmatic reaction in certain sensitive individuals (Taylor *et al.*, 1986). The first reports about sulfite-induced asthma were by Kochen (1976) and Freedman (1977). The simultaneous reports from Allen and Collett (1981) and Stevenson and Simon (1981) initiated a great deal of research thereafter. Various types of adverse reactions have been observed, such as anaphylactic shock, hypotension, headaches, abdominal pain, nausea, dizziness, hives, urticaria and pruritis, eczema, angioedema, laryngel edema, tachycardia, clammy skin, and dyspnea (Sullivan and Smith, 1985; Taylor *et al.*, 1986). However, the most threatening effect of sulfites in foods is their ability to induce asthma in certain asthmatics. The asthma reaction in some sulfite-sensitive individuals can be sufficiently severe to be life-threatening. According to Sullivan and Smith (1985), of more than 300 reports of adverse reactions to sulfites that the FDA (Food and Drug Administration, U.S.A.) received, 6 involved deaths. The American Academy of Allergy and Immunology estimated that up to 17 fatalities have been attributed to sulfite ingestion in susceptible asthmatics (Bush, 1986).

Adverse reactions to sulfites are extremely rare in normal individuals (Bush, 1986). Even among asthmatics, only a small fraction (4.6-8%) is sensitive to the ingestion of

sulfites (Taylor and Bush, 1986). However, the safety problem of sulfites has received much attention from organizations such as the FDA.

Since 1959, the FDA has listed the following six sulfiting agents as GRAS (Generally Recognized As Safe): sulfur dioxide, sodium sulfite, sodium and potassium bisulfite, and sodium and potassium metabisulfite (Sullivan and Smith, 1985). On August 14, 1985, the FDA proposed a regulation to rescind the GRAS status for sulfites used on fruits and vegetables (except potatoes) which were intended for consumption in the raw state, particularly in salad bars. This proposal became effective on August 8, 1986 (FDA, 1986). The final action on the GRAS status of sulfiting agents for use in potato products has been scheduled for June, 1987 (Semling, 1987).

A labelling regulation was also proposed by the FDA on April 3, 1985, requiring the declaration of sulfites on the label when the residual sulfite content, as total SO_2, exceeds 10 ppm (FDA, 1985). The final action of the FDA (1986) requested that this labelling rule become effective on January 9, 1987.

A troublesome point in carrying out the labelling rule might be the unsuitability of the present analytical methodology for sulfites. The AOAC method, i.e. the Monier-Williams method, was reported not only to be very time consuming but also inaccurate at a sulfite level below 60 ppm (Sullivan and Smith, 1985; Moylan *et al.*, 1986). Upon

recognizing this unsuitability, the FDA has made some refinements to the Monier-Williams method, which include using a more dilute titrant and a minor modification to the apparatus. The modified method was reported to give suitable accuracy and reproducibility at the 10 ppm level (FDA, 1986). A recently developed ion chromatographic (IC) method for sulfite determination was reported to be both rapid and accurate at a sulfite level below 10 ppm (Sullivan and Smith, 1985; Moylan *et al.*, 1986).

The regulations regarding sulfiting agents will no doubt drive food scientists and manufacturers to seek substitutes. However, according to Taylor *et al.* (1986), it is virtually impossible to find a complete substitute for sulfiting agents. The potential substitutes are also stated to be less effective and more costly in most cases. The problems also lie in the fact that no other single preservative is as versatile as sulfites in its preservative functions. For example, one may be effective against enzymatic browning, but not nonenzymatic browning or microorganisms, or vice versa. Therefore, several types of preservatives must be found to replace sulfites in their various functions. To prevent nonenzymatic browning, for instance, ascorbic and citric acids have been used with some success. However, they are not as effective as sulfites and are more expensive. Other methods or substitutes still need to be found. One of these methods may be the use of glucose oxidase to reduce the concentration of glucose, one of the

major reactants in browning reactions.

2.7 Glucose Oxidase

2.7.1 General information

Glucose oxidase (EC 1.1.3.4, β-D-glucose : oxygen 1-oxidoreductase) is a flavoprotein which carries out the overall reaction of Equation 2.1:

$$C_6H_{12}O_6 + O_2 + H_2O \rightarrow C_6H_{12}O_7 + H_2O_2 \qquad (2.1)$$

The above conversion of glucose to gluconic acid in bacteria was first observed in 1878 by Boutroux with *Acetobacter aceti* (Bentley, 1963). Beginning in 1928, Muller made an extensive study on glucose oxidase from both *Aspergillus niger* and *Penicillium glaucum*, therefore it was generally believed that the glucose oxidase enzyme was discovered by Muller (Nakamura and Ogura, 1968; Whitaker, 1985).

Glucose oxidase has also been demonstrated in other fungi, including *Aspergillus oryzae*, *Penicillium notatum*, *P. amagasakiense* and *P. vital* (Scott, 1975a). It has not been found in higher plants or in animals (Whitaker, 1985).

2.7.2 Preparation of glucose oxidase

According to Scott (1975a) and Schwimmer (1981), glucose oxidase is prepared from the preferred fungal sources in different countries. The enzyme is prepared from *Aspergillus niger* in the United States, and from *Penicillium vital* in the U.S.S.R. In Japan, the preferred organism is *P.*

amagasakiense, with only a small production from *A. niger*. The enzyme from *A. niger* is considered to be an intracellular enzyme which can be obtained from the mycelium of the organisms. The enzyme from *Penicillium* species is purified from the fermentation medium, therefore it is considered as an extracellular enzyme (Scott, 1975a).

Two types of commercial enzyme preparations are available. One is a clear amber solution, stable under refrigeration for several years and at room temperature for many months (Scott, 1975a). Another is a light tan, amorphous dry powder. The liquid preparation is claimed to have a better solubility, more convenience in applying, low cost and greater stability, when compared to the powder form (Scott, 1975a; Fermco Biochemics, a).

The commercial preparations of glucose oxidase contain not only glucose oxidase enzyme, but also catalase (EC 1.11.1.6; hydrogen-peroxide ; hydrogen peroxide oxido-reductase) (Scott, 1975a; Fermco Biochemics, a; Miles Lab., 1985). Catalase catalyzes the decomposition of hydrogen peroxide (Equation 2.2):

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2 \qquad (2.2)$$

Catalase can be derived from animal livers, plants and microorganisms (Whitaker, 1985). The catalase in commercial glucose oxidase preparation can be obtained from *Aspergillus niger* (Miles Lab., 1985). Therefore, the overall reaction of commercial glucose oxidase-catalase preparation can be presented in Equation 2.3:

$$2C_6H_{12}O_6 + O_2 \rightarrow 2C_6H_{12}O_7 \qquad (2.3)$$

Glucose Gluconic acid

2.7.3 Mechanisms and molecular properties

2.7.3.1 Mechanisms

Two important papers on kinetic mechanisms of glucose oxidase were published by Nakamura and Oguar (1962), using enzymes from *P. amagaskinense*, and by Gibson *et al.* (1964), with enzymes from *A. niger*. A general scheme which could accommodate the reactions of glucose oxidase with all kinds of substrates (sugars) was proposed by Gibson *et al.* (1964), and is presented in Scheme I.

$$E_o + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E_o\text{-}S \xrightarrow{k_2} E_r P_1 \xrightarrow{k_3} E_r + P_1$$

$$E_r + O_2 \xrightarrow{k_4} E_o\text{-}H_2O_2 \xrightarrow{k_5} E_o + H_2O_2$$

Scheme I

where, E_o = enzyme in oxidized form; E_r = enzyme in reduced form; S = substrate; P_1 = oxidized substrates, i.e., δ-lactones.

When β-D-glucose was used as the substrate, the above general scheme was reduced to Scheme II (Gibson *et al.*, 1964):

$$E_o + G \xrightarrow{k_1} E_r\text{-}P_1 \xrightarrow{k_2} E_r + H_2O_2$$

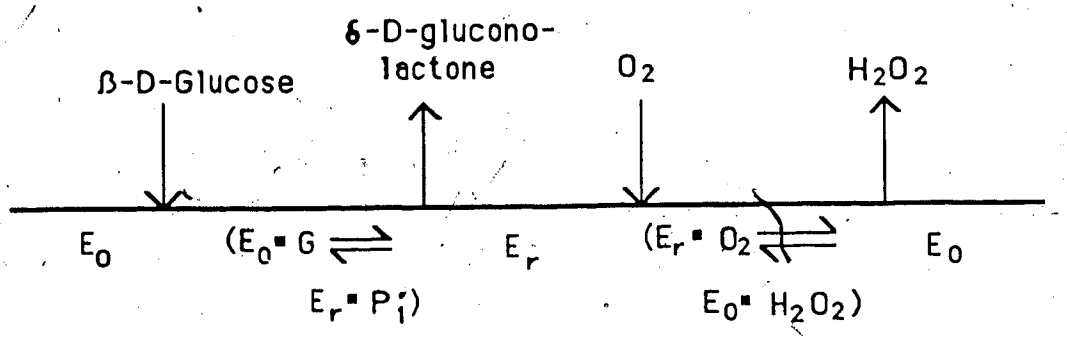
$$E_r + O_2 \xrightarrow{k_3} E_o\text{-}H_2O_2 \xrightarrow{k_4} E_o + H_2O_2$$

Scheme II

where, G = β-D-glucose; P_1 = δ-D-gluconolactone; E_o, E_r = enzyme in oxidized and reduced forms, respectively.

The δ-D-gluconolactone generated in Scheme II is hydrolyzed to gluconic acid, slowly and nonenzymatically (Whitaker, 1985).

Based on the experimental data of Gibson *et al.* (1964), Whitaker (1985) concluded that the mechanism of glucose oxidation by glucose oxidase was a ping-pong bi-bi mechanism, which is presented as Scheme III:



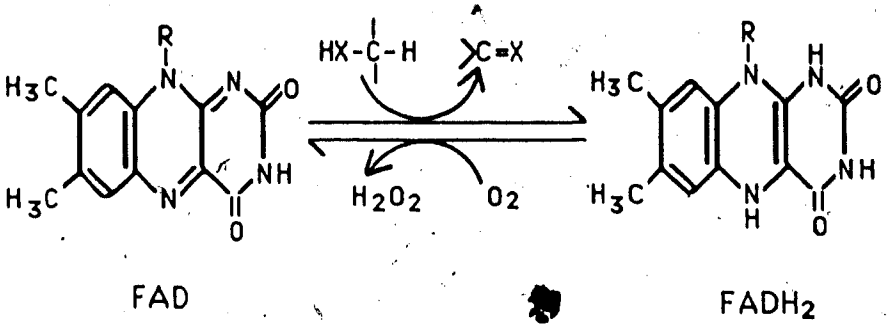
Scheme III

2.7.3.2 Molecular properties

Glucose oxidases from various fungal sources not only catalyze the same reactions, but also are substantially similar in molecular properties.

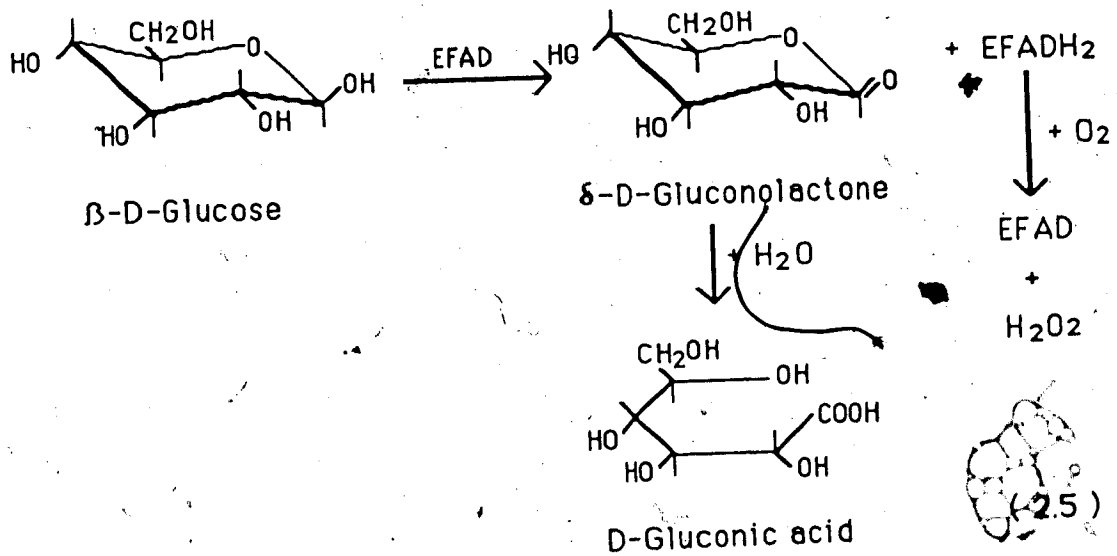
1. Glucose oxidase is a flavin enzyme, containing two molecules of flavin adenine dinucleotide (FAD) per enzyme molecule. The existence of FAD as the prosthetic group of glucose oxidase was first proved by Kellin and Hartree (1946). In glucose oxidase catalyzed reactions, the FAD moiety can exist in two redox states, i.e., fully oxidized

FAD and fully reduced ($FADH_2$), as shown by Equation 2.4 (Bright, 1974).



FAD $FADH_2$ (2.4)

Therefore, the FAD moiety is the center of oxidation and reduction in the enzyme reactions. The oxidation of glucose by the enzyme can be written as Equation 2.5 (Whitaker, 1985).



ß-D-Glucose δ-D-Gluconolactone

D-Gluconic acid (2.5)

The molecular weight of the enzyme from various fungal sources is around 160,000-180,000 (Table 2.2).

Table 2.2 Molecular weight of glucose oxidase (various reports).

Reference	*A. niger*	*P. amagasakiense*
Whitaker, 1965	186,000	———
Scott, 1975a	192,000	154,000
Nakamura and Fujiki, 1968	152,000	150,000

2. Glucose oxidase is also a glycoprotein, containing mannose, galactose, glucose and hexosamine. The experimental results of the carbohydrate contents in glucose oxidase enzyme have been summarized in Table 2.3.

The carbohydrate contents of glucose oxidase vary from production lot to lot (Nakamura *et al.*, 1976) as well as different fungal sources (Nakamura and Fujiki, 1968). It was postulated that the carbohydrate moiety of the enzyme provided a protective effect, which increased the stability of the protein moiety against denaturing agents (Nakamura *et al.*, 1976; Nakamura and Hayashi, 1974).

2.7.4 Activity assay and unit

The multiplicity of units and activity assay methods is one of the most confusing things confronting those who deal with enzymes. Various assay procedures have also been employed in the study of glucose oxidase. The overall reactions of glucose oxidase acting on glucose could follow either Equation 2.1 or Equation 2.3, as described earlier, depending on the absence or presence of catalase in the enzyme preparations. The activity assay, therefore, can be based on:

(1) formation of H_2O_2;

(2) consumption of O_2 or O_2 substitute;

(3) formation of gluconic acid.

The hydrogen peroxide (H_2O_2) produced in an enzymatic reaction has been utilized for activity assay, either in the

Table 2.3 Carbohydrate content of glucose oxidase.

Fungal Source	Carbohydrate Content (%)					Reference
	Mannose	Glucose	Galactose	Hexos-amine	Total	
A. niger	13.9	—	0.3	2.0	16.2	Pazur *et al.* 1965
	9.6	0.2	0.7	1.7	12.2	Nakamura *et al.* 1976
	7.9	0.1	1.7	0.8	10.5	Nakamura and Hayashi, 1974
	13.2	0.7	—	2.1	16.0	Nakamura and Fujiki, 1968
P. amagasakiense	8.3	1.1	—	1.7	11.1	Nakamura and Fujiki, 1968

presence or in the absence of catalase (Ciucu and Patroescu, 1984). Some compounds, such as o-dianisidine, o-toluidine, or 2,6-dichlorobenzenone-indophenol, can be oxidized by H_2O_2 and catalase from the colorless reduced forms to the colored oxidized forms (Equation 2.6), therefore making spectrometric assay feasible (Huggett and Nixon, 1957; Dobrick, 1958).

$$\text{Reactant} + H_2O_2 \xrightarrow{\text{catalase}} H_2O + \text{Product} \qquad (2.6)$$

reduced, colorless — oxidized, colored

Without the assistance of catalase, hydrogen peroxide rapidly oxidizes iodide to iodine in the presence of Mo(VI) (Pardue *et al.*, 1964; Pardue and Simon, 1964). The iodine formed can be detected either by potentiometry (Pardue *et al.*, 1964) or by amperometry (Pardue and Simon, 1964).

Ciucu and Patroescu (1984) described a spectrometric assay method, using benzoquinone to substitute O_2. The formation of hydroquinone was measured at 290 nm. The method was reported to be direct, rapid and could be used to determine the initial velocity of enzyme reaction.

Based on the consumption of oxygen in Equations 2.7.1 and 2.7.3, the reaction velocity can be determined by manometry or polarography. The manometric assay for glucose oxidase was first described by Scott (1953). The consumption of oxygen was measured as the decrease of air pressure inside a Warburg flask fitted with a manometer. The amount of oxygen consumed in 30 minutes was used to calculate the

reaction velocity and activity.

The manometric method has been superseded by the oxygen electrode technique, a polarographic method (Estabrook, 1967; Bright, 1974). Several reviews have been published regarding this technique (Fatt, 1976; Gnaiger and Forstner, 1983). The heart of the instrument is an oxygen probe. A cell, consisting of a platinum cathode and a silver anode, is separated from the surrounding sample solution by a specific membrane which is permeable only to gases such as oxygen. When a suitable polarizing voltage is applied across the cell, oxygen undergoes an electric reduction at the cathode, causing a current to flow through the cell. The magnitude of current generated is proportional to the amount of oxygen present, which, in turn, depends on the oxygen pressure in the surrounding solution. By recording the electric current generated, the oxygen pressure in the solution can be traced continuously. In the study of glucose oxidase, the most widely used probe is the Clark-type polarographic electrode (Nakamura and Hayashi, 1974; Nakamura and Ougura, 1962; Weibel and Bright, 1971).

A titrimetric method (Underkofler, 1958) has been employed by some of the enzyme producers (Fermco Biochemics, b; Miles Lab., 1981). The gluconic acid produced in the enzyme reactions in 15 minutes is back-titrated to obtain the enzyme reaction rate and activity.

Various units have been used in the activity assay of glucose oxidase. The unit used for commercial preparation is

the manometric unit in GOU/g or GOU/mL, where GOU stands for Glucose Oxidase Unit. It was defined by Scott (1953) as "the quantity of enzyme that will cause an oxygen uptake of 10 mm^3 per minute under assay conditions". In the titrimetric method, the unit was defined as the equivalent of glucose oxidase to produce 1 mL of 0.05 N gluconic acid (Fermco Biochemics, b). The glucose oxidase activity obtained through the titrimetric method was expressed as the manometric unit. An arbitrary factor, 3, was used to convert the titrimetric unit to the manometric unit. It was obtained by regressing the experimental results of the same samples with the manometric and titrimetric methods (Underkofler, 1958).

When manometric or polarographic methods are used, the already confounding units can become even more puzzling in some cases. Some suppliers define their glucose oxidase units as being determined in the absence of catalase, therefore doubling the oxygen uptake, making the activities of different suppliers uncomparable (Scott, 1975a).

In order to bring uniformity into the chaos of expressing the enzyme activity, the International Union of Biochemistry, Enzyme Commission, proposed the definition of "Unit" (IUB, 1964) as: "One Unit (U) of any enzyme is that amount which will catalyse the transformation of 1 micromole of the substrate per minute under standard conditions".

It was also suggested that the measurement be carried out under optimum conditions and the "enzyme assays should

be based wherever possible on measurements of initial rate of reaction, and not on amounts of substrate changed by the end of a period of time unless it is known that the velocity remains constant throughout the "period". Obviously, the titrimetric method does not meet this criterion, while the polarographic method is able to measure the initial reaction velocity.

2.7.5 Reaction properties of glucose oxidase

2.7.5.1 Substrate specificity

The substrate specificity of glucose oxidase has been well studied and some of the results were summarized by Whitaker (1985) and are presented in Table 2.4. Glucose oxidase has a very high specificity for β-D-glucose. However, Taylor *et al.* (1975) reported that glucose oxidase from *Pseudomonas fluorescens* oxidized β-D-glucose and 3-deoxy-3-fluoro-D-glucose at the same initial rate at a substrate concentration up to 200 μmole.

2.7.5.2 Michaelis constant (K_m)

The Michaelis constant, also referred to as Michaelis-Menten constant (K_m) is defined as "the concentration of the specific substrate at which a given enzyme yields one-half its maximum velocity" (Lehninger, 1982). It reflects the affinity characteristics of an enzyme for a specific substrate under certain reaction conditions. The reported Michaelis constants of glucose oxidase for glucose are

Table 2.4 Substrate specificity of glucose oxidase (adapted from Whitaker, 1985).

Compound	Relative Rate
ß-D-Glucose	100
∂-D-Glucose	0.64
1.5-Anhydro-ß-D-glucitol	0
2-Deoxy-ß-D-glucose	3.3
D-Mannose	0.98
2-O-Methyl-ß-D-glucose	0
3-Deoxy-ß-D-glucose	1
ß-D-Galactose	0.5
4-Deoxy-ß-D-glucose	2
5-Deoxy-ß-D-glucose	0.05
L-ß-D-glucose	0
6-Deoxy-ß-D-glucose	10
Xylose	0.98

presented in Table 2.5. A large variance among the reported K_m values can be observed. For example, the K_m of *Aspergillus* enzyme ranges from 0.026-0.11 M, even though the assay conditions are almost the same.

2.7.5.3 pH dependence of the enzyme reaction

Glucose oxidase from both *Aspergillus niger* and *Penicillium* strains has a wide range of effective pH values between 4.5 to 7.0, with optimum pH around 5.6 (Scott, 1975a; Bentley, 1963). At lower pH values, the rate limiting step of overall turn-over was reported to be the rate of formation of enzyme-substrate complex (E_o-S), while at a higher pH range, the limiting step was the combination of reduced-form glucose oxidase (E_r) with oxygen to form E_o-H_2O_2 complex (Bright and Appleby, 1969).

Glucose oxidase has also been reported to be quite unstable at pH values greater than 8 (Bentley, 1963). However, the enzyme could be stabilized by the presence of its substrate, such as glucose (Scott, 1975a).

2.7.5.4 Effect of temperature on the reaction rate

The elevation of temperature will affect the reaction rate of glucose oxidase in three aspects. The high temperature will: (1) lower the activation energy for the reaction, thus accelerating the reaction rate; (2) decrease the solubility of oxygen in the solution; and (3) accelerate the heat denaturation of the enzyme. Balanced by these three factors, the reaction rate of glucose oxidase is found to be

Table 2.5 Michaelis constants of glucose oxidase.

Fungi Sources	Temp. (^{0}C)	pH	K_m For Glucose(M)	Reference
A. niger	15	5.5	0.05	Nakamura and Ogura, 1968
A. niger	25	5.5	0.064	Nakamura and Ogura , 1968
A. niger	27	5.6	0.11	Gibson *et al.* 1964
A. niger	25	5.6	0.033	Swoboda and Massey, 1964
A. niger	25	5.5	0.026	Nakamura and Hayashi, 1974
P. amaga-sakiense	30	5.6	0.015	Swoboda and Massey, 1964
P. notatum	20	5.6	0.096	Swoboda and Massey, 1964
Not reported	25	5.0	0.034	Ciucu and Petroescu, 1984

relatively unchanged from 30 to 60°C, and does not decrease significantly when temperature is well below that range (Scott, 1975a). Actually, the offset resulting from the decrease of oxygen solubility at higher temperature is so severe that the reaction rate of the enzyme at 90°F (32°C) is substantially the same as the rate at 50°F (10°C) (Scott, 1975a). On the other hand, Ohlmeyer (1957) reported that the activity of glucose oxidase increased as the temperature increased, up to a limit where the enzyme was rapidly denatured, at 87°C.

2.7.5.5 Inhibitors of glucose oxidase

Glucose oxidases from various sources are inhibited by metal ions such as Cu^{2+} and Hg^{2+} (Nakamura and Ogura, 1968) and partially by chemicals such as sodium bisulfite (Bentley, 1963). The product of enzyme reaction, D-gluconoδ-lactone, was found to be an inhibitor of glucose oxidase, possibly by binding itself in some way to the active center of the enzyme (Nakamura and Ogura, 1962; Gibson *et al.*, 1964).

2.7.6 The applications of glucose oxidase in food processing

Glucose oxidase, alone or coupled with catalase, has found wide applications in food science and technology because of its versatile functions. These numerous applications can be divided into five main categories:

1. to determine the glucose content of foodstuffs;

2. to form hydrogen peroxide;
3. to produce gluconic acid;
4. to remove glucose;
5. to remove oxygen.

2.7.6.1 Enzymatic determination of glucose

Because of its high substrate specificity, glucose oxidase, free or bound, has been used to determine the glucose contents of foodstuffs (Valentova *et al.*, 1983). A glucose oxidase impregnated test tape was adapted from clinical use to the potato chip industry by Smith (1960). The so-called "Chip-Color Tester" changes its color from yellow to various shades of green when it is in contact with raw potato slices. The glucose content of the potatoes can be read directly. The color of potato chips could be predetermined successfully from the reading of the Chip-Color Tester.

2.7.6.2 Formation of hydrogen peroxide

Some chemical oxidants, such as bromates, calcium peroxide, ascorbic acid, and chlorine dioxide, have long been used in the bread making industry to mature or bleach flour (Pomeranz and Shallenberger, 1971). The improving effect of ascorbic acid on the quality of baking flour was reported to be enhanced by adding glucose oxidase (Maltha, 1955). A USSR patent also described the addition of glucose oxidase and ascorbic acid to dough (Kretovich *et al.*, 1969). Scott (1975b) postulated that the enzyme removed free oxygen

in the dough, thus saving ascorbic acid, leaving it for its improving effect on baking properties of flour.

Glucose oxidase from *A. niger*, combined with lactoperoxidase, can preserve a contaminated milk product against a mixed microbial challenge (Banks and Board, 1985). The role of glucose oxidase enzyme was believed to be generating the required hydrogen peroxide.

2.7.6.3 Production of gluconic acid

The gluconic acid produced by glucose oxidase can be used to acidify milk to meet the requirement for milk curdling (Rand, 1972). Either glucose or lactose has to be present to provide sufficient substrate for glucose oxidase.

The utilization of glucose oxidase to extend the shelf-life of fresh seafood was reported recently (Wesley, 1982; Field *et al.*, 1986). The application could be accomplished by: (1) dipping fresh fish in enzyme-glucose solution; (2) packing fish with crushed enzyme-substrate containing ice; or (3) by storing glucose-solution-dipped fish and enzyme-impregnated algin blankets in alternating layers (Field *et al.*, 1986). The method nearly doubled the shelf-life of fillets and reduced drip loss in storage. It was believed that the gluconic acid generated during the treatment would reduce the pH of the surface of the fish, inhibiting the growth of spoilage microorganisms which were acclimatized to neutral or slightly alkaline marine environments. Gluconic acid itself could also be bacteriostatic, as could the hydrogen peroxide which might be

produced (Field *et al.*, 1986).

2.7.6.4 Removal of glucose

Glucose can, in some cases, present problems in food processing and preservation. Glucose oxidase has been used to remove glucose from foodstuffs such as corn syrup, invert sugar, dehydrated meat and dried egg powder.

Glucose in invert sugars and corn syrups is not as desirable as fructose due to its lower sweet equivalence, possible slower assimilation in digestion (Scott, 1975b), and facile crystallizing characteristics (Mermelstein, 1975). To remove glucose from invert sugar, glucose has been first oxidized by glucose oxidase to gluconic acid, which was separated by passage through an ion-exchange column (Scott, 1975b). Glucose oxidase has also been applied to produce the low glucose corn syrup which has an dextrose equivalent (DE) value equal to ordinary corn syrup, but contains a lesser amount of monosaccharides (Mermelstein, 1975).

The glucose content of meat may increase because of glycolysis activity (Sharp, 1957). The discoloration of dehydrated meat during storage is believed to be caused by the Maillard reaction between amino groups and glucose or its breakdown products (Henrickson *et al.*, 1956). Glucose oxidase enzyme was used by Henrickson *et al.* (1956) with hydrogen peroxide to treat meat before dehydration. They found that enzyme-treated pork was more stable and lighter in color than the control.

The greatest success of commercial application of glucose oxidase in the food industry is in egg drying. The natural glucose in eggs can deteriorate the quality of dried powders by either condensation with amino groups or by reacting with cephalin to produce off-flavors (Hill and Sebring, 1977; Berquist, 1977).

The removal of glucose from eggs, referred to as "desugaring", used to be achieved by fermentation with contaminating microorganisms or by controlled bacteria and yeast. The first application of glucose oxidase enzyme in a desugaring process was reported by Baldwin *et al.* (1953). Egg white, containing 3.4% glucose on a dry basis, was acidified with dilute hydrochloric acid to lower the pH from 9.0 to 7.3. After treating with 0.5% glucose oxidase-catalase enzymes for about 15 hours at 80°F (26.7°C), the glucose content dropped to 0.1% (dry basis), and the egg white was suitable for drying. Their results were corroborated by Carlin and Ayres (1953).

Scott (1953) developed an empirical relationship for glucose level, time, enzyme level and hydrogen peroxide demanded for enzyme desugarization in preparation of albumen solids. Yolk and whole egg were also desugarized by glucose oxidase-catalase enzyme system. The glucose content dropped from 1.2% (dry basis) to 0.1% after treatment with 0.3% enzyme for 4 hours.

The quality of enzyme-desugarized egg white, yolk and whole egg powders has been the subject of several studies.

The enzyme-treated powders were compared with control samples (Paul *et al.*, 1957; Song *et al.*, 1984), or with microorganism-fermented samples (Kline *et al.*, 1954; Darwish and Sadek, 1978). Enzyme treatment improved the storage stability of the egg yolk solids (Paul *et al.*, 1957), and resulted in higher foaming ability of egg white than the control (Song *et al.*, 1984). The enzyme-desugared egg white had a better foaming capacity than the yeast-desugared sample (Darwish and Sadek, 1978). The storage stability of egg powders prepared by the enzyme or yeast desugaring methods were equivalent as appraised by chemical, functional and flavor tests (Kline *et al.*, 1954).

The desugarization of egg white by co-immobilized glucose oxidase and catalase was reported by Kobayshi *et al.* (1978). Glucose oxidase and catalase were immobilized together or separately by using polyacrylamide gel. Oxidation of glucose to gluconic acid followed Michaelis-Menten kinetics.

2.7.6.5 Removal of oxygen

For many years, food scientists have been aware of the deteriorating effects of oxygen during food processing and food preservation. Various methods of removing oxygen from foodstuffs have been developed, such as vacuum or nitrogen packaging. Glucose oxidase-catalase enzymes have also been applied to many food commodities to achieve this goal.

During the storage of lipids, the oxidative rancidity resulting from exposure to oxygen is the most common and

most troublesome problem (Hamilton, 1983). Mitsyk *et al.* (1968) applied a glucose oxidase preparation from *Penicillium vitals* to prolong the shelf-life of fats. The surface of the fat was covered by a film of a mixture of enzyme and acetate buffer, glucose, and ascorbic acid. Osadehaya (1971) added glucose oxidase and catalase into melted pork and beef fat and kept the mixture hermetically-sealed in glass jars. The fat thus treated remained usable for more than 18 months.

Dehydrated foods, because of their tremendous increase of the surface area exposed to oxygen (air), are especially susceptible to autoxidative rancidity. The direct application of enzyme system to these foods, however, is impossible, as the moisture content of the foods is too low to permit enzyme activation (Scott, 1975b). However, the free oxygen in packages or containers of foods can be removed by means of a dispersion of water, glucose and enzyme system of glucose oxidase and catalase (Scott, 1956). The enzyme dispersion was atomized onto suitable carriers, such as sawdust, corn coles, polystyrene or clay, and this deoxygenating mixture was separated from the package contents with an oxygen-permeable barrier such as paper or polyethylene (Scott and Hammer, 1962, 1963). Levina *et al.* (1968) were also able to prevent dry foods, such as dry milk, coffee and candy, from autoxidation by adding a mixture of glucose oxidase, catalase, glucose, buffer, antibiotics and a filler, such as starch.

In many cases, foods are either hot-filled or heated after sealing. Glucose oxidase and catalase have to survive the heat treatment for their function of deoxygenation. Scott (1975b) coated the dry enzyme with a hot-water insoluble material, such as methyl cellulose, or a time-release coating, and thus was able to stabilize the enzyme through the heat treatment.

The deterioration and spoilage of beer during storage, caused by oxidation reactions, can be inhibited by addition of glucose oxidase-catalase enzymes (Ohlmeyer, 1957).

The glucose oxidase-catalase system was applied by Barton *et al.* (1955) to protect canned soft drinks from fading of sensitive colors and picking up of iron. The enzymes have also been utilized to stabilize fruit drinks and concentrates, such as orange juice, lemon juice and grapefruit drinks (Scott, 1975b).

Apple wine is susceptible to oxidation and to the development of acetic acid. Yang (1955) applied the glucose oxidase-catalase system to remove oxygen from the wine, and thus prevented development of volatile acids. According to Scott (1975b), a considerable amount of work has been done by Ough and his colleagues on the stabilization of white wine with glucose oxidase-catalase system and favorable results have been achieved.

A few more applications are worth mentioning. A glucose oxidase-catalase impregnated plastic wrapper was able to prevent browning ring in the loaves of cheeses (Scott,

1975b). The enzymes can also be used to prevent oxidative rancidity in mayonnaise (Scott, 1958), to prevent discoloration in precooked frozen shrimp (Kelley, 1974), and to protect ascorbic acid (Chogovadaze and Baturadze, 1972).

2.7.6.6 Recent research activities

Recently, the research activities on glucose oxidase seem to emphasize immobilization of the enzyme, alone or with catalase, and the application of immobilized enzymes. Examples of such research endeavors include immobilization of glucose oxidase by irreversible adsorption on the surface of a graphite electrode (Ikeda *et al.*, 1984); by entrapment in gels (Freeman *et al.*, 1983); on a collagen membrane (Gozia *et al.*, 1983); and on dimethyl-amiated nylon gels (Miyama *et al.*, 1985).

3. EXPERIMENTAL

3.1 Materials

3.1.1 Potatoes

Potatoes of Superior and Shepody varieties were obtained from I & S Produce Ltd., a local food processor. Norland potatoes were purchased from Centennial Acres, a local potato supplier. Potatoes were either stored at 4°C, or reconditioned at room temperature to achieve suitable reducing sugar content.

3.1.2 Glucose oxidase

Two commercial preparations of glucose oxidase enzyme from *Aspergillus niger* were used:

A. DeeO 1500, in powder form, 1500 Glucose Oxidase Units (GOU)/g (Miles Laboratories, Elkhart, IN).

B. Fermcozyme CBB-750, in liquid form, 750 GOU/mL, (Fermco Biochemics, Elk Grove Village, IL).

3.1.3 Chemicals

Monobasic (KH_2PO_4) and dibasic (K_2HPO_4) potassium phosphate, 85% phosphoric acid, and anhydrous dextrose (D-glucose), all certified A.C.S. grade (Fisher Scientific Co., Fair Lawn, NJ).

Reagent grade chloroform and anhydrous ethyl ether (Caledon Labs., Georgeton, Ont.).

3.1.4 Equipment

Model 53 biological oxygen monitor and YSI Model 27 industrial analyzer (Yellow Springs Instruments, Yellow Springs, OH).

Hobart food cutter and dicer (The Hobart Manufacturing Co., Troy, OH).

General Electric fryer, Model CK40 (General Electric Co., Chicago Heights, IL).

HunterLab Model D25M/L-2 colorimeter (Hunter Assoc. Lab., Fairfax, VA).

Beckman J-21B centrifuge (Beckman Instr., Spinco Div., Palo Alto, CA).

Model 5851 vacuum oven (National Appliance Co., Portland, OR).

3.2 Studies on Glucose Oxidase

3.2.1 Preparation of phosphate buffer and various solutions

Phosphate buffers of various pH values were prepared by mixing the KH_2PO_4 and K_2HPO_4 solutions (both 0.1 M or 0.15 M) to obtain the desired pH values. A Model 230 pH/ion meter (Fisher Scientific, Fair Lawn, NJ) was used to measure pH. For buffer of pH lower than 4.5, phosphoric acid was used to bring KH_2PO_4 solution to the desired pH.

Glucose solution, 1 M either in phosphate buffer or in Milli-Q water, was kept at room temperature overnight before use.

Glucose oxidase solutions were prepared by dissolving the enzyme either in phosphate buffer or in Milli-Q water prior to the experiment. The enzyme concentration was expressed as percentage in solution, w/v for powder enzyme and v/v for liquid enzyme.

3.2.2 Activity assay procedures

A total of 2.95 mL of glucose solution and phosphate buffer was pipetted into the reaction cell of the oxygen monitor and stirred for 3-5 min to achieve temperature equilibrium and air saturation. The oxygen probe was inserted carefully to expel any air bubbles that might attach to it. Glucose oxidase solution (50 μL) was added with a syringe through the slot on the oxygen probe. The oxygen pressure of the solution was recorded with a 10 in Beckman recorder at a chart speed of 1 in/min. An example of the graph obtained is presented in Figure 3.1.

To check the effect of glucose concentration on the enzyme reaction rate, the experiments were carried out at 25.0°C, as recommended by IUB (1964), and at pH 5.5, which was reported to be the optimum by Bentley (1963). The ratio of glucose solution (1.0 M in buffer) to phosphate buffer was adjusted to obtain the desired glucose concentrations in the final solution.

To carry out the measurement at different pH's, 0.90 mL 1.0 M glucose in water and 2.05 mL 0.15 M phosphate buffer were used for each determination so that the buffer

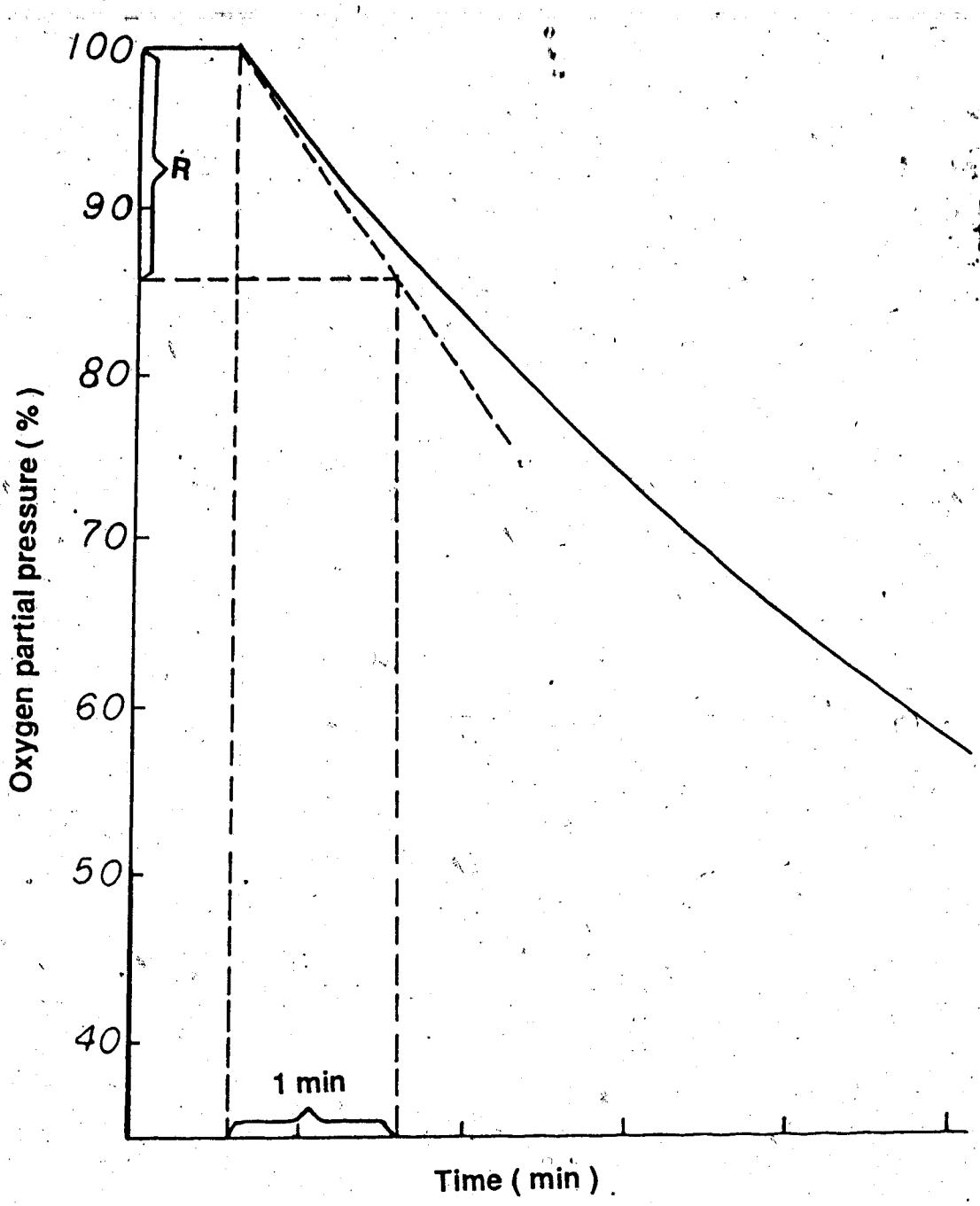


Figure 3.1 Example graph of oxygen partial pressure traced by the Model 53 Biological Oxygen Monitor. The measurement was carried out at 40°C, 0.3 M glucose, with chart speed of 1 in/min.

concentration of the final solution was kept at 0.1 M.

To test the effect of reaction temperature on the reaction rate, the experiments were carried out at various temperatures (25 to 40°C), at pH 5.5 and glucose concentration of 0.3 M, which was found to be the minimum glucose concentration for activity assay.

To study the thermal stability of the enzyme, about 2 mL of enzyme solution (in 0.1 M phosphate buffer, pH 5.5) was put into a test tube and held in a water bath at specific temperatures. The test tube was taken out after a certain time and cooled in cold water. The remaining activity was assayed at 25°C at pH 5.5 and glucose concentration of 0.3 M.

3.2.3 Calculation of reaction rates

To calculate the initial velocity of the reaction, a straight line was drawn tangentially to the recorded curve as illustrated in Figure 3.1. The reduction of oxygen pressure in 1 min, R, was obtained from the graph. The velocity of the enzyme reaction, ν, was calculated according to Equation 3.1.

$$\nu = \frac{S_T \times R \times V}{22.4 \times 100} \times 2 \qquad (3.1)$$

where:

ν: reaction velocity in μmole/min;

S_T: oxygen solubility of the solution at temperature T in μL O_2/mL;

R: oxygen pressure reduction (%) in 1 min;

V: total volume (mL) of the solution, which was 3.0 mL in these experiments.

The activity of the enzyme preparations was calculated with Equation 3.2:

$$\frac{IU}{g \text{ or } mL} = \frac{\nu}{E} \qquad (3.2)$$

where:

IU: International Unit;

ν: reaction velocity calculated from Eq. 3.1;

E: total amount of enzyme used in measurement in g or mL.

The oxygen solubility at temperature T (S_T in Eq. 3.1) was calculated from Eq. 3.3:

$$S_T = S_T^{O} \times e^{-kc} - D_{buffer} \qquad (3.3)$$

where:

S_T^{O}: oxygen solubility in pure water at temperature T ($\mu L\ O_2/mL$);

k: constant, 0.0156 (Zander, 1976);

C: glucose concentration (%).

D_{buffer}: decrease of oxygen solubility caused by 0.1 M phosphate buffer at temperature T.

S_T^{O} values could be found from previous data, and D_{buffer} was calculated from the data given by Robinson and

Cooper (1970).

3.3 Processing and Enzyme Treatment of Potato Chips and French Fries

3.3.1 Processing of potato chips

Potatoes of Superior and Shepody varieties were used for chip making. They were either from cold storage (4°C) or reconditioned at ambient temperature for a suitable length of time to achieve various reducing sugar contents.

Potatoes were washed, hand peeled, and sliced with the Hobart Food Cutter and Dicer to about 1/20 in (1.3 mm) thickness. Samples of the slices were taken for glucose and moisture content determinations. After washing in cold water to get rid of surface starch, the slices were either dried on paper towel or treated with enzyme solution. They were then fried in hot oil at 180°C. Frying was terminated when bubbles ceased to rise from the slices. The chips obtained were packed in polyethylene bags for later analyses.

3.3.2 Enzyme treatment of potato chips

About 250 g of the cold water-washed slices were immersed in 1 L of water or enzyme solutions at various temperatures for various times. The enzyme concentration in the solutions ranged from 0.005% to 1.5% (w/v for powder enzyme and v/v for liquid enzyme). The treatment was carried out at 20, 30 and 40°C, with the dipping time varying from

30 to 90 min. During dipping, the samples were occasionally stirred with glass rod. After dipping, slices were dried on paper towel and samples of the slices were taken for glucose and moisture content determinations.

To check the effect of aeration on the enzyme reaction on potato slices, compressed air (approx. 21% O_2, 78% N_2) was bubbled through the solution during the enzyme treatment at a rate of about 5000 cc air/min (Lab Crest Series 100 Century Flowmeter; Fisher Scientific Co., Fair Lawn, NJ).

3.3.3 Processing of French fries

Norland potatoes from cold storage were washed, peeled and sliced with the Hobart Food Cutter and Dicer into strips of 3/8 in^2 (1 cm^2) cross section. Samples of the strips were taken for glucose and moisture content determinations. Strips were then washed in cold water twice. Pre-frying treatments of the strips, such as blanching, were carried out, followed by the enzyme treatment. Strips were then dried on paper towel and fried in oil at 185°C for 3 min. French fries thus obtained were kept for various analyses and evaluations.

3.3.4 Pre-frying treatments for French fries

Part of the raw potato strips were fried directly without further treatment. The remaining strips were hot water blanched or steam blanched prior to further treatments. Hot water blanching was performed at 80°C for 15

min, as recommended by Brown and Morales (1970). Live steam at atmospheric pressure for 7 min was used in steam blanching of potato strips according to Strong (1968) and Wilder (1972).

3.3.5 Enzyme treatment of potato strips

About 450 g of potato strips, blanched or unblanched, were immersed in 1 L of either water or enzyme solutions kept at various temperatures. The enzyme concentrations ranged from 0.05% to 1.5% (w/v for powder enzyme and v/v for liquid enzyme). The dipping was carried out at 25 or 35°C for 5-20 min. During the treatment, solutions were stirred every 2-3 min. After treatment, the strips were dried on paper towel and samples of the strips were taken for glucose and moisture content determinations.

3.4 Analysis and Sensory Evaluation

3.4.1 Moisture content determination

The AOAC (1980) method with a conventional oven at 105°C was used for moisture determination.

3.4.2 Glucose content determination

3.4.2.1 Raw potato samples

Chopped raw potatoes (50.0 g) were blended with 100.0 mL distilled water at high speed for 2 min. The mixture was then filtered through a Whatman No. 4 paper in a Buchner

funnel attached to a water aspirator. A portion of the filtrate was placed in a test tube and heated in 80°C water for 10 min, cooled, and refiltered through a Whatman No. 4 paper. A 25 μL aliquot of filtrate was then injected into the YSI Glucose Analyzer. Glucose content of the sample was calculated from the following formula:

$$\% \text{ Glucose} = \frac{Rd}{1000} \times 3$$

where Rd was the direct reading from the instrument in mg/dL.

3.4.2.2 Cooked (blanched) samples

Chopped samples (25.0 g) were blended with 100 mL of distilled water at high speed for 2 min. The slurry was then centrifuged at 25,400 G for 15 min. A 25 μL aliquot of the supernatant was injected into the YSI Glucose Analyzer. Glucose content of the sample was calculated from:

$$\% \text{ Glucose} = \frac{Rd}{1000} \times 5$$

3.4.3 Color measurement of potato chips and French fries

3.4.3.1 Potato chips

About 40 g of chips were crushed in polyethylene bags so that they could be put into the blending jar. Samples were blended at low speed for a few minutes and sifted through a 10 mesh sieve. The sifted samples were filled into the Hunterlab sample holder for color measurement. The surface of the sample was smoothed with a spatula.

The Hunterlab Colorimeter was standardized with a standard yellow plate (L=77.9, a=-1.4, b=23.3). The color of duplicate samples was then measured and average readings of L, a and b were obtained.

3.4.3.2 French fries

For color measurement of French fries the Hunterlab was also standardized with the yellow plate. Samples of French fries were placed side by side on the sample holder. The samples were then covered with a black aluminum plate in which a hole of 4 cm diameter was cut in the center. The color of samples was measured through the hole. Three different locations on the samples were measured to obtain the average values.

The color of French fries was also evaluated subjectively by comparing the sample color with the USDA color standards (USDA, 1972).

3.4.4 Determination of fat content of potato chips and French fries

The fat content of potato chips was determined with the Soxhlet extraction method as described by Lees (1975).

The AOAC (1980) method was used for the determination of fat content in French fries. Samples were vacuum dried at 60°C and 100 mm Hg for 24 h, followed by Soxhlet extraction with anhydrous ethyl ether for 5 h.

3.4.5 Sensory evaluation

Sensory evaluation was carried out to compare the flavor of French fries prepared with hot water blanching and steam blanching. Water blanching was carried out at 80°C for 15 min, while in steam blanching, strips were steamed under atmospheric pressure for 7 min and dipped in water at 35°C for 15 min before frying. Samples from these two treatments were served to the panelists together immediately after frying. Panelists, recruited from the graduate and undergraduate students and technicians from the Department of Food Science, were asked to taste and compare the samples. The evaluation sheet is shown in Figure 3.2.

The sensory evaluation was also carried out to determine whether glucose oxidase treatment imparted any off-flavors to potato chips and French fries. Samples of potato chips were prepared by dipping the slices in water or 0.1% liquid enzyme solution at 40°C for 60 min before frying. French fries were prepared by dipping the steam blanched potato strips in water or 0.1% liquid enzyme solution at 35°C for 15 min before frying. The control and enzyme treated samples were served together to the panelists. An evaluation sheet is shown in Figure 3.3.

Sensory Evaluation For French Fries

Judge:_____ Date:_____,1987

1. Definitions

1) Flavor: the typical flavor of potato French fries

2) Texture: good texture means:

external surfaces: moderately crispy; no noticeble separation from the inner portion; not excessively oily

interior portions: well cooked; tender; practically free from sogginess

2. Instructions

Taste the samples and check the appropriate small boxes beside.
If you cannot decide, guess please.
Do not take the color into consideration.

3. Flavor

Which sample has a stronger flavor?

X☐ Y☐

4. Texture

Which, according to the definitions, has a better texture?

X☐ Y☐

Figure 3.2 Sensory evaluation sheet for French fries prepared with water blanching and steam blanching.

Sensory Evaluation of Potato Products

Judge_____ Date:_____, 1987

Sample type: potato chips ☐ French fries ☐

I. Instructions:

Taste the provided samples and check the appropriate box.

II. Question :

Did you detect any difference in flavor between the two samples?

YES ☐ NO ☐

If YES, please describe :

Figure 3.3 Sensory evaluation sheet for potato chips and French fries of enzyme treated and control samples.

4. RESULTS AND DISCUSSION

4.1 Reaction Properties of Two Commercial Preparations of Glucose Oxidase

4.1.1 Oxygen solubility in the system

In the calculation of the enzyme reaction velocity and activity, an unknown variable in Eq. 3.1 was S_T, the oxygen solubility in the solution at temperature T. As there were no available data for the phosphate-glucose-water system, the oxygen solubilities under various glucose concentrations and temperatures were calculated using Eq. 3.3, and are presented in Tables 4.1 and 4.2, respectively.

S_T^o values were taken from Stephen and Stephen (1963). D_{buffer} values were calculated from the graph given by Robinson and Cooper (1970), as shown in Figure 4.1. The k value was taken as 0.0156 from Zander (1976).

The oxygen solubility of a solution is affected by factors such as oxygen partial pressure above the solution (Henry's Law), temperature and the types and concentrations of solutes. As the experiments were carried out at atmospheric pressure of air, the partial pressure of oxygen was quite stable and exerted little effect on the oxygen solubility in different solutions. The oxygen solubility at different temperatures could be obtained from the previous data (Table 4.2 and Figure 4.1).

Table 4.1 Oxygen solubility in solutions of various glucose concentrations at 25°C, calculated using Eq. 3.3.

Glucose Concentration		S_T^{o} [1]	D_{buffer} [2]	S_T [3]
M	%	µL / mL	µL / mL	µL / mL
0.02	0.36	5.75	0.22	5.50
0.04	0.72			5.47
0.06	1.08			5.43
0.08	1.44			5.50
0.10	1.80			5.37
0.20	3.60			5.22
0.30	5.40			5.07
0.40	7.20			4.92
0.50	9.00			4.78

1 adapted from Stephen and Stephen, 1963

2 adapted from Robinson and Cooper, 1970

3 calculated using Eq. 3.3

Table 4.2 Oxygen solubility in solutions at various temperatures with glucose concentration at 0.3 M.

Temperature (^{o}C)	S_T^{o} [1] µL / mL	D_{buffer} [2] µL / mL	S_T [3] µL / mL
25	5.75	0.22	5.07
30	5.24	0.25	4.57
35	4.86	0.28	4.19
40	4.48	0.34	3.78

1 adapted from Stephen and Stephen, 1963

2 adapted from Robinson and Cooper, 1970

3 calculated using Eq. 3.3

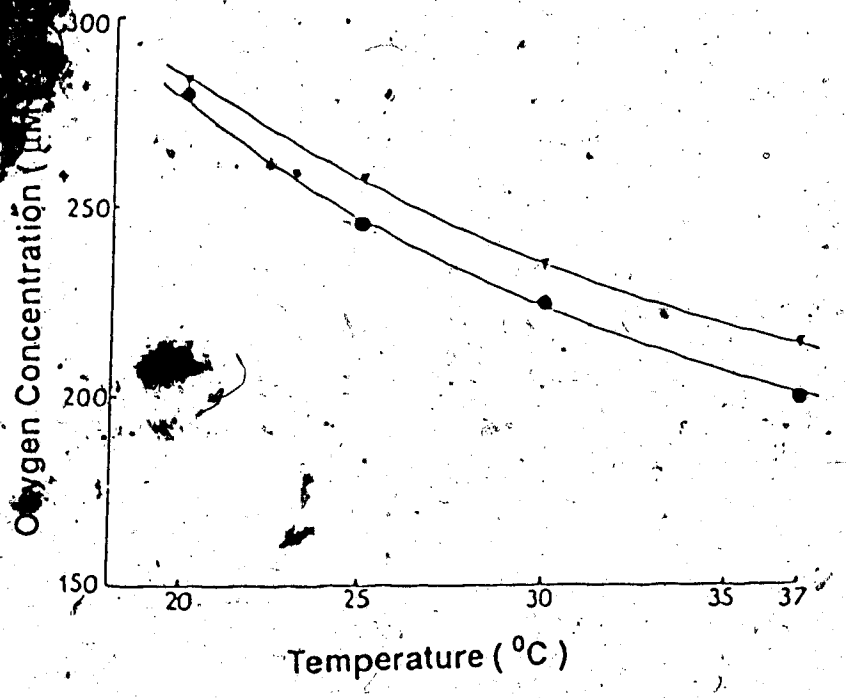


Figure 4.1 Oxygen solubility at different temperatures in pure water (▼) and in 0.1 M phosphate buffer (●) at pH 7.4 (adapted from Robinson and Cooper, 1970).

The oxygen solubility in the solution was also affected by glucose content and phosphate buffer. The increase of the solubility caused by 0.1 M phosphate buffer has been reported by Robinson and Cooper (1970) and their data were used in the present calculations as D_{buffer}. The effect of glucose on the solubility, however, could not be easily obtained from previous work.

The oxygen solubility in glucose-water system could be calculated from the Setschenow equation (Eq. 4.1) (Zander, 1976).

$$ST = ST^{o} \times e^{-kc} \qquad (4.1)$$

All variables in the equation are the same as in Eq. 3.3.

The constant k at 37°C was reported to be 0.0156 by Zander (1976). As k values were not available for other temperatures, this k value was used in calculating the oxygen solubility at other temperatures in the range of 25-40°C. The calculated oxygen solubility at different temperatures was compared with the reported data, as presented in Table 4.3.

At temperatures as low as 21.2°C, the oxygen solubilities calculated and reported were in good agreement. Therefore, by using k=0.0156, the oxygen solubility between 21.2 and 37°C could be calculated quite accurately.

As the salt effect of solutes in a solution contributed additively (Zander, 1976), the overall oxygen solubility of glucose-phosphate-water system could be calculated using Eq. 3.3.

Table 4.3 Oxygen solubility calculated from Setschenow equation (k=0.0156) and reported data.

Temp. (°C)	Glucose Conc. (%)	Calculated S_T (µL/mL)	Reported S_T (µL/mL)	Error from Reported (%)
21.2	10.89	25.70	26.50 [1]	± 3.1
21.5	20.70	21.92	22.02 [1]	± 0.1
37.0	10.00	20.62	20.60 [2]	± 0.1

1 adapted from IUPAC, 1979

2 adapted from Zander, 1976

The oxygen solubility in diluted aqueous solution of acids and bases was reported by Guseva *et al.* (1972) to be independent of pH in the range of pH 2-12 at 20-40°C. Therefore, the oxygen solubility calculated with Eq. 3.3 would also apply to solutions at various pH's.

4.1.2 Effect of glucose concentration on enzyme reaction velocity

The reaction velocities of the two commercial preparations of glucose oxidase at various concentrations are presented in Table 4.4 and Figure 4.2.

From the data, several basic properties of the two preparations of glucose oxidase, such as activity, minimum glucose concentration for activity assay, and the Michaelis-Menten constant could be obtained.

4.1.2.1 Michaelis-Menten constant

The Michaelis-Menten constant, K_m, is defined as "the concentration of the specific substrate at which a given enzyme yields one-half its maximum velocity" (Lehninger, 1982). It reflects the affinity characteristics of an enzyme for a specific substrate.

Several methods are available for calculation of K_m. The statistical method developed by Wilkinson (1961) was reported to be more accurate than the widely used graphic methods, such as the double-reciprocal Lineweaver plot. The K_m values of the two preparations calculated by the computer-assisted Wilkinson method are shown in Table 4.5.

Table 4.4. Enzyme reaction velocity (μmole/min) at various glucose concentrations.

Glucose Conc.(M)	Powder Enzyme		Liquid Enzyme	
	R (%)[1]	Velocity	R (%)[1]	Velocity
0.02	4.5	0.066	4.9	0.072
0.04	6.1	0.089	7.0	0.103
0.06	7.3	0.106	8.0	0.116
0.08	8.0	0.116	8.9	0.129
0.10	8.6	0.124	9.8	0.141
0.20	9.7	0.136	11.0	0.154
0.30	10.6	0.144	11.5	0.156
0.40	10.9	0.144	12.0	0.158
0.50	11.8	0.143	12.5	0.160

[1] reduction of oxygen partial pressure in 1 min.

average of triplicates with std. dev. ranging from 0.10% to 0.15 %

Table 4.5 The Michaelis constants for both enzyme preparations.

Enzyme Prepration	K_m (M Glucose)
Powder Enzyme	0.027
Liquid Enzyme	0.026

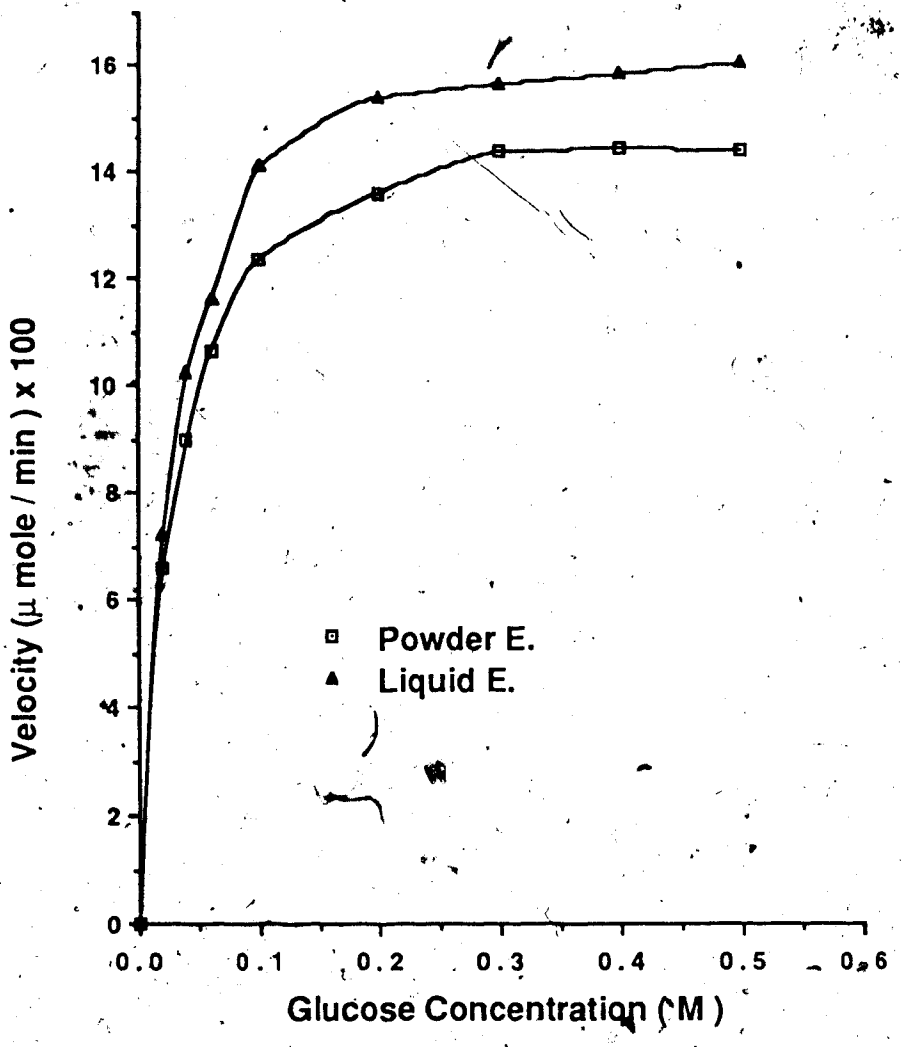


Figure 4.2 Effect of glucose concentration on [illegible]
at 25°C, pH 5.5.

These results corroborated the K_m value reported by Nakamura and Ogura (1962). The reported K_m values for glucose oxidase from *A. niger* vary from 0.02 M (Nakamura and Ogura, 1962) to 0.11 M (Gibson *et al.*, [illegible] although the conditions under which they were measured were almost the same. This difference might be attributed to the assay methods. Nakamura and Ogura (1962) used the polarographic method, while Gibson *et al.* (1964) employed the manometric method.

4.1.2.2 Minimum glucose concentration for activity assay

At low substrate concentration, the initial velocity of an enzyme reaction is directly proportional to the substrate concentration until the substrate concentration reaches 10 K_m or higher (Guilbault, 1984). Therefore, the activity assay should be carried out at substrate concentrations greater than 10 K_m.

Figure 4.2 shows that the reaction velocity of both powder and liquid enzymes increased sharply from 0 to about 12-14 x 10^{-2} μmole/min as glucose concentration increased from 0 to about 0.1 M, then slowly thereafter until glucose concentration reached 0.25 M and higher, when the velocity became almost constant. Therefore, the activity assay for glucose oxidase should be carried out with glucose concentration at 0.25 M or higher. In the present experiments, the enzyme activity was assayed with a glucose concentration of 0.3 M (5.4%).

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GLUCOSE OXIDASE PRETREATMENT TO MINIMIZE NONENZYMATIC BROWNING IN POTATO CHIPS AND FRENCH FRIES

by

ZHIRONG JIANG

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled **GLUCOSE OXIDASE PRETREATMENT TO MINIMIZE NONENZYMATIC BROWNING IN POTATO CHIPS AND FRENCH FRIES** submitted by **ZHIRONG JIANG** in partial fulfilment of the requirements for the degree of **Master of Science in Food Processing.**

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Supervisor

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Date.... June 15, 1987

Dedicated to

My Beloved Parents

ABSTRACT

The reaction properties of two commercial preparations of glucose oxidase, DeeO 1500 in powder form and Fercozyme CBB-750 in liquid form, were studied using a polarographic method. The experimental activities of both preparations were much higher than declared. The Michaelis constant was 0.27 M and 0.26 M glucose for powder and liquid enzymes, respectively. Temperature had no appreciable effect on the reaction rate in the range studied (25 to 40°C). Both enzyme preparations had a relatively broad effective range of pH, from 4 to 7, with optimal pH's around 5.0 and 5.5 for powder and liquid enzymes, respectively. The liquid enzyme had a superior thermal stability to the powder enzyme.

The two glucose oxidase preparations were applied to minimize the undesirable nonenzymatic browning in potato chips and French fries. Apparent improvement of lightness and uniformity of color of potato chips was achieved, with potatoes marginally suitable for chipping (glucose content ≈ 0.3%), by dipping potato slices in 0.05% (w/v) powder enzyme or 0.10% (v/v) liquid enzyme at ≤40°C for 30 min or longer. In the French fry process, the steam blanching (7 min under atmospheric pressure) followed by water leaching (≤35°C for up to 15 min) of potato strips retained more flavor in French fries than the recommended hot water blanching (85°C for 15 min). Dipping of steam-blanched potato strips in 0.2% (v/v) liquid enzyme or 0.1% (w/v) powder enzyme at ≤35°C for up to 15 min improved, to a limited extent, the lightness

and uniformity of the color of French fries when compared to the control (dipping in tap water).

The oil content of potato chips was increased by the dipping treatment, either in tap water or in enzyme solution. Steam blanching followed by dipping in water or enzyme solution produced French fries of lower oil content than when hot water blanching was used. The glucose oxidase treatment imparted no off-flavor to potato chips or French fries.

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1. INTRODUCTION

The color of some potato products, such as potato chips and French fries, is of primary importance in determining the acceptance of the products. The discoloration of these potato products could be caused both enzymatically and nonenzymatically. Enzymatic browning, which occurs in peeled or cut potatoes when exposed to air, results from the activity of polyphenol oxidase on some phenols to form dark polymers (Lerner and Fitzpatrick, 1950; Mondy *et al.*, 1960). Nonenzymatic browning, also known as the Maillard reaction, is "a group of chemical reactions involving the amino and carbonyl functions present in foodstuffs and leading to browning and flavor production" (Mauron, 1981). The discoloration in fried potato products can be attributed mainly to nonenzymatic browning.

As reducing sugars are the limiting reactants in nonenzymatic browning, potatoes of appropriate variety, maturity and post-harvest handling with low reducing sugar contents, are therefore chosen for making fried products. In some cases, potatoes of high sugar content from low temperature storage could be reconditioned by holding the tubers at room temperature in order to produce acceptable products. In normal practice, sodium bisulfite, alone or with phosphoric or citric acid, is employed to treat potato slices or strips to prevent undesirable discoloration during frying (Smith, 1975b). Sulfiting agents, such as sodium bisulfite, are capable of inhibiting both enzymatic and

nonenzymatic browning.

Despite the long history of wide applications of sulfites in foods, the safety of sulfiting agents in foods has recently been questioned on the basis of their role in the initiation of asthmatic reactions in certain sensitive individuals (Taylor *et al.*, 1986). A series of actions has been taken by the U.S. Food and Drug Administration (FDA) to limit the use of sulfites in foods. In 1986, the FDA lifted the GRAS (Generally Recognized As Safe) status of sulfites used in fresh vegetables and fruits (except potatoes) intended for consumption in raw state, particularly in salad bars. Another FDA regulation came into effect in 1987, requesting the declaration of sulfites when the residual sulfite content, as total SO_2, exceeds 10 ppm. The final action on the GRAS status of sulfiting agents for use on potato products has been scheduled for June 1987 (Semling, 1987).

The safety problem of sulfites and the regulations regarding their use created a practical necessity for new approaches to prevent undesirable browning in foods. Glucose oxidase enzyme could be an agent to replace sulfites. By oxidizing glucose, one of the reactants in the Maillard reaction, to the unreactive gluconic acid, the enzyme is able to block nonenzymatic browning. The glucose oxidase/catalase system has been used successfully to prevent the browning of dried egg powder (Baldwin *et al.*, 1953; Scott, 1953).

Although it was stated as early as 1953 that the application of glucose oxidase would "no doubt be made to other products such as dehydrated mashed potatoes" (Scott, 1953), the enzyme has never been reported to have been applied to such products. The recent limitation on the use of sulfite in foods has presented major problems to the production of some color-sensitive potato products, such as potato granules, potato chips and French fries. Therefore, it is logical that the effectiveness of glucose oxidase in preventing nonenzymatic browning in these products should now be investigated. In this project, the focus was on the prevention or minimization of browning in potato chips and French fries with glucose oxidase. A parallel study was also performed on potato granules by other researchers in this laboratory.

The objectives of the present research were:

1. to study the chemical and kinetic properties of the commercial glucose oxidase preparations;

2. to establish the optimum conditions for glucose oxidase treatment of potatoes;

3. to determine the effect of glucose oxidase treatment on the color and general acceptability of potato chips and French fries.

2. LITERATURE REVIEW

2.1 Potato Production, Consumption and Processing

The white potato (*Solanum tuberosum*, L.) is one of the few crops capable of nourishing a significant portion of the world population. Despite the tremendous increase in quantities and varieties of food sources, the annual per-capita consumption of the potato in the United States and Canada has remained quite constant (Table 2.1). However, the consumption pattern of potatoes has been changing. In the United States, the consumption of fresh potatoes decreased from 1960 to 1980, while the consumption of processed potatoes almost tripled in the same period. Potato chips and French fries are the two major potato products, accounting for almost two-thirds of the processed potatoes (Figure 2.1).

2.2 Potato Chips

The production of potato chips in the United States has increased slowly but constantly (Figure 2.1). Potato chips (crisps) are the most important processed potato products in the United Kingdom (Smith and Davis, 1977). Because of convenience in serving and variety in flavoring, potato chips are expected to remain popular.

Table 2.1 Annual per-capita consumption of potatoes in U.S.A. and in Canada.

U. S. A.[1]		Canada[2]	
Year	Consumption (kg)	Year	Consumption (kg)
1945	55.39	1978	72.54
1950	48.12	1979	78.77
1955	49.49	1980	70.87
1960	49.03	1981	64.79
1965	49.03	1982	66.66
1970	53.57	1983	72.08
1975	54.93	1984	60.69
1980	53.57	1985	67.68

1: U.S.D.A., Agricultural Statistcs, 1972; 1985

2: Statistics Canada, 1979-1985

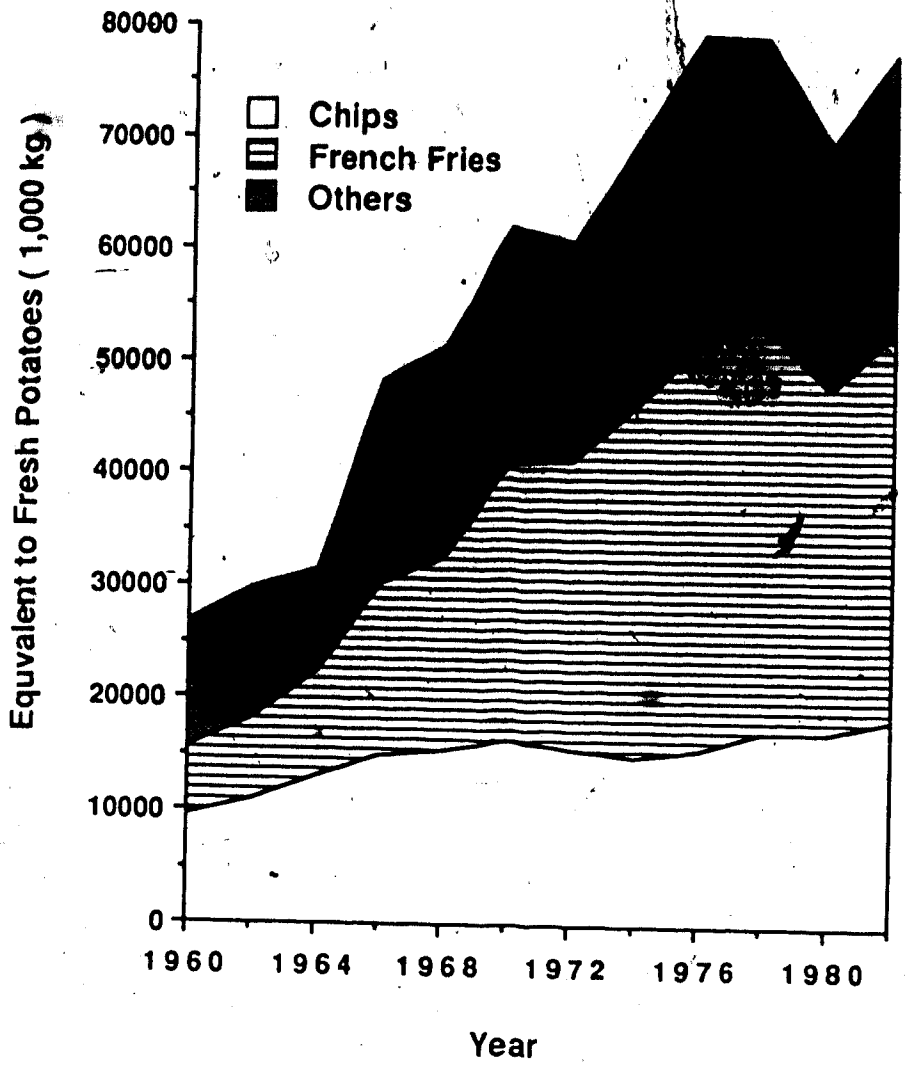


Figure 2.1 U.S.A. production of potato chips (including shoestrings), frozen French fries, and others (including dehydrated potatoes, other frozen products, canned potatoes and potatoes for starch and flour).

2.2.1 Processing procedures

The most commonly practised processing procedures for potato chips were described by Smith (1975b). After washing and peeling, potato tubers are sliced to a thickness of 1/15 to 1/30 in (1.70 to 0.85 mm). The slices are then washed in cold water to remove surface starch to prevent adhesion of the slices during frying. Treatment to improve the color of potato chips can be carried out at this stage. The slices are then partially dried and fried in oils at 350-375°F (177-191°C) until bubbling stops. The chips are now ready for salting and flavoring, inspection and packaging. A flow chart for potato chip processing is presented in Figure 2.2.

2.2.2 Quality of potato chips

The quality of potato chips can be evaluated from their physical appearance and their flavor. The most important factor in determining the quality of potato chips is the color of the product. The desired product has a uniform, light-yellow color.

2.3 French Fries

French fries, in a partially-fried frozen form, have become the most popular processed potato products in North America. In the United States, the production of French fries in 1981 was almost six times that of 1960 (USDA, 1966-1985). About half of the processed potatoes since 1972 was in the form of frozen French fries.

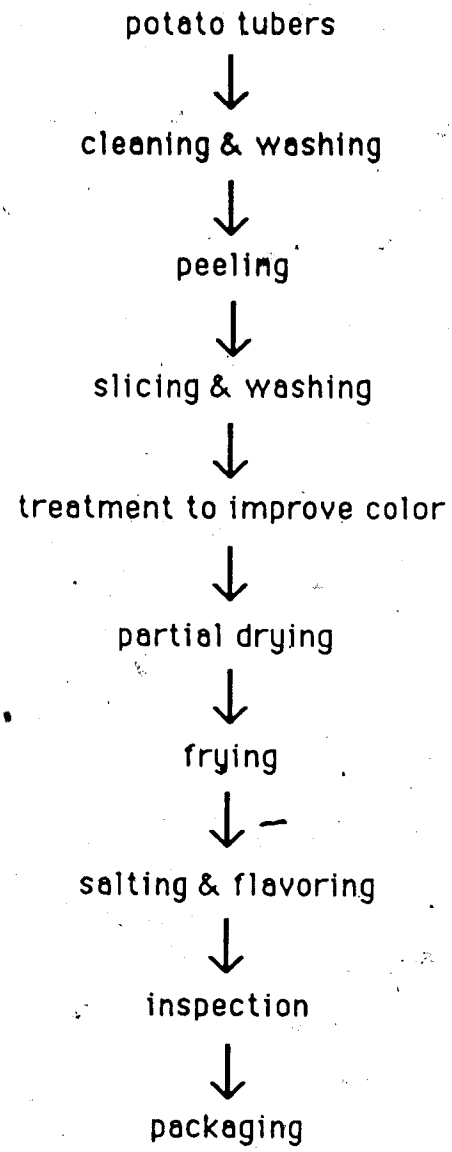


Figure 2.2 The processing procedures of potato chips.

2.3.1 Processing procedures of French fries

French fries can be produced either by finish frying potato strips or by partial frying the strips for a short time before freezing, producing the so-called par-fried frozen French fries. The general processing procedures for frozen French fries are presented in Figure 2.3. After washing and peeling, the potato tubers are sliced to generate strips, usually 1/4 or 3/8 in² in cross section. The strips are washed in cold water to remove surface starch. Blanching, using various methods, is then performed, followed by treatment, if any, to improve the quality of the final product. The strips are then partially dried and partially fried in oils at about 180°C for a short period of time, usually 30-90 sec. The partially fried potato strips are quickly frozen at -30°C and kept frozen until serving (Weaver *et al.*, 1975). The frozen French fries can be prepared for serving by finish frying in hot oil, by conventional oven heating, by microwave heating (Weaver *et al.*, 1975; Bushway *et al.*, 1984), or by infrared heating (Mohr *et al.*, 1960). In some restaurants, the potato strips, blanched or unblanched, are fried until they are ready for serving (Martino, 1969).

Novel methods of producing extruded French fries from freshly cooked and mashed potatoes (Weaver *et al.*, 1974) or from dehydrated potato granules (Jadhav *et al.*, 1976) have also been described.

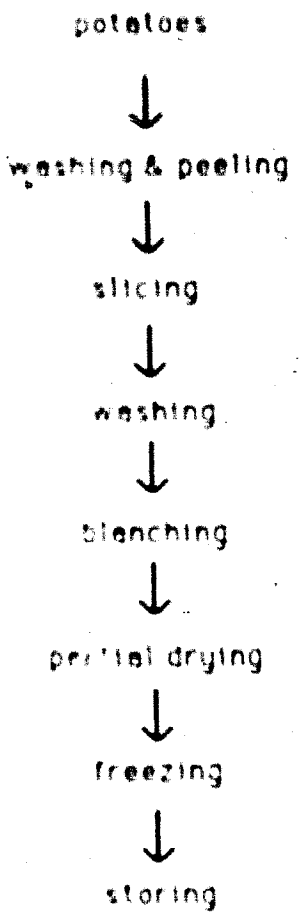


Figure 2 Common processing procedures for frozen French fries

2.3.2 Blanching methods for French fries

Potato strips are usually blanched before frying. The advantages of blanching are: (1) reduction of fat adsorption through gelatinization of the surface layer of starch; (2) shortened frying time since potato strips are partially cooked by blanching; (3) inactivation of certain enzymes so that undesirable flavor and color are avoided during storage and subsequent handling; (4) improvement of the texture and flavor of the final product; and (5) removal of certain solutes, in water blanching, to prevent excess browning (Weaver *et al.*, 1975; Brown and Morales, 1970).

Several blanching methods are practised by the French fry industry, including hot water, hot air, steam and oil blanching. The most commonly practised method is hot water blanching. The temperature and time may vary from 66-88°C and 3-15 min, respectively (Vahlsing, 1965; Van Patten and Freck, 1973), with the optimum conditions recommended at 80°C and 15 min (Brown and Morales, 1970). Two or even more blanchers are commonly operated in series for greater flexibility and more effective control of color of the products (Weaver *et al.*, 1975). The first blancher can be used for hot water leaching, while the second may contain a dilute glucose solution to produce the desired golden brown color during frying. In some processing plants, more stages are used. The strips are blanched at 82°C (180°F) for 8 min, cooled in water at 45-50°C for 6 min, blanched at the same temperature for 8 min, and dipped in a dilute glucose

solution at 180°F for a short time (≃10 sec) before frying (Iwabuchi, 1986). However, the hot water blanching process can also result in the loss of desirable flavor constituents from potato strips, and serious impairment of texture and flavor is often encountered. In addition, the method has been reported to not always be effective in avoiding darkening (Weaver and Nonaka, 1976).

A technique, referred to as dry blanching, is also employed by the industry. It uses hot air to heat the potato strips to achieve blanching effect, and the product is reported to be more crisp and rigid (Weaver *et al.*, 1975).

Steam blanching is preferred in some processing procedures (Strong, 1968; Wilder, 1972). Potato strips are blanched at atmospheric pressure for 2-10 min, preferably about 7 min. This process, with no solutes removed from the potato strips, avoids the possible loss of flavor components encountered in hot water blanching.

Recently, a new process using hot oil to blanch starchy vegetables, such as potatoes, has been reported (Fan and Arce, 1985). The potato cuts are dipped in a hot edible vegetable oil at 50-60°C for a short time before frying. The claimed advantages include less oil absorption, less potato solids loss, and improved flavor and texture when compared with that of water-blanched products.

2.3.3 Quality of French fries

The quality of frozen French fries can be evaluated according to the following scheme (Weaver *et al.*, 1975):

Factors	Maximum Score
Color	30
Uniformity of size and symmetry	20
Defects	20
Texture	30

Among the listed factors, the color of the French fries is the most sensitive and important parameter. The desired gold-brown colored French fries can be made from potatoes of proper variety, maturity and post-harvest handling. However, the discoloration of French fries is the most troublesome problem that often confronts processors.

2.4 Browning Problems in Potato Products

2.4.1 Types and occurrence of browning

There are three distinct types of discoloration that are associated with the browning of potato products (Smith and Davis, 1977): (1) enzymatic discoloration; (2) after-cooking darkening; and (3) nonenzymatic browning, also known as the Maillard reaction. The enzymatic browning which occurs in peeled or cut potatoes when exposed to air results from the activity of polyphenol oxidase on some phenols to form dark polymers (Lerner and Fitzpatrick, 1950; Mondy *et*

al., 1960). The cause of after-cooking darkening of cooked potatoes is generally believed to be the reaction of certain ortho-diphenols, such as chlorogenic acid, with the ferrous ions of potato tubers, forming a colorless complex, which is oxidized to yield a deeply-colored ferric compound (Hughes *et al.*, 1962; Hughes and Swain, 1962). More recently, ascorbic acid-Fe^{3+} complex was suggested as another cause of after-cooking darkening of potatoes (Muneta and Kaisaki, 1985). However, the above two types of browning are not important in potato chips and French fries. The discoloration of potato chips and French fries is attributed mainly to the nonenzymatic browning reaction.

2.4.2 Nonenzymatic browning

Nonenzymatic browning, also known as the Maillard reaction, is "a group of chemical reactions involving the amino and carbonyl functions present in foodstuffs and leading to browning and flavor production" (Mauron, 1981). The first coherent scheme of the Maillard reaction was put forward by Hodge (1953) in a comprehensive review. The Maillard reaction has also been the subject of several other reviews (Reynolds, 1963, 1965; Mauron, 1981; Kawamura, 1983; Danehy, 1986).

The complicated Maillard reaction can be divided into three stages: early, advanced and final reactions (Hodge, 1953; Mauron, 1981). The early stage involves a simple condensation process between the carbonyl group of reducing

sugars and the free amino group of the amino acids or proteins, followed by the Amadori rearrangement, a step which isomerizes an aldose derivative to a keto derivative. The advanced Maillard reaction, starting from the Amadori compounds, generates flavor components which are responsible for the roasted, bready and nutty flavor of heated foods. The final Maillard reaction produces the brown melanoidin pigments formed by polymerization of many highly reactive compounds generated during the advanced stage. More recently, Namiki and Hayashi (1983) reported a new pathway which involves the cleavage of sugar molecules to generate highly active two-carbon fragments prior to the Amadori rearrangement.

Among the parameters that affect the rate of the Maillard reaction, temperature and water conte are by far the most important. The Q_{10} value of the browning reaction in dehydrated vegetables was reported to be 5-8.4 (Legault *et al.*, 1947), which was exceptionally high. The browning rate is near zero in the anhydrous state. It reaches a maximum at about 30% water content, and then decreases gradually as the moisture increases. The reaction stops at about 90% water content (Wolfrom and Rooney, 1953).

2.4.3 Browning of potato chips and French fries

The browning of potato chips and French fries had been associated with sugar content of potato tubers no later than 1930. Sweetman (1930) reported the correlation between the

brown color of potato chips and the high sugar content of tubers. He concluded that the formation of brown color was due to the caramelization of sugars at high temperature. Later, Thornton (1940) pointed out that the dark brown color of potato chips was caused by high reducing sugar, not by sucrose or total sugar content of potato tubers. Several years later, however, the browning of chips and other potato products was attributed, at least in part, to the Maillard reaction between reducing sugars and amino acids (Legault *et al.*, 1947). Fitzpatrick and coworkers (Fitzpatrick *et al.*, 1965; Fitzpatrick and Porter, 1966) were able to substantiate the above theory by tracing the changes in reducing sugars and amino acids during chip frying. The ratio of the consumption of free amino acid nitrogen to reducing sugars was 9:1 in low reducing sugar and 1:1.35 in high reducing sugar potato chips. The browning of French fries was also believed to be caused by the Maillard reaction between reducing sugars and amino acids.

2.4.4 Potato composition and browning

Although the discoloration of potato chips and French fries can be mainly attributed to the Maillard reaction, the relationships between the color of the products and the chemical components, such as sucrose, glucose and fructose, amino acids and dry matter, are very complicated. Wunsch and Schaller (1972) reported that the discoloration was essentially dependent on four groups of components: total

amino acids, glucose, fructose, and tyrosine and proline. Yada *et al.* (1985) found that approximately 60% of the variability seen in chip color could be accounted for by examining the fructose, glucose and sucrose content of potato tubers.

Habib and Brown (1956, 1957) obtained the correlation coefficients for both reducing sugars and amino acids with potato chip color. Both coefficients were significant at the 1% level. They also showed that there was a positive correlation between nonreducing sugar (sucrose) content and the lightness of potato chip color. However, Shallenberger *et al.* (1959) demonstrated that chip color was a function of both reducing sugar and sucrose contents of tubers. They postulated that sucrose must first be hydrolyzed, during frying, to participate in the nonenzymatic discoloration of chips.

Although both reducing sugars and amino acids participate in the Maillard reaction, amino acids and other nonprotein nitrogenous compounds do not correlate well with chip color (Miller, 1972). The limiting factor of the browning reaction was proposed to be the reducing sugar content (Marquez and Anon, 1986). Miller (1972) reported that glucose was the major determinant of chip color, while fructose appeared to be unimportant in browning. Sucrose had a minor, but significant role in color development, probably as a result of hydrolysis during frying. Miller *et al.* (1975) were able to substantiate those results.

2.4.5 Factors affecting the color of the products

The color of potato chips and French fries is determined by the chemical composition of potato tubers which, in turn, is affected by many factors during potato cultivation and post-harvest handling.

The cultivation conditions related to the color of potato chips and French fries include variety and maturity of potatoes, irrigation and soil moisture, and application of fertilizers and some other chemicals. Some well known varieties that usually produce chips of good to excellent color are Monona, Kennebec, Norchip, Russet Rural, Superior and Katahdin (Smith, 1975b). Several new good chipping varieties released in recent years are Rosa (Plaisted *et al.*, 1981), Simcoe (Johnson and Rowberry, 1982), Yankee Chipper (Reeves *et al.*, 1984a) and Islander (Reeves *et al.*, 1984b). Varieties which produce light-colored potato chips were found to contain the lowest amount of reducing sugars (Shallenberger, 1955).

Maturity of potato tubers when harvested is another well-known factor affecting the color of potato products. The more mature the potatoes, the easier they can be handled, stored and reconditioned to produce light-colored chips (Smith, 1975b). Shallenberger (1955) found that more mature tubers produced light-colored chips not only on the day of harvest, but also after storage at different temperatures. The reducing sugar content was found, in general, to be higher in immature tubers than in more mature

ones.

Several other cultural conditions during the growing season may also affect the color of potato products. Kushman *et al.* (1959) reported that the darker color of chips made from potatoes grown under high soil moisture content was always apparent after storage at 60°F (15.6°C), and sometimes also appeared immediately after harvest. A heavy application of nitrogen during growing was often regarded as a factor that makes stored potatoes more likely to produce dark chips (Smith, 1975a). However, Kunkel and Holstad (1972), after an extensive 11-year study, reported that potato chip color was almost unaffected by nutritional balance or total amount of fertilizers used. Whenever the soil temperature at a depth of 4 inches goes below 4.4°C (40°F) for several days or nights, the potatoes subsequently harvested will produce dark chips (Smith, 1959b).

The effect of transit and storage conditions of tubers on the color of potato chips can not be overemphasized. The accumulation of sugars during low temperature storage of potatoes was reported as long ago as 1882 (Smith, 1975a). Early work by Sweetman (1930) showed that chips made from tubers stored at 32-37°F (0-2.8°C) were darker than those made from tubers stored at 40-55°F (4.4-12.8°C). Habib and Brown (1957) found that glucose content of potatoes was almost doubled after storage at 40°F (4.4°C) for 4 weeks, while the fructose and sucrose contents did not change significantly. Ewing *et al.* (1981) reported that glucose and

fructose content showed a similar increase in potatoes exposed to 1°C for 4 days or longer, except that the glucose level was almost always much higher, roughly twice that of fructose.

The sugars accumulated during low temperature storage of potatoes disappear when the tubers are held at temperatures of 18°C or higher for 1-3 weeks (Weaver *et al.*, 1975). This process, referred to as conditioning or reconditioning, has been practised extensively in the potato processing industry to produce properly colored chips and French fries from potatoes stored at low temperature.

However, not all potatoes respond to cold storage and warm reconditioning to the same extent. Potato varieties that accumulate less sugars in cold storage and respond more readily in warm conditioning should be selected for processing of potato chips and French fries. Also, more mature potatoes accumulate less sugars during storage and respond to reconditioning more readily (Shallenberger, 1955; Burton, 1965).

The change of potato sugar content has been associated with the activities of certain enzymes, such as invertase, phosphohexose isomerase and aldolase. The accumulation of reducing sugars was found to occur with concomitant formation of the enzyme invertase (Pressey and Shaw, 1966). During the initial period of cold treatment, when reducing sugars increased rapidly, invertase formation proceeded until the level of enzyme exceeded that of a proteinaceous

invertase inhibitor, resulting in a basal invertase activity. On transfer of cold stored potatoes to warmer temperatures, sugar content and invertase level decreased sharply, and a large excess of inhibitor developed.

Pressey (1969) pointed out that invertase participated in reducing sugar formation, but other factors were responsible for the regulation of starch-sugar conversion in potatoes during storage. A possible sequence of events in cold stored potatoes was proposed by Tishel and Mazelis (1966). The low temperature appeared to induce in the tubers (1) a temporary decrease in aldolase activity, (2) an increase in sucrose, (3) increased invertase activity, (4) accumulation of reducing sugars, and (5) a lowering of the activity of phosphohexose isomerase.

2.5 Various Methods of Improving the Color of Potato Chips and French Fries

It may often be almost impossible to make potato chips and French fries of acceptable color without some treatments of raw potatoes or potato cuts. The following are some of the treatments that have been used or tried.

2.5.1 Reconditioning of potatoes stored at low temperature

A common practice to improve the color of potato chips and French fries from low-temperature stored potatoes is the reconditioning process. Potatoes are removed from low temperature storage and held at room temperature (18°C or

higher) for 1-3 weeks before processing. However, as stated previously, some varieties do not respond readily to conditioning and, even in potato varieties that normally respond well to reconditioning, disorders such as sugar end can sometimes occur (Weaver *et al.*, 1975). In addition, reconditioning is expensive as it involves extra handling costs, ties up large quantities of potatoes, and requires large storage space and facilities for the treatment (Weaver and Nonaka, 1976). Nonetheless, the technique is widely used in industry.

2.5.2 Leaching techniques

Various leaching techniques have been tested to prevent the discoloration in potato chips and French fries by removing the solutes from the potato cuts.

Light-colored potato chips could be made from cold stored potatoes, without reconditioning, by dipping the slices in hot water at 153-163°F (67.2-72.8°C) for 6-7 minutes before frying (Townsley, 1952). Dexter and Salunkhe (1952a) reported that immersion in water at 70°C for 1.5 minutes, followed by soaking in cold water for 15 minutes, produced chips of excellent color.

Hot water blanching is a powerful leaching process for French fry production. The leaching of reactants by hot water blanching is so extensive that glucose has to be added back to produce the proper light golden color typical of French fries. However, the drastic water treatments often

remove all flavor, in addition to their undesired effects of extracting nutrients and giving rise to waste water disposal problems (Weaver *et al.*, 1974).

Some surface treatments of potato cuts to accelerate the leaching process have been reported. Weaver and Hautala (1972) patented a process for making French fries, wherein potato strips were dipped in a liquid refrigerant, such as liquid nitrogen or dichlorodifluoromethane, for a short period (about 7 seconds), followed by leaching in hot water at 50-60°C for 1-5 minutes. Another process, patented by Weaver and Nonaka (1976), described the production of fried potato products of improved texture, flavor and color from raw stock that exhibits excessive browning tendencies. Typically, potato cuts were prefried in edible oil for a short period, then leached with water. The treatment was reported to be applicable to both French fries and potato chips.

2.5.3 Microwave heating and other processing methods

The Maillard reaction is usually accelerated at a later stage in frying of potato products, when moisture content is relatively low and the temperature of potato cuts increases, as less water is evaporated (Sijbring and Van de Velde, 1969). To avoid excessive discoloration of chips from potatoes with high reducing sugar content, the slices can be partially fried and then subjected to other methods of drying, such as hot air drying at 250°F (121.1°C) (Smith,

[illegible] infrared heating (Smith, 1975b), vacuum frying [illegible]ng and Van de Velde, 1969), and microwave heating [illegible] Fitzpatrick and Porter, 1968). The preparation [illegible] French fries suitable for microwave heating has also been [illegible]ted (Buckley et al., 1984).

[illegible] Chemical treatments of potatoes and potato cuts

Various aqueous solutions, such as ether, alcohol, salt and acid were tested by Dexter and Salunkhe (1952b) to [illegible]ly remove reducing sugars from potato slices. [illegible] 0.1% phosphoric or hydrochloric acid at pH 1.9 for [illegible] minutes followed by washing in water for 3.5 minutes, produced chips of excellent color. Patton (1948) patented a process of dipping slices for various lengths of time in hot aqueous solutions of alkaline earth salts ($CaCl_2$, $MgCl_2$, [illegible] sulfamate of concentrations from 0.005-0.1 M before frying. Simon et al. (1955) reported that retardation of non-enzymatic browning in dehydrated white potatoes by [illegible] with calcium chloride.

Sodium bisulfite solution has been used to treat potato slices from low stores of high reducing sugars (Smith, 1972). Attractive, light colored chips could be produced by dipping the slices for 1 minute either in 0.25% sodium bisulfite at 180-200°F (82.2-93.3°C), or in a solution of sodium citrate, sodium bisulfite and phosphoric acid held at [illegible]°F ([illegible]-82.2°C). Xander (1952) claimed that uniformly colored and flavored chips could be produced year

round, regardless of the variety used or the reducing sugar content, if slices were immersed in an SO_2 bath and then washed with water to remove the residual SO_2 and reducing sugars.

Whole potato tubers were also treated with SO_2 gas (Smith, 1959a). The treated potatoes resulted in chips of lighter color for tubers stored as long as three months at 4.4°C (40°F) than untreated tubers.

2.6 Sulfites in Foods: Uses and Safety Problems

2.6.1 Applications of sulfites in foods

Sulfiting agents, in various forms, alone or with phosphoric acid, sodium citrate or citric acid, have been employed in industry to treat potatoes and potato cuts to prevent nonenzymatic discoloration during frying (Smith, 1975b). As a group of versatile food additives, sulfiting agents have found wide applications in various foods for different technical purposes, such as inhibition of nonenzymatic and enzymatic browning, inhibition and control of microorganisms, and as reducing agents and antioxidants. They have been widely used to prevent enzymatic browning in peeled or sliced vegetables and fruits such as prepeeled potatoes, sliced potatoes, cut apples and cut lettuce (Taylor *et al.*, 1986). The mechanism of sulfite action is not clear, but sulfites appear to both inactivate enzyme phenolase and interrupt the subsequent reactions leading to

colored products (Haisman, 1974). The sulfites play crucial roles in the inhibition and control of microorganisms in several food processes, such as in preserving soft fruits, in jam making, in sausage production, and in wine making (Roberts and McWeeny, 1972). As an antioxidant, sulfur dioxide has been shown to be effective in preventing loss of ascorbic acid during processing and storage of products such as dehydrated cabbage, fruit cordials and grape juice. It has also been shown to be a reasonably effective antioxidant for protection of lipids in lipid-protein-water emulsion, of essential oils and carotenoids in citrus juices (Roberts and McWeeny, 1972). Sulfites are also widely used as dough conditioners in the baking industry for biscuits, crackers, cookies and frozen pizza doughs, where they act as reducing agents (Taylor *et al.*, 1986).

Sulfites find wide use as inhibitors of nonenzymatic browning. They have been used for this purpose to control discoloration of dehydrated potatoes, vegetables and fruits, white grape juice and other fruit juices and drinks (Taylor *et al.*, 1986). Detailed mechanisms by which the sulfites exert their anti-browning action in foods are still largely unknown. It was proposed by McWeeny *et al.* (1974) that sulfites exhibited their anti-browning action by their ability to react with various carbonyl intermediates formed during the nonenzymatic browning reaction. The theory is illustrated in Figure 2.4.

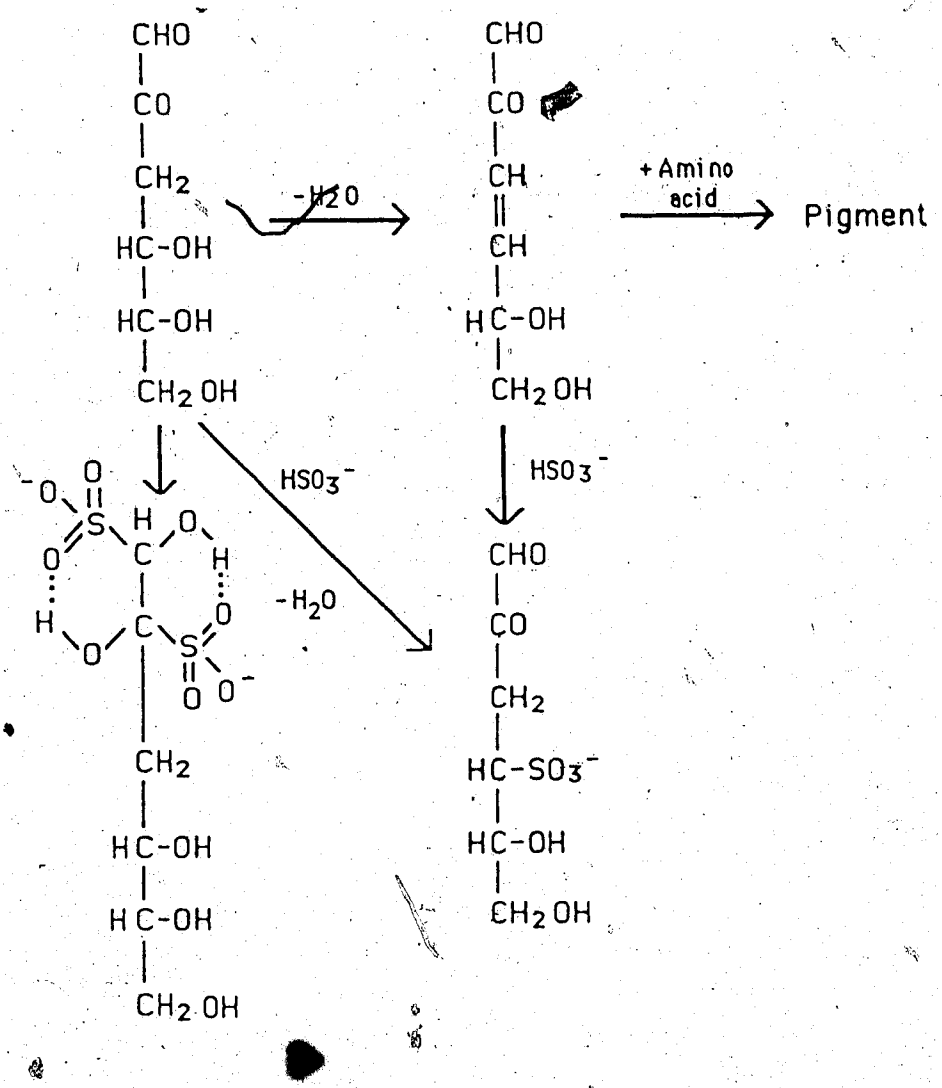


Figure 2.4 Reactions of sulfites with carbonyl intermediates (adapted from McWeeny *et al.*, 1974).

2.6.2 Safety problems of sulfites in foods

Recently, the safety of sulfites in foods has been questioned based mainly on their alleged role in the initiation of asthmatic reaction in certain sensitive individuals (Taylor *et al.*, 1986). The first reports about sulfite-induced asthma were by Kochen (1976) and Freedman (1977). The simultaneous reports from Allen and Collett (1981) and Stevenson and Simon (1981) initiated a great deal of research thereafter. Various types of adverse reactions have been observed, such as anaphylactic shock, hypotension, headaches, abdominal pain, nausea, dizziness, hives, urticaria and pruritis, eczema, angioedema, laryngel edema, tachycardia, clammy skin, and dyspnea (Sullivan and Smith, 1985; Taylor *et al.*, 1986). However, the most threatening effect of sulfites in foods is their ability to induce asthma in certain asthmatics. The asthma reaction in some sulfite-sensitive individuals can be sufficiently severe to be life-threatening. According to Sullivan and Smith (1985), of more than 300 reports of adverse reactions to sulfites that the FDA (Food and Drug Administration, U.S.A.) received, 6 involved deaths. The American Academy of Allergy and Immunology estimated that up to 17 fatalities have been attributed to sulfite ingestion in susceptible asthmatics (Bush, 1986).

Adverse reactions to sulfites are extremely rare in normal individuals (Bush, 1986). Even among asthmatics, only a small fraction (4.6-8%) is sensitive to the ingestion of

sulfites (Taylor and Bush, 1986). However, the safety problem of sulfites has received much attention from organizations such as the FDA.

Since 1959, the FDA has listed the following six sulfiting agents as GRAS (Generally Recognized As Safe): sulfur dioxide, sodium sulfite, sodium and potassium bisulfite, and sodium and potassium metabisulfite (Sullivan and Smith, 1985). On August 14, 1985, the FDA proposed a regulation to rescind the GRAS status for sulfites used on fruits and vegetables (except potatoes) which were intended for consumption in the raw state, particularly in salad bars. This proposal became effective on August 8, 1986 (FDA, 1986). The final action on the GRAS status of sulfiting agents for use in potato products has been scheduled for June, 1987 (Semling, 1987).

A labelling regulation was also proposed by the FDA on April 3, 1985, requiring the declaration of sulfites on the label when the residual sulfite content, as total SO_2, exceeds 10 ppm (FDA, 1985). The final action of the FDA (1986) requested that this labelling rule become effective on January 9, 1987.

A troublesome point in carrying out the labelling rule might be the unsuitability of the present analytical methodology for sulfites. The AOAC method, i.e. the Monier-Williams method, was reported not only to be very time consuming but also inaccurate at a sulfite level below 60 ppm (Sullivan and Smith, 1985; Moylan *et al.*, 1986). Upon

recognizing this unsuitability, the FDA has made some refinements to the Monier-Williams method, which include using a more dilute titrant and a minor modification to the apparatus. The modified method was reported to give suitable accuracy and reproducibility at the 10 ppm level (FDA, 1986). A recently developed ion chromatographic (IC) method for sulfite determination was reported to be both rapid and accurate at a sulfite level below 10 ppm (Sullivan and Smith, 1985; Moylan *et al.*, 1986).

The regulations regarding sulfiting agents will no doubt drive food scientists and manufacturers to seek substitutes. However, according to Taylor *et al.* (1986), it is virtually impossible to find a complete substitute for sulfiting agents. The potential substitutes are also stated to be less effective and more costly in most cases. The problems also lie in the fact that no other single preservative is as versatile as sulfites in its preservative functions. For example, one may be effective against enzymatic browning, but not nonenzymatic browning or microorganisms, or vice versa. Therefore, several types of preservatives must be found to replace sulfites in their various functions. To prevent nonenzymatic browning, for instance, ascorbic and citric acids have been used with some success. However, they are not as effective as sulfites and are more expensive. Other methods or substitutes still need to be found. One of these methods may be the use of glucose oxidase to reduce the concentration of glucose, one of the

major reactants in browning reactions.

2.7 Glucose Oxidase

2.7.1 General information

Glucose oxidase (EC 1.1.3.4, β-D-glucose : oxygen 1-oxidoreductase) is a flavoprotein which carries out the overall reaction of Equation 2.1:

$$C_6H_{12}O_6 + O_2 + H_2O \rightarrow C_6H_{12}O_7 + H_2O_2 \qquad (2.1)$$

The above conversion of glucose to gluconic acid in bacteria was first observed in 1878 by Boutroux with *Acetobacter aceti* (Bentley, 1963). Beginning in 1928, Muller made an extensive study on glucose oxidase from both *Aspergillus niger* and *Penicillium glaucum*, therefore it was generally believed that the glucose oxidase enzyme was discovered by Muller (Nakamura and Ogura, 1968; Whitaker, 1985).

Glucose oxidase has also been demonstrated in other fungi, including *Aspergillus oryzae*, *Penicillium notatum*, *P. amagasakiense* and *P. vital* (Scott, 1975a). It has not been found in higher plants or in animals (Whitaker, 1985).

2.7.2 Preparation of glucose oxidase

According to Scott (1975a) and Schwimmer (1981), glucose oxidase is prepared from the preferred fungal sources in different countries. The enzyme is prepared from *Aspergillus niger* in the United States, and from *Penicillium vital* in the U.S.S.R. In Japan, the preferred organism is *P.*

amagasaklense, with only a small production from *A. niger*. The enzyme from *A. niger* is considered to be an intracellular enzyme which can be obtained from the mycelium of the organisms. The enzyme from *Penicillium* species is purified from the fermentation medium, therefore it is considered as an extracellular enzyme (Scott, 1975a).

Two types of commercial enzyme preparations are available. One is a clear amber solution, stable under refrigeration for several years and at room temperature for many months (Scott, 1975a). Another is a light tan, amorphous dry powder. The liquid preparation is claimed to have a better solubility, more convenience in applying, low cost and greater stability, when compared to the powder form (Scott, 1975a; Fermco Biochemics, a).

The commercial preparations of glucose oxidase contain not only glucose oxidase enzyme, but also catalase (EC 1.11.1.6; hydrogen-peroxide : hydrogen peroxide oxidoreductase) (Scott, 1975a; Fermco Biochemics, a; Miles Lab., 1985). Catalase catalyzes the decomposition of hydrogen peroxide (Equation 2.2):

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2 \qquad (2.2)$$

Catalase can be derived from animal livers, plants and microorganisms (Whitaker, 1985). The catalase in commercial glucose oxidase preparation can be obtained from *Aspergillus niger* (Miles Lab., 1985). Therefore, the overall reaction of commercial glucose oxidase-catalase preparation can be presented in Equation 2.3:

$$2C_6H_{12}O_6 + O_2 \rightarrow 2C_6H_{12}O_7 \qquad (2.3)$$

Glucose Gluconic acid

2.7.3 Mechanisms and molecular properties

2.7.3.1 Mechanisms

Two important papers on kinetic mechanisms of glucose oxidase were published by Nakamura and Oguar (1962), using enzymes from *P. amagaskinense*, and by Gibson *et al.* (1964), with enzymes from *A. niger*. A general scheme which could accommodate the reactions of glucose oxidase with all kinds of substrates (sugars) was proposed by Gibson *et al.* (1964), and is presented in Scheme I.

$$E_o + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E_o\text{-}S \xrightarrow{k_2} E_r P_1 \xrightarrow{k_3} E_r + P_1$$

$$E_r + O_2 \xrightarrow{k_4} E_o\text{-}H_2O_2 \xrightarrow{k_5} E_o + H_2O_2$$

Scheme I

where, E_o = enzyme in oxidized form; E_r = enzyme in reduced form; S = substrate; P_1 = oxidized substrates, i.e., δ-lactones.

When β-D-glucose was used as the substrate, the above general scheme was reduced to Scheme II (Gibson *et al.*, 1964):

$$E_o + G \xrightarrow{k_1} E_r\text{-}P_1 \xrightarrow{k_2} E_r + H_2O_2$$

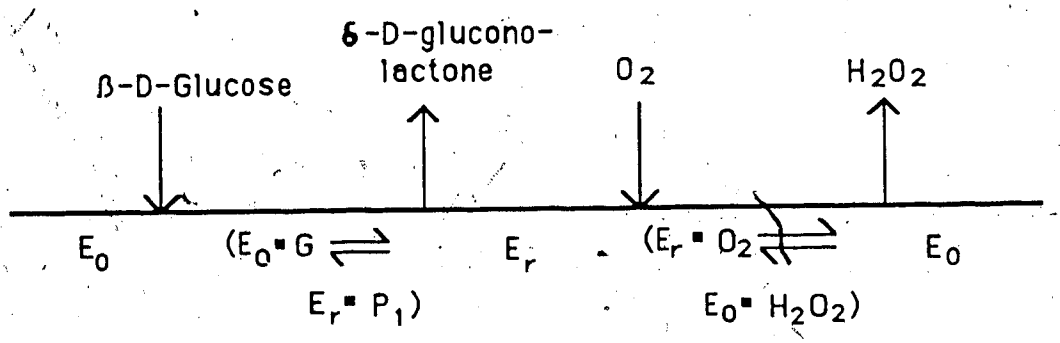
$$E_r + O_2 \xrightarrow{k_3} E_o\text{-}H_2O_2 \xrightarrow{k_4} E_o + H_2O_2$$

Scheme II

where, G = β-D-glucose; P_1 = δ-D-gluconolactone; E_o, E_r = enzyme in oxidized and reduced forms, respectively.

The δ-D-gluconolactone generated in Scheme II is hydrolyzed to gluconic acid, slowly and nonenzymatically (Whitaker, 1985).

Based on the experimental data of Gibson *et al.* (1964), Whitaker (1985) concluded that the mechanism of glucose oxidation by glucose oxidase was a ping-pong bi-bi mechanism, which is presented as Scheme III:



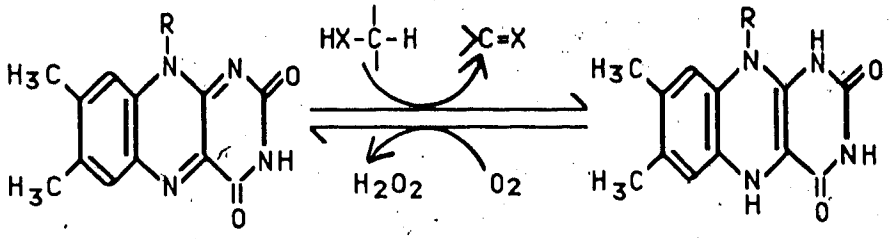
Scheme III

2.7.3.2 Molecular properties

Glucose oxidases from various fungal sources not only catalyze the same reactions, but also are substantially similar in molecular properties.

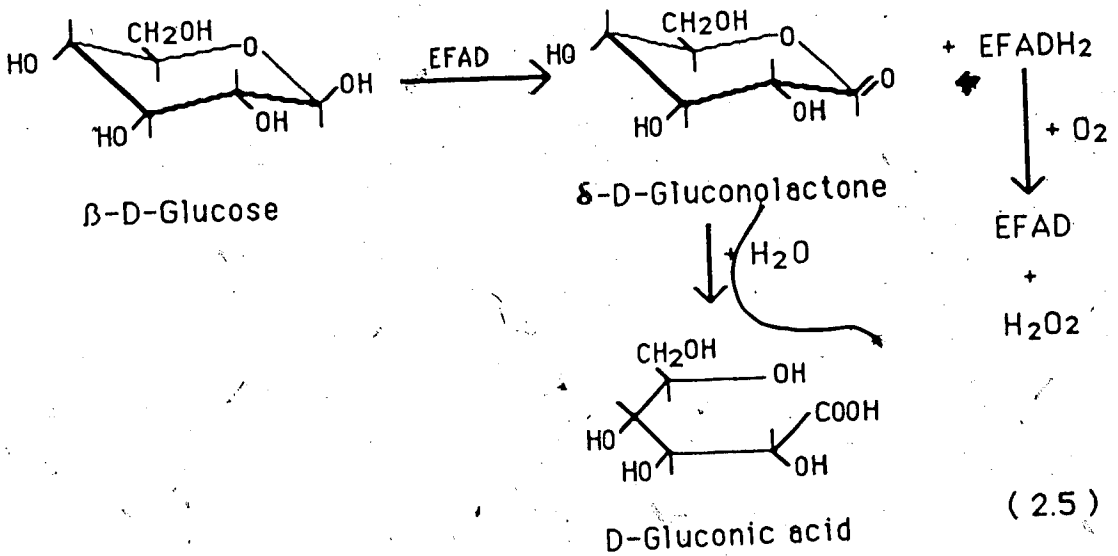
1. Glucose oxidase is a flavin enzyme, containing two molecules of flavin adenine dinucleotide (FAD) per enzyme molecule. The existence of FAD as the prosthetic group of glucose oxidase was first proved by Kellin and Hartree (1946). In glucose oxidase catalyzed reactions, the FAD moiety can exist in two redox states, i.e., fully oxidized

FAD and fully reduced ($FADH_2$), as shown by Equation 2.4 (Bright, 1974).



FAD $FADH_2$ (2.4)

Therefore, the FAD moiety is the center of oxidation and reduction in the enzyme reactions. The oxidation of glucose by the enzyme can be written as Equation 2.5 (Whitaker, 1985).



ß-D-Glucose

δ-D-Gluconolactone

D-Gluconic acid

(2.5)

The molecular weight of the enzyme from various fungal sources is around 160,000-180,000 (Table 2.2).

Table 2.2 Molecular weight of glucose oxidase (various reports).

Reference	*A. niger*	*P. amagasakiense*
Whitaker, 1965	186,000	———
Scott, 1975a	192,000	154,000
Nakamura and Fujiki, 1968	152,000	150,000

2. Glucose oxidase is also a glycoprotein, containing mannose, galactose, glucose and hexosamine. The experimental results of the carbohydrate contents in glucose oxidase enzyme have been summarized in Table 2.3.

The carbohydrate contents of glucose oxidase vary from production lot to lot (Nakamura *et al.*, 1976) as well as different fungal sources (Nakamura and Fujiki, 1968). It was postulated that the carbohydrate moiety of the enzyme provided a protective effect, which increased the stability of the protein moiety against denaturing agents (Nakamura *et al.*, 1976; Nakamura and Hayashi, 1974).

2.7.4 Activity assay and unit

The multiplicity of units and activity assay methods is one of the most confusing things confronting those who deal with enzymes. Various assay procedures have also been employed in the study of glucose oxidase. The overall reactions of glucose oxidase acting on glucose could follow either Equation 2.1 or Equation 2.3, as described earlier, depending on the absence or presence of catalase in the enzyme preparations. The activity assay, therefore, can be based on:

(1) formation of H_2O_2;

(2) consumption of O_2 or O_2 substitute;

(3) formation of gluconic acid.

The hydrogen peroxide (H_2O_2) produced in an enzymatic reaction has been utilized for activity assay, either in the

Table 2 Carbohydrate contents of [illegible]

Fungal Source	Carbohydrate Content (%)					Reference
	Mannitol	Glucose	Galactose	Insoluble glucan	Total	
A. niger	13.9	—	0.3	2.0	16.2	Peitur *et al.* 1955
	9.6	0.2	0.7	1.7	12.2	Nakamura *et al.* 1976
	7.9	0.1	1.7	0.8	10.5	Nakamura and Hagashi 1974
	13.2	0.7	—	2.1	16.0	Nakamura and Fujiki 1968
A. awamori-sokiense	8.3	1.1	—	1.7	11.1	Nakamura and Fujiki 1968

presence or in the absence of catalase (Ciucu and Patroescu, 1984). Some compounds, such as o-dianisidine, o-toluidine, or 2,6-dichlorobenzenone-indophenol, can be oxidized by H_2O_2 and catalase from the colorless reduced forms to the colored oxidized forms (Equation 2.6), therefore making spectrometric assay feasible (Huggett and Nixon, 1957; Dobrick, 1958).

$$\text{Reactant} + H_2O_2 \xrightarrow{\text{catalase}} H_2O + \text{Product} \qquad (2.6)$$

reduced, colorless — oxidized, colored

Without the assistance of catalase, hydrogen peroxide rapidly oxidizes iodide to iodine in the presence of Mo(VI) (Pardue *et al.*, 1964; Pardue and Simon, 1964). The iodine formed can be detected either by potentiometry (Pardue *et al.*, 1964) or by amperometry (Pardue and Simon, 1964).

Ciucu and Patroescu (1984) described a spectrometric assay method, using benzoquinone to substitute O_2. The formation of hydroquinone was measured at 290 nm. The method was reported to be direct, rapid and could be used to determine the initial velocity of enzyme reaction.

Based on the consumption of oxygen in Equations 2.7.1 and 2.7.3, the reaction velocity can be determined by manometry or polarography. The manometric assay for glucose oxidase was first described by Scott (1953). The consumption of oxygen was measured as the decrease of air pressure inside a Warburg flask fitted with a manometer. The amount of oxygen consumed in 30 minutes was used to calculate the

reaction velocity and activity.

The manometric method has been superseded by the oxygen electrode technique, a polarographic method (Estabrook, 1967; Bright, 1974). Several reviews have been published regarding this technique (Fatt, 1976; Gnaiger and Forstner, 1983). The heart of the instrument is an oxygen probe. A cell, consisting of a platinum cathode and a silver anode, is separated from the surrounding sample solution by a specific membrane which is permeable only to gases such as oxygen. When a suitable polarizing voltage is applied across the cell, oxygen undergoes an electric reduction at the cathode, causing a current to flow through the cell. The magnitude of current generated is proportional to the amount of oxygen present, which, in turn, depends on the oxygen pressure in the surrounding solution. By recording the electric current generated, the oxygen pressure in the solution can be traced continuously. In the study of glucose oxidase, the most widely used probe is the Clark-type polarographic electrode (Nakamura and Hayashi, 1974; Nakamura and Ougura, 1962; Weibel and Bright, 1971).

A titrimetric method (Underkofler, 1958) has been employed by some of the enzyme producers (Fermco Biochemics, b; Miles Lab., 1981). The gluconic acid produced in the enzyme reactions in 15 minutes is back-titrated to obtain the enzyme reaction rate and activity.

Various units have been used in the activity assay of glucose oxidase. The unit used for commercial pr[redacted]cation i

the manometric unit in GOU/g or GOU/mL, where GOU stands for Glucose Oxidase Unit. It was defined by Scott (1953) as "the quantity of enzyme that will cause an oxygen uptake of 10 mm^3 per minute under assay conditions". In the titrimetric method, the unit was defined as the equivalent of glucose oxidase to produce 1 mL of 0.05 N gluconic acid (Fermco Biochemics, b). The glucose oxidase activity obtained through the titrimetric method was expressed as the manometric unit. An arbitrary factor, 3, was used to convert the titrimetric unit to the manometric unit. It was obtained by regressing the experimental results of the same samples with the manometric and titrimetric methods (Underkofler, 1958).

When manometric or polarographic methods are used, the already confounding units can become even more puzzling in some cases. Some suppliers define their glucose oxidase units as being determined in the absence of catalase, therefore doubling the oxygen uptake, making the activities of different suppliers uncomparable (Scott, 1975a).

In order to bring uniformity into the chaos of expressing the enzyme activity, the International Union of Biochemistry, Enzyme Commission, proposed the definition of "Unit" (IUB, 1964) as: "One Unit (U) of any enzyme is that amount which will catalyse the transformation of 1 micromole of the substrate per minute under standard conditions".

It was also suggested that the measurement be carried out under optimum conditions and the "enzyme assays should

be based wherever possible on measurements of initial rate of reaction, and not on amounts of substrate changed by the end of a period of time unless it is known that the velocity remains constant throughout the period". Obviously, the titrimetric method does not meet this criterion, while the polarographic method is able to measure the initial reaction velocity.

2.7.5 Reaction properties of glucose oxidase

2.7.5.1 Substrate specificity

The substrate specificity of glucose oxidase has been well studied and some of the results were summarized by Whitaker (1985) and are presented in Table 2.4. Glucose oxidase has a very high specificity for β-D-glucose. However, Taylor *et al.* (1975) reported that glucose oxidase from *Pseudomonas fluorescens* oxidized β-D-glucose and 3-deoxy-3-fluoro-D-glucose at the same initial rate at a substrate concentration up to 200 μmole.

2.7.5.2 Michaelis constant (K_m)

The Michaelis constant, also referred to as Michaelis-Menten constant (K_m) is defined as "the concentration of the specific substrate at which a given enzyme yields one-half its maximum velocity" (Lehninger, 1982). It reflects the affinity characteristics of an enzyme for a specific substrate under certain reaction conditions. The reported Michaelis constants of glucose oxidase for glucose are

Table 2.4 Substrate specificity of glucose oxidase (adapted from Whitaker, 1985).

Compound	Relative Rate
ß-D-Glucose	100
∂-D-Glucose	0.64
1.5-Anhydro-ß-D-glucitol	0
2-Deoxy-ß-D-glucose	3.3
D-Mannose	0.98
2-O-Methyl-ß-D-glucose	0
3-Deoxy-ß-D-glucose	1
ß-D-Galactose	0.5
4-Deoxy-ß-D-glucose	2
5-Deoxy-ß-D-glucose	0.05
L-ß-D-glucose	0
6-Deoxy-ß-D-glucose	10
Xylose	0.98

presented in Table 2.5. A large variance among the reported K_m values can be observed. For example, the K_m of *Aspergillus* enzyme ranges from 0.026-0.11 M, even though the assay conditions are almost the same.

2.7.5.3 pH dependence of the enzyme reaction

Glucose oxidase from both *Aspergillus niger* and *Penicillium* strains has a wide range of effective pH values between 4.5 to 7.0, with optimum pH around 5.6 (Scott, 1975a; Bentley, 1963). At lower pH values, the rate limiting step of overall turn-over was reported to be the rate of formation of enzyme-substrate complex (E_o-S), while at a higher pH range, the limiting step was the combination of reduced-form glucose oxidase (E_r) with oxygen to form E_o-H_2O_2 complex (Bright and Appleby, 1969).

Glucose oxidase has also been reported to be quite unstable at pH values greater than 8 (Bentley, 1963). However, the enzyme could be stabilized by the presence of its substrate, such as glucose (Scott, 1975a).

2.7.5.4 Effect of temperature on the reaction rate

The elevation of temperature will affect the reaction rate of glucose oxidase in three aspects. The high temperature will: (1) lower the activation energy for the reaction, thus accelerating the reaction rate; (2) decrease the solubility of oxygen in the solution; and (3) accelerate the heat denaturation of the enzyme. Balanced by these three factors, the reaction rate of glucose oxidase is found to be

Table 2.5 Michaelis constants of glucose oxidase.

Fungi Sources	Temp. (^{o}C)	pH	K_m For Glucose(M)	Reference
A. niger	15	5.5	0.05	Nakamura and Ogura, 1968
A. niger	25	5.5	0.064	Nakamura and Ogura, 1968
A. niger	27	5.6	0.11	Gibson *et al.* 1964
A. niger	25	5.6	0.033	Swoboda and Massey, 1964
A. niger	25	5.5	0.026	Nakamura and Hayashi, 1974
P. amaga-sakiense	30	5.6	0.015	Swoboda and Massey, 1964
P. notatum	20	5.6	0.0096	Swoboda and Massey, 1964
Not reported	25	5.0	0.034	Ciucu and Patroescu, 1984

relatively unchanged from 30 to 60°C, and does not decrease significantly when temperature is well below that range (Scott, 1975a). Actually, the offset resulting from the decrease of oxygen solubility at higher temperature is so severe that the reaction rate of the enzyme at 90°F (32°C) is substantially the same as the rate at 50°F (10°C) (Scott, 1975a). On the other hand, Ohlmeyer (1957) reported that the activity of glucose oxidase increased as the temperature increased, up to a limit where the enzyme was rapidly denatured, at 87°C.

2.7.5.5 Inhibitors of glucose oxidase

Glucose oxidases from various sources are inhibited by metal ions such as Cu^{2+} and Hg^{2+} (Nakamura and Ogura, 1968) and partially by chemicals such as sodium bisulfite (Bentley, 1963). The product of enzyme reaction, D-glucono-δ-lactone, was found to be an inhibitor of glucose oxidase, possibly by binding itself in some way to the active center of the enzyme (Nakamura and Ogura, 1962; Gibson *et al.*, 1964).

2.7.6 The applications of glucose oxidase in food processing

Glucose oxidase, alone or coupled with catalase, has found wide applications in food science and technology because of its versatile functions. These numerous applications can be divided into five main categories:

1. to determine the glucose content of foodstuffs;

2. to form hydrogen peroxide;
3. to produce gluconic acid;
4. to remove glucose;
5. to remove oxygen.

2.7.6.1 Enzymatic determination of glucose

Because of its high substrate specificity, glucose oxidase, free or bound, has been used to determine the glucose contents of foodstuffs (Valentova *et al.*, 1983). A glucose oxidase impregnated test tape was adapted from clinical use to the potato chip industry by Smith (1960). The so-called "Chip-Color Tester" changes its color from yellow to various shades of green when it is in contact with raw potato slices. The glucose content of the potatoes can be read directly. The color of potato chips could be predetermined successfully from the reading of the Chip-Color Tester.

2.7.6.2 Formation of hydrogen peroxide

Some chemical oxidants, such as bromates, calcium peroxide, ascorbic acid, and chlorine dioxide, have long been used in the bread making industry to mature or bleach flour (Pomeranz and Shallenberger, 1971). The improving effect of ascorbic acid on the quality of baking flour was reported to be enhanced by adding glucose oxidase (Maltha, 1955). A USSR patent also described the addition of glucose oxidase and ascorbic acid to dough (Kretovich *et al.*, 1969). Scott (1975b) postulated that the enzyme removed free oxygen

in the dough, thus saving ascorbic acid, leaving it for its improving effect on baking properties of flour.

Glucose oxidase from *A. niger*, combined with lactoperoxidase, can preserve a contaminated milk product against a mixed microbial challenge (Banks and Board, 1985). The role of glucose oxidase enzyme was believed to be generating the required hydrogen peroxide.

2.7.6.3 Production of gluconic acid

The gluconic acid produced by glucose oxidase can be used to acidify milk to meet the requirement for milk curdling (Rand, 1972). Either glucose or lactose has to be present to provide sufficient substrate for glucose oxidase.

The utilization of glucose oxidase to extend the shelf-life of fresh seafood was reported recently (Wesley, 1982; Field *et al.*, 1986). The application could be accomplished by: (1) dipping fresh fish in enzyme-glucose solution; (2) packing fish with crushed enzyme-substrate containing ice; or (3) by storing glucose-solution-dipped fish and enzyme-impregnated algin blankets in alternating layers (Field *et al.*, 1986). The method nearly doubled the shelf-life of fillets and reduced drip loss in storage. It was believed that the gluconic acid generated during the treatment would reduce the pH of the surface of the fish, inhibiting the growth of spoilage microorganisms which were acclimatized to neutral or slightly alkaline marine environments. Gluconic acid itself could also be bacteriostatic, as could the hydrogen peroxide which might be

produced (Field *et al.*, 1986).

2.7.6.4 Removal of glucose

Glucose can, in some cases, present problems in food processing and preservation. Glucose oxidase has been used to remove glucose from foodstuffs such as corn syrup, invert sugar, dehydrated meat and dried egg powder.

Glucose in invert sugars and corn syrups is not as desirable as fructose due to its lower sweet equivalence, possible slower assimilation in digestion (Scott, 1975b), and facile crystallizing characteristics (Mermelstein, 1975). To remove glucose from invert sugar, glucose has been first oxidized by glucose oxidase to gluconic acid, which was separated by passage through an ion-exchange column (Scott, 1975b). Glucose oxidase has also been applied to produce the low glucose corn syrup which has an dextrose equivalent (DE) value equal to ordinary corn syrup, but contains a lesser amount of monosaccharides (Mermelstein, 1975).

The glucose content of meat may increase because of glycolysis activity (Sharp, 1957). The discoloration of dehydrated meat during storage is believed to be caused by the Maillard reaction between amino groups and glucose or its breakdown products (Henrickson *et al.*, 1956). Glucose oxidase enzyme was used by Henrickson *et al.* (1956) with hydrogen peroxide to treat meat before dehydration. They found that enzyme-treated pork was more stable and lighter in color than the control.

The greatest success of commercial application of glucose oxidase in the food industry is in egg drying. The natural glucose in eggs can deteriorate the quality of dried powders by either condensation with amino groups or by reacting with cephalin to produce off-flavors (Hill and Sebring, 1977; Berquist, 1977).

The removal of glucose from eggs, referred to as "desugaring", used to be achieved by fermentation with contaminating microorganisms or by controlled bacteria and yeast. The first application of glucose oxidase enzyme in a desugaring process was reported by Baldwin *et al.* (1953). Egg white, containing 3.4% glucose on a dry basis, was acidified with dilute hydrochloric acid to lower the pH from 9.0 to 7.3. After treating with 0.5% glucose oxidase-catalase enzymes for about 15 hours at 80°F (26.7°C), the glucose content dropped to 0.1% (dry basis), and the egg white was suitable for drying. Their results were corroborated by Carlin and Ayres (1953).

Scott (1953) developed an empirical relationship for glucose level, time, enzyme level and hydrogen peroxide demanded for enzyme desugarization in preparation of albumen solids. Yolk and whole egg were also desugarized by glucose oxidase-catalase enzyme system. The glucose content dropped from 1.2% (dry basis) to 0.1% after treatment with 0.3% enzyme for 4 hours.

The quality of enzyme-desugarized egg white, yolk and whole egg powders has been the subject of several studies.

The enzyme-treated powders were compared with control samples (Paul *et al.*, 1957; Song *et al.*, 1984), or with microorganism-fermented samples (Kline *et al.*, 1954; Darwish and Sadek, 1978). Enzyme treatment improved the storage stability of the egg yolk solids (Paul *et al.*, 1957), and resulted in higher foaming ability of egg white than the control (Song *et al.*, 1984). The enzyme-desugared egg white had a better foaming capacity than the yeast-desugared sample (Darwish and Sadek, 1978). The storage stability of egg powders prepared by the enzyme or yeast desugaring methods were equivalent as appraised by chemical, functional and flavor tests (Kline *et al.*, 1954).

The desugarization of egg white by co-immobilized glucose oxidase and catalase was reported by Kobayshi *et al.* (1978). Glucose oxidase and catalase were immobilized together or separately by using polyacrylamide gel. Oxidation of glucose to gluconic acid followed Michaelis-Menten kinetics.

2.7.6.5 Removal of oxygen

For many years, food scientists have been aware of the deteriorating effects of oxygen during food processing and food preservation. Various methods of removing oxygen from foodstuffs have been developed, such as vacuum or nitrogen packaging. Glucose oxidase-catalase enzymes have also been applied to many food commodities to achieve this goal.

During the storage of lipids, the oxidative rancidity resulting from exposure to oxygen is the most common and

[illegible] problem (Hamilton, 1961). Mitsyk *et al.* [illegible] applied a glucose oxidase preparation from [illegible] vitale to prolong the shelf life of fats. The surface of the fat was covered by a film of a mixture of enzyme and acetate buffer, glucose, and ascorbic acid. [illegible] added glucose oxidase and catalase into melted pork and beef fat and kept the mixture hermetically sealed in glass jars. The fat thus treated remained usable for more than 6 months.

Dehydrated foods because of their tremendous increase in the surface area exposed to oxygen (air), are especially susceptible to autoxidative rancidity. The direct application of enzyme system to these foods, however, is impossible as the moisture content of the foods is too low to permit enzyme activation (Scott, 1956b). However, the free oxygen or packages or containers of foods can be removed by means of a dispersion of water, glucose and enzyme system of glucose oxidase and catalase (Scott, 1956). The enzyme dispersion was atomized onto suitable carriers, such as sawdust, corn cobs, polystyrene or clay, and this dehydrating mixture was separated from the package contents with an oxygen permeable barrier such as paper or polyethylene (Scott and Hammer, 1962, 1963). Levins *et al.* (1960) were also able to prevent dry foods, such as dry milk, coffee and candy, from autoxidation by adding a mixture of glucose oxidase, catalase, glucose, buffer, antibiotics and a filler, such as starch.

In many cases, foods are either hot-filled or heated after sealing. Glucose oxidase and catalase have to survive the heat treatment for their function of deoxygenation. Scott (1975b) coated the dry enzyme with a hot-water insoluble material, such as methyl cellulose, or a time-release coating, and thus was able to stabilize the enzyme through the heat treatment.

The deterioration and spoilage of beer during storage, caused by oxidation reactions, can be inhibited by addition of glucose oxidase-catalase enzymes (Ohlmeyer, 1957).

The glucose oxidase-catalase system was applied by Barton *et al.* (1955) to protect canned soft drinks from fading of sensitive colors and picking up of iron. The enzymes have also been utilized to stabilize fruit drinks and concentrates, such as orange juice, lemon juice and grapefruit drinks (Scott, 1975b).

Apple wine is susceptible to oxidation and to the development of acetic acid. Yang (1955) applied the glucose oxidase-catalase system to remove oxygen from the wine, and thus prevented development of volatile acids. According to Scott (1975b), a considerable amount of work has been done by Ough and his colleagues on the stabilization of white wine with glucose oxidase-catalase system and favorable results have been achieved.

A few more applications are worth mentioning. A glucose oxidase-catalase impregnated plastic wrapper was able to prevent browning ring in the loaves of cheeses (Scott,

1975b). The enzymes can also be used to prevent oxidative rancidity in mayonnaise (Scott, 1958), to prevent discoloration in precooked fro[redacted]imp (Kelley, 1974), and to protect ascorbic acid (Chogov[redacted]aze and Bakuradze, 1972).

2.7.6.6 Recent research activities

Recently, the research activities on glucose oxidase seem to emphasize immobilization of the enzyme, alone or with catalase, and the application of immobilized enzymes. Examples of such research endeavors include immobilization of glucose oxidase by irreversible adsorption on the surface of a graphite electrode (Ikeda *et al.*, 1984); by entrapment in gels (Freeman *et al.*, 1983); on a collagen membrane (Gozia *et al.*, 1983); and on dimethyl-amiated nylon gels (Miyama *et al.*, 1985).

3. EXPERIMENTAL

3.1 Materials

3.1.1 Potatoes

Potatoes of Superior and Shepody varieties were obtained from I & S Produce Ltd., a local food processor. Norland potatoes were purchased from Centennial Acres, a local potato supplier. Potatoes were either stored at 4°C, or reconditioned at room temperature to achieve suitable reducing sugar content.

3.1.2 Glucose oxidase

Two commercial preparations of glucose oxidase enzyme from *Aspergillus niger* were used:

A. DeeO 1500, in powder form, 1500 Glucose Oxidase Units (GOU)/g (Miles Laboratories, Elkhart, IN).

B. Fermcozyme CBB-750, in liquid form, 750 GOU/mL, (Fermco Biochemics, Elk Grove Village, IL).

3.1.3 Chemicals

Monobasic (KH_2PO_4) and dibasic (K_2HPO_4) potassium phosphate, 85% phosphoric acid, and anhydrous dextrose (D-glucose), all certified A.C.S. grade (Fisher Scientific Co., Fair Lawn, NJ).

Reagent grade chloroform and anhydrous ethyl ether (Caledon Labs., Georgeton, Ont.).

3.1.4 Equipment

Model 53 biological oxygen monitor and YSI Model 27 industrial analyzer (Yellow Springs Instruments, Yellow Springs, OH).

Hobart food cutter and dicer (The Hobart Manufacturing Co., Troy, OH).

General Electric fryer, Model CK40 (General Electric Co., Chicago Heights, IL).

HunterLab Model D25M/L-2 colorimeter (Hunter Assoc. Lab., Fairfax, VA).

Beckman J-21B centrifuge (Beckman Instr., Spinco Div., Palo Alto, CA).

Model 5851 vacuum oven (National Appliance Co., Portland, OR).

3.2 Studies on Glucose Oxidase

3.2.1 Preparation of phosphate buffer and various solutions

Phosphate buffers of various pH values were prepared by mixing the KH_2PO_4 and K_2HPO_4 solutions (both 0.1 M or 0.15 M) to obtain the desired pH values. A Model 230 pH/ion meter (Fisher Scientific, Fair Lawn, NJ) was used to measure pH. For buffer of pH lower than 4.5, phosphoric acid was used to bring KH_2PO_4 solution to the desired pH.

Glucose solution, 1 M either in phosphate buffer or in Milli-Q water, was kept at room temperature overnight before use.

Glucose oxidase solutions were prepared by dissolving the enzyme either in phosphate buffer or in Milli-Q water prior to the experiment. The enzyme concentration was expressed as percentage in solution, w/v for powder enzyme and v/v for liquid enzyme.

3.2.2 Activity assay procedures

A total of 2.95 mL of glucose solution and phosphate buffer was pipetted into the reaction cell of the oxygen monitor and stirred for 3-5 min to achieve temperature equilibrium and air saturation. The oxygen probe was inserted carefully to expel any air bubbles that might attach to it. Glucose oxidase solution (50 μL) was added with a syringe through the slot on the oxygen probe. The oxygen pressure of the solution was recorded with a 10 in Beckman recorder at a chart speed of 1 in/min. An example of the graph obtained is presented in Figure 3.1.

To check the effect of glucose concentration on the enzyme reaction rate, the experiments were carried out at 25.0°C, as recommended by IUB (1964), and at pH 5.5, which was reported to be the optimum by Bentley (1963). The ratio of glucose solution (1.0 M in buffer) to phosphate buffer was adjusted to obtain the desired glucose concentrations in the final solution.

To carry out the measurement at different pH's, 0.90 mL 1.0 M glucose in water and 2.05 mL 0.15 M phosphate buffer were used for each determination so that the buffer

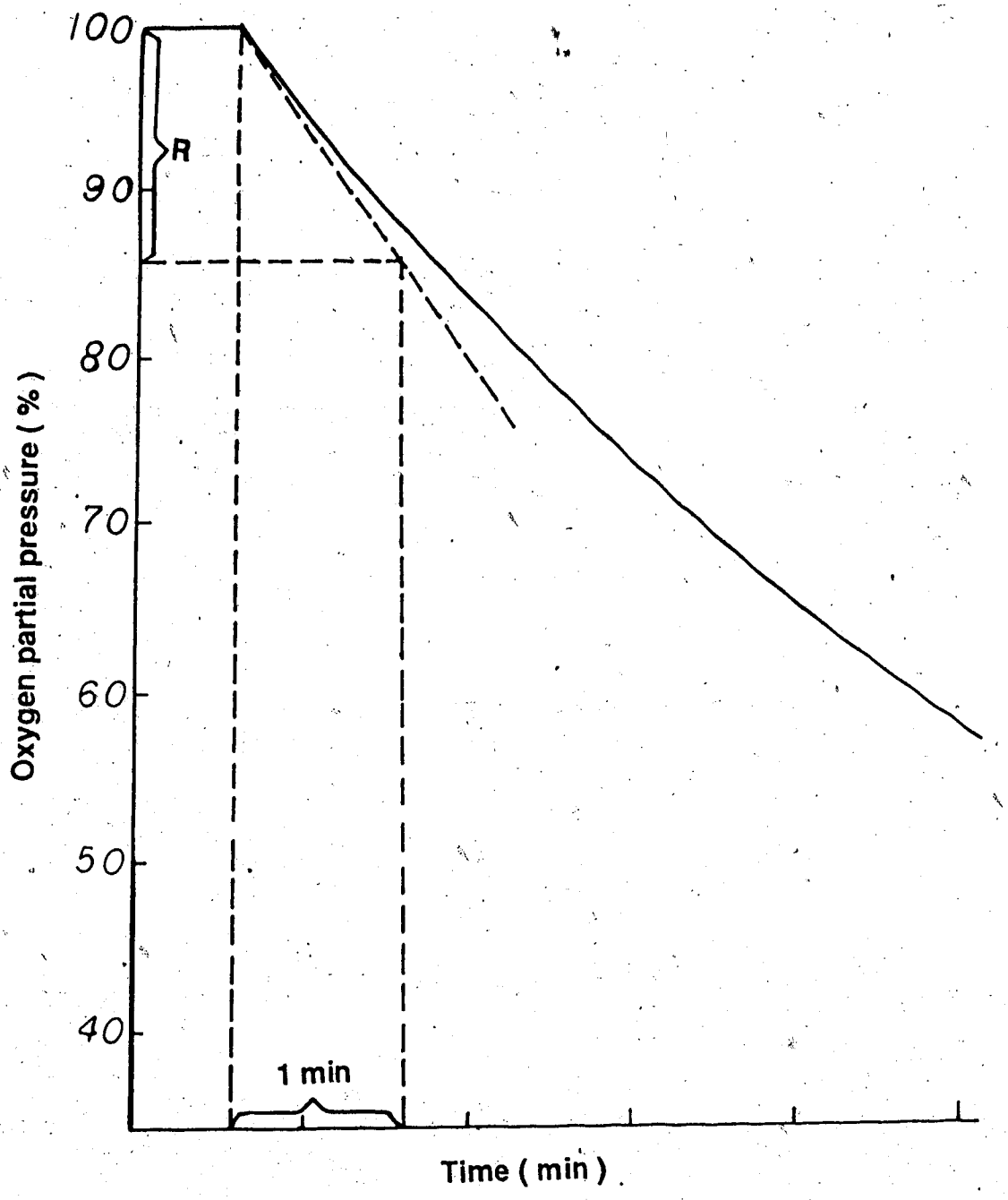


Figure 3.1 Example graph of oxygen partial pressure traced by the Model 53 Biological Oxygen Monitor. The measurement was carried out at 40°C, 0.3 M glucose, with chart speed of 1 in/min.

concentration of the final solution was kept at 0.1 M.

To test the effect of reaction temperature on the reaction rate, the experiments were carried out at various temperatures (25 to 40°C), at pH 5.5 and glucose concentration of 0.3 M, which was found to be the minimum glucose concentration for activity assay.

To study the thermal stability of the enzyme, about 2 mL of enzyme solution (in 0.1 M phosphate buffer, pH 5.5) was put into a test tube and held in a water bath at specific temperatures. The test tube was taken out after a certain time and cooled in cold water. The remaining activity was assayed at 25°C at pH 5.5 and glucose concentration of 0.3 M.

3.2.3 Calculation of reaction rates

To calculate the initial velocity of the reaction, a straight line was drawn tangentially to the recorded curve as illustrated in Figure 3.1. The reduction of oxygen pressure in 1 min, R, was obtained from the graph. The velocity of the enzyme reaction, ν, was calculated according to Equation 3.1.

$$\nu = \frac{S_T \times R \times V}{22.4 \times 100} \times 2 \qquad (3.1)$$

where:

ν: reaction velocity in μmole/min;

S_T: oxygen solubility of the solution at temperature T in μL O_2/mL;

R: oxygen pressure reduction (%) in 1 min;

V: total volume (mL) of the solution, which was 3.0 mL in these experiments.

The activity of the enzyme preparations was calculated with Equation 3.2:

$$\frac{IU}{g \text{ or } mL} = \frac{\nu}{E} \qquad (3.2)$$

where:

IU: International Unit;

ν: reaction velocity calculated from Eq. 3.1;

E: total amount of enzyme used in measurement in g or mL.

The oxygen solubility at temperature T (S_T in Eq. 3.1) was calculated from Eq. 3.3:

$$S_T = S_T^{o} \times e^{-kc} - D_{buffer} \qquad (3.3)$$

where:

S_T^{o}: oxygen solubility in pure water at temperature T ($\mu L\ O_2/mL$);

k: constant, 0.0156 (Zander, 1976);

C: glucose concentration (%).

D_{buffer}: decrease of oxygen solubility caused by 0.1 M phosphate buffer at temperature T.

S_T^{o} values could be found from previous data, and D_{buffer} was calculated from the data given by Robinson and

Cooper (1970).

3.3 Processing and Enzyme Treatment of Potato Chips and French Fries

3.3.1 Processing of potato chips

Potatoes of Superior and Shepody varieties were used for chip making. They were either from cold storage (4°C) or reconditioned at ambient temperature for a suitable length of time to achieve various reducing sugar contents.

Potatoes were washed, hand peeled, and sliced with the Hobart Food Cutter and Dicer to about 1/20 in (1.3 mm) thickness. Samples of the slices were taken for glucose and moisture content determinations. After washing in cold water to get rid of surface starch, the slices were either dried on paper towel or treated with enzyme solution. They were then fried in hot oil at 180°C. Frying was terminated when bubbles ceased to rise from the slices. The chips obtained were packed in polyethylene bags for later analyses.

3.3.2 Enzyme treatment of potato chips

About 250 g of the cold water-washed slices were immersed in 1 L of water or enzyme solutions at various temperatures for various times. The enzyme concentration in the solutions ranged from 0.005% to 1.5% (w/v for powder enzyme and v/v for liquid enzyme). The treatment was carried out at 20, 30 and 40°C, with the dipping time varying from

30 to 90 min. During dipping, the samples were occasionally stirred with glass rod. After dipping, slices were dried on paper towel and samples of the slices were taken for glucose and moisture content determinations.

To check the effect of aeration on the enzyme reaction on potato slices, compressed air (approx. 21% O_2, 78% N_2) was bubbled through the solution during the enzyme treatment at a rate of about 5000 cc air/min (Lab Crest Series 100 Century Flowmeter; Fisher Scientific Co., Fair Lawn, NJ).

3.3.3 Processing of French fries

Norland potatoes from cold storage were washed, peeled and sliced with the Hobart Food Cutter and Dicer into strips of 3/8 in^2 (1 cm^2) cross section. Samples of the strips were taken for glucose and moisture content determinations. Strips were then washed in cold water twice. Pre-frying treatments of the strips, such as blanching, were carried out, followed by the enzyme treatment. Strips were then dried on paper towel and fried in oil at 185°C for 3 min. French fries thus obtained were kept for various analyses and evaluations.

3.3.4 Pre-frying treatments for French fries

Part of the raw potato strips were fried directly without further treatment. The remaining strips were hot water blanched or steam blanched prior to further treatments. Hot water blanching was performed at 80°C for 15

min, as recommended by Brown and Morales (1970). Live steam at atmospheric pressure for 7 min was used in steam blanching of potato strips according to Strong (1968) and Wilder (1972).

3.3.5 Enzyme treatment of potato strips

About 450 g of potato strips, blanched or unblanched, were immersed in 1 L of either water or enzyme solutions kept at various temperatures. The enzyme concentrations ranged from 0.05% to 1.5% (w/v for powder enzyme and v/v for liquid enzyme). The dipping was carried out at 25 or 35°C for 5-20 min. During the treatment, solutions were stirred every 2-3 min. After treatment, the strips were dried on paper towel and samples of the strips were taken for glucose and moisture content determinations.

3.4 Analysis and Sensory Evaluation

3.4.1 Moisture content determination

The AOAC (1980) method with a conventional oven at 105°C was used for moisture determination.

3.4.2 Glucose content determination

3.4.2.1 Raw potato samples

Chopped raw potatoes (50.0 g) were blended with 100.0 mL distilled water at high speed for 2 min. The mixture was then filtered through a Whatman No. 4 paper in a Buchner

funnel attached to a water aspirator: A portion of the filtrate was placed in a test tube and heated in 80°C water for 10 min, cooled, and refiltered through a Whatman No. 4 paper. A 25 μL aliquot of filtrate was then injected into the YSI Glucose Analyzer. Glucose content of the sample was calculated from the following formula:

$$\% \text{ Glucose} = \frac{Rd}{1000} \times 3$$

where Rd was the direct reading from the instrument in mg/dL.

3.4.2.2 Cooked (blanched) samples

Chopped samples (25.0 g) were blended with 100 mL of distilled water at high speed for 2 min. The slurry was then centrifuged at 25,400 G for 15 min. A 25 μL aliquot of the supernatant was injected into the YSI Glucose Analyzer. Glucose content of the sample was calculated from:

$$\% \text{ Glucose} = \frac{Rd}{1000} \times 5$$

3.4.3 Color measurement of potato chips and French fries

3.4.3.1 Potato chips

About 40 g of chips were crushed in polyethylene bags so that they could be put into the blending jar. Samples were blended at low speed for a few minutes and sifted through a 10 mesh sieve. The sifted samples were filled into the Hunterlab sample holder for color measurement. The surface of the sample was smoothed with a spatula.

The Hunterlab Colorimeter was standardized with a standard yellow plate (L=77.9, a=-1.4, b=23.3). The color of duplicate samples was then measured and average readings of L, a and b were obtained.

3.4.3.2 French fries

For color measurement of French fries the Hunterlab was also standardized with the yellow plate. Samples of French fries were placed side by side on the sample holder. The samples were then covered with a black aluminum plate in which a hole of 4 cm diameter was cut in the center. The color of samples was measured through the hole. Three different locations on the samples were measured to obtain the average values.

The color of French fries was also evaluated subjectively by comparing the sample color with the USDA color standards (USDA, 1972).

3.4.4 Determination of fat content of potato chips and French fries

The fat content of potato chips was determined with the Soxhlet extraction method as described by Lees (1975).

The AOAC (1980) method was used for the determination of fat content in French fries. Samples were vacuum dried at 60°C and 100 mm Hg for 24 h, followed by Soxhlet extraction with anhydrous ethyl ether for 5 h.

3.8.3 Sensory evaluation

Sensory evaluation was carried out to compare the flavour of French fries prepared with hot water blanching and steam blanching. Water blanching was carried out at 80°C for 2 min while in steam blanching, strips were steamed under atmospheric pressure for 7 min and dipped in water at 35°C for 12 min before frying. Samples from these two treatments were served to the panelists together immediately after frying. Panelists recruited from the graduate and under graduate students and technicians from the Department of Food Science were asked to taste and compare the samples. The evaluation sheet is shown in Figure 3.2.

The sensory evaluation was also carried out to determine whether glucose oxidase treatment imparted any off flavors to potato chips and French fries. Samples of potato chips were prepared by dipping the slices in water or 1 % liquid enzyme solution at 60°C for 60 min before frying. French fries were prepared by dipping the steam blanched potato strips in water or 0.1 % liquid enzyme solution at 35°C for 12 min before frying. The control and enzyme treated samples were served together to the panelists. An evaluation sheet is shown in Figure 3.3.

Sensory Evaluation For French Fries

Judge:_____ Date:_____,1987

1. Definitions

1) Flavor: the typical flavor of potato French fries

2) Texture: good texture means:

external surfaces: moderately crispy; no noticeble separation from the inner portion; not excessively oily
interior portions: well cooked; tender; practically free from sogginess

2. Instructions

Taste the samples and check the appropriate small boxes beside.
If you cannot decide, guess please.
Do not take the color into consideration.

3: Flavor

Which sample has a stronger flavor?

X☐ Y☐

4. Texture

Which, according to the definitions, has a better texture?

X☐ Y☐

Figure 3.2 Sensory evaluation sheet for French fries prepared with water blanching and steam blanching.

Sensory Evaluation of Potato Products

Judge_____ Date:_____, 1987

Sample type: potato chips ☐ French fries ☐

I. Instructions:

Taste the provided samples and check the appropriate box.

II. Question :

Did you detect any difference in flavor between the two samples?

YES ☐ NO ☐

If YES, please describe :

Figure 3.3 Sensory evaluation sheet for potato chips and French fries of enzyme treated and control samples.

4. RESULTS AND DISCUSSION

4.1 Reaction Properties of Two Commercial Preparations of Glucose Oxidase

4.1.1 Oxygen solubility in the system

In the calculation of the enzyme reaction velocity and activity, an unknown variable in Eq. 3.1 was S_T, the oxygen solubility in the solution at temperature T. As there were no available data for the phosphate-glucose-water system, the oxygen solubilities under various glucose concentrations and temperatures were calculated using Eq. 3.3, and are presented in Tables 4.1 and 4.2, respectively.

S_T^o values were taken from Stephen and Stephen (1963). D_{buffer} values were calculated from the graph given by Robinson and Cooper (1970), as shown in Figure 4.1. The k value was taken as 0.0156 from Zander (1976).

The oxygen solubility of a solution is affected by factors such as oxygen partial pressure above the solution (Henry's Law), temperature and the types and concentrations of solutes. As the experiments were carried out at atmospheric pressure of air, the partial pressure of oxygen was quite stable and exerted little effect on the oxygen solubility in different solutions. The oxygen solubility at different temperatures could be obtained from the previous data (Table 4.2 and Figure 4.1).

Table 4.1 Oxygen solubility in solutions of various glucose concentrations at 25°C, calculated using Eq. 3.3.

Glucose Concentration		$S_T{}^0$ [1]	D_{buffer} [2]	S_T [3]
M	%	µL / mL	µL / mL	µL / mL
0.02	0.36	5.75	0.22	5.50
0.04	0.72			5.47
0.06	1.08			5.43
0.08	1.44			5.50
0.10	1.80			5.37
0.20	3.60			5.22
0.30	5.40			5.07
0.40	7.20			4.92
0.50	9.00			4.78

1 adapted from Stephen and Stephen, 1963

2 adapted from Robinson and Cooper, 1970

3 calculated using Eq. 3.3

Table 4.2 Oxygen solubility in solutions at various temperatures with glucose concentration at 0.3 M.

Temperature (^{o}C)	S_T^{o} [1] µL / mL	D_{buffer} [2] µL / mL	S_T [3] µL / mL
25	5.75	0.22	5.07
30	5.24	0.25	4.57
35	4.86	0.28	4.19
40	4.48	0.34	3.78

1 adapted from Stephen and Stephen, 1963

2 adapted from Robinson and Cooper, 1970

3 calculated using Eq. 3.3

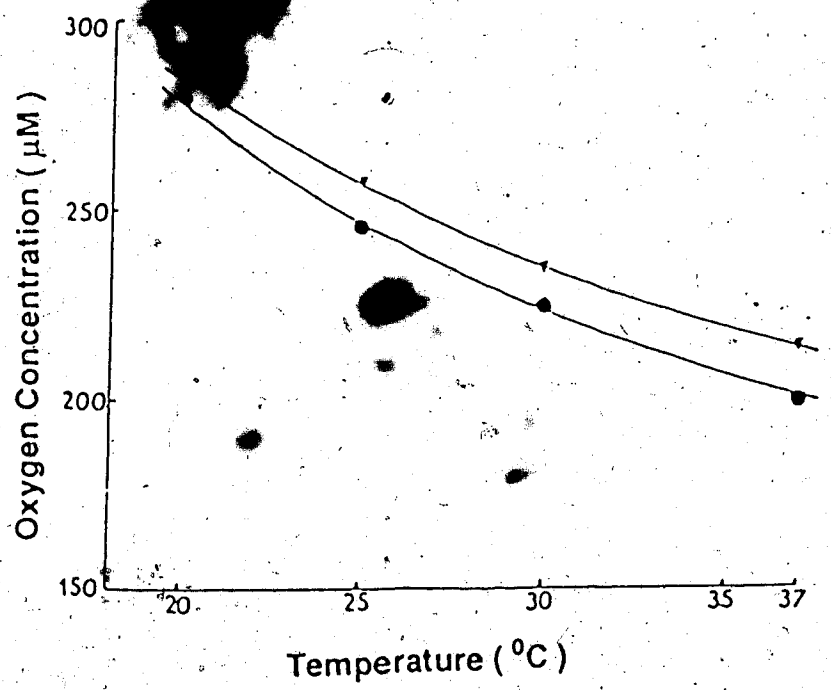


Figure 4.1 Oxygen solubility at different temperatures in pure water (▼) and in 0.1 M phosphate buffer (•) at pH 7.4 (adapted from Robinson and Cooper, 1970).

The oxygen solubility in the solution was also affected by glucose content and phosphate buffer. The decrease of the solubility caused by 0.1 M phosphate buffer has been reported by Robinson and Cooper (1970) and their data were used in the present calculations as D_{buffer}. The effect of glucose on the solubility, however, could not be easily obtained from previous work.

The oxygen solubility in glucose-water system could be calculated from the Setschenow equation (Eq. 4.1) (Zander, 1976).

$$S_T = S_T^o \times e^{-kc} \qquad (4.1)$$

All variables in the equation are the same as in Eq. 3.3.

The constant k at 37°C was reported to be 0.0156 by Zander (1976). As k values were not available for other temperatures, this k value was used in calculating the oxygen solubility at other temperatures in the range of 25-40°C. The calculated oxygen solubility at different temperatures was compared with the reported data, as presented in Table 4.3.

At temperatures as low as 21.2°C, the oxygen solubilities calculated and reported were in good agreement. Therefore, by using k=0.0156, the oxygen solubility between 21.2 and 37°C could be calculated quite accurately.

As the salt effect of solutes in a solution contributed additively (Zander, 1976), the overall oxygen solubility of glucose-phosphate-water system could be calculated using Eq. 3.3.

Table 4.3 Oxygen solubility calculated from Setschenow equation (k=0.0156) and reported data.

Temp. (°C)	Glucose Conc. (%)	Calculated S_T (µL/mL)	Reported S_T (µL/mL)	Error from Reported (%)
21.2	10.89	25.70	26.50 [1]	± 3.1
21.5	20.70	21.92	22.02 [1]	± 0.1
37.0	10.00	20.62	20.60 [2]	± 0.1

1 adapted from IUPAC, 1979

2 adapted from Zander, 1976

The oxygen solubility in diluted aqueous solution of acids and bases was reported by Guseva *et al.* (1972) to be independent of pH in the range of pH 2-12 at 20-40°C. Therefore, the oxygen solubility calculated with Eq. 3.3 would also apply to solutions at various pH's.

4.1.2 Effect of glucose concentration on enzyme reaction velocity

The reaction velocities of the two commercial preparations of glucose oxidase at various concentrations are presented in Table 4.4 and Figure 4.2.

From the data, several basic properties of the two preparations of glucose oxidase, such as activity, minimum glucose concentration for activity assay, and the Michaelis-Menten constant could be obtained.

4.1.2.1 Michaelis-Menten constant

The Michaelis-Menten constant, K_m, is defined as "the concentration of the specific substrate at which a given enzyme yields one-half its maximum velocity" (Lehninger, 1982). It reflects the affinity characteristics of an enzyme for a specific substrate.

Several methods are available for calculation of K_m. The statistical method developed by Wilkinson (1961) was reported to be more accurate than the widely used graphic methods, such as the double-reciprocal Lineweaver plot. The K_m values of the two preparations calculated by the computer-assisted Wilkinson method are shown in Table 4.5.

Table 4.4 Enzyme reaction velocity (μmole/min) at various glucose concentrations.

Glucose Conc.(M)	Powder Enzyme		Liquid Enzyme	
	R (%)[1]	Velocity	R (%)[1]	Velocity
0.02	4.5	0.066	4.9	0.072
0.04	6.1	0.089	7.0	0.103
0.06	7.3	0.106	8.0	0.116
0.08	8.0	0.116	8.9	0.129
0.10	8.6	0.124	9.8	0.141
0.20	9.7	0.136	11.0	0.154
0.30	10.6	0.144	11.5	0.156
0.40	10.9	0.144	12.0	0.158
0.50	11.2	0.143	12.5	0.160

[1] reduction of oxygen partial pressure in 1 min.

average of triplicates with std. dev. ranging from 0.10% to 0.15 %

Table 4.5 The Michaelis constants for both enzyme preparations.

Enzyme Prepration	K_m (M Glucose)
Powder Enzyme	0.027
Liquid Enzyme	0.026

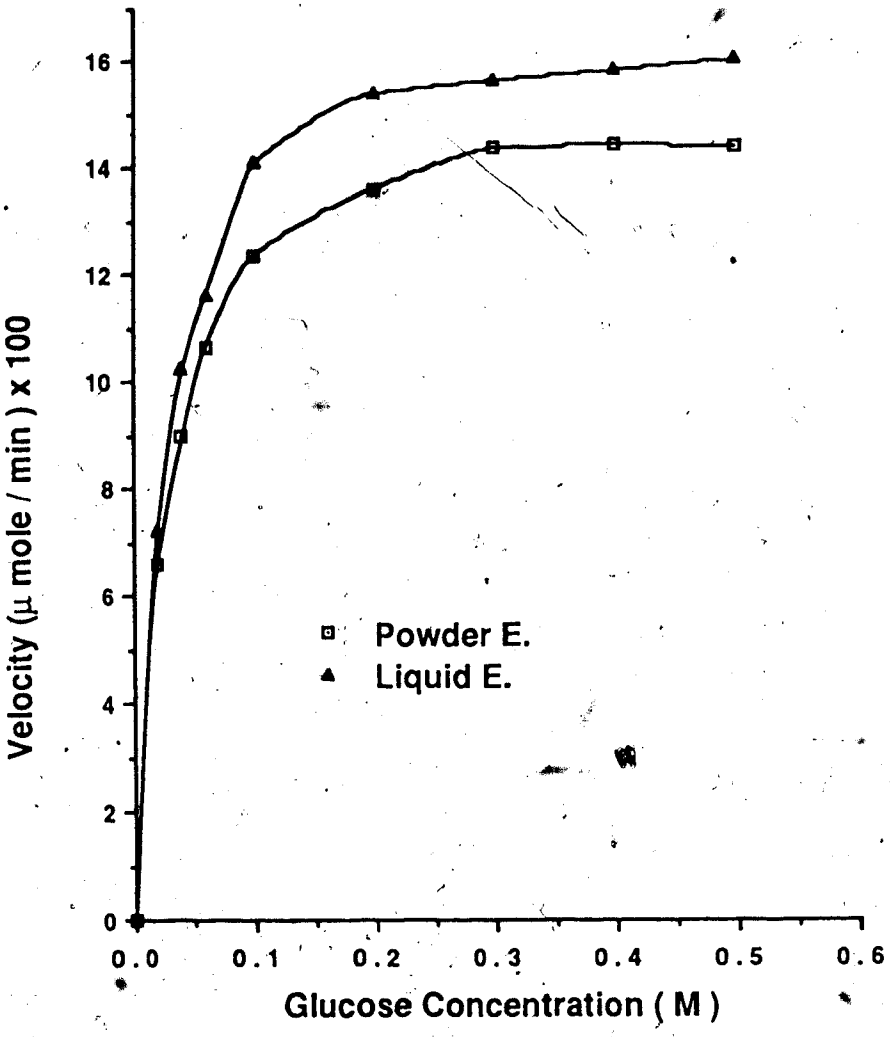


Figure 4.2 Effect of glucose concentration on reaction rate at 25°C, pH 5.5.

These results corroborated the K_m value reported by Nakamura and Ogura (1962). The reported K_m values for glucose oxidase from *A. niger* vary from 0.026 M (Nakamura and Ogura, 1962) to 0.11 M (Gibson *et al.*, 1964), although the conditions under which they were measured were not the same. This difference might be attributed to the assay methods. Nakamura and Ogura (1962) used the polarographic method, while Gibson *et al.* (1964) employed the manometric method.

4.1.2.2 Minimum glucose concentration for activity assay

At low substrate concentration, the initial velocity of an enzyme reaction is directly proportional to the substrate concentration until the substrate concentration reaches 10 K_m or higher (Guilbault, 1984). Therefore, the activity assay should be carried out at substrate concentrations greater than 10 K_m.

Figure 4.2 shows that the reaction velocity of both powder and liquid enzymes increased sharply from 0 to about 12-14 x 10^{-2} μmole/min as glucose concentration increased from 0 to about 0.1 M, then slowly thereafter until glucose concentration reached 0.25 M and higher, when the velocity became almost constant. Therefore, the activity assay for glucose oxidase should be carried out with glucose concentration at 0.25 M or higher. In the present experiments, the enzyme activity was assayed with a glucose concentration of 0.3 M (5.4%).

Table 4.6 Activities of the two commercial enzyme preparations.

Enzyme Preparation	Experimental Result IU / g or mL	Declared Activity	
		GOU /g or mL	IU / g or mL
Powder Enzyme	2870	1500	1188
Liquid Enzyme	1560	750	593

4.1.2.3 Activity of the two commercial enzyme preparations

The activities of the two commercial enzyme preparations obtained in the present experiments are shown in Table 4.6. The declared activities are also shown in Table 4.6, in both Glucose Oxidase Units (GOU) and International Units (IU).

The IU is defined as the quantity of enzyme that transforms 1 micromole of substrate in one minute under optimum assay conditions (IUB, 1964). GOU was defined by Scott (1953) as the amount of enzyme that could cause an oxygen uptake of 10 mm^3 per min under assay conditions. GOU can be converted into IU through the following calculations:

$$1\ GOU = 10\ mm^3\ O_2/min$$

$$= \frac{10}{22.4} \times \frac{273.15}{308.15}\ \mu mole\ O_2/min$$

$$= 0.396\ \mu mole\ O_2/min$$

$$= 0.79\ \mu mole\ glucose/min$$

$$= 0.79\ IU$$

The activities obtained in the present experiments were more than twice the declared activities when compared in IU/g or IU/mL. The difference may be due to the different assay methods used.

Both manufacturers employed the titrimetric method for activity assay (Miles Labs., 1981; Fermco Biochemics, b). When compared with the polarographic method used in the present experiments, several observations may be made which explain the differences between the experimental and declared activities:

1. The confusion in the unit conversion from titrimetric unit to manometric unit. Both manufacturers expressed their glucose oxidase activity in manometric units, i.e., GOU/g or GOU/mL, although the activities were assayed with the titrimetric method. In titrimetric assay, one unit is defined as the amount of enzyme that "produces 1 mL 0.05 N gluconic acid" under assay conditions in 15 minutes (Fermco Biochemics, b). According to the definition, one titrimetric unit is equal to 4.2 manometric units. Calculations are shown below.

1 titrimetric unit = 1 mL 0.05 N gluconic acid/15 min
= 1 mL 0.05 M glucose/15 min
= 50 μmole glucose/15 min
= 3.33 μmole/min
= 3.33 IU
= 4.2 GOU

However, when the titrimetric method was released, an arbitrary factor of 3 was used to convert the titrimetric unit to manometric unit (Underkofler, 1958), and this factor was used by both enzyme suppliers. Therefore, the labelled activity could be only about 70% of the actual activity.

2. The substrate concentration in the titrimetric assay was lower than 10 K_m. In the assay procedures described by the two suppliers, the glucose concentration was 0.15 M, which was one-half of the glucose concentration used in the present experiments. Based on the glucose concentration-velocity plot in Figure 4.2, the initial

velocity was only about 90% of the maximum velocity. Thus, the titrimetric method employed by both suppliers could give only 90% of the actual activity of glucose oxidase.

3. The titrimetric method assays the activity by measuring the average velocity of enzyme reaction in 15 min, while the polarographic method measures the initial velocity. As recommended by the International Union of Biochemistry (IUB, 1964), the activity assay "should be based wherever possible on measurements of inital rates of reaction, and not on amounts of substrate changed by the end of a period of time unless it is known that the velocity remains constant throughout this period". The titrimetric method, by measuring the average velocity in 15 min, does not meet this criterion. The reaction conditions could change so much during that time that they might shift away from the optimum conditions as required by activity assay.

The glucose concentration could get even lower. In the procedures described for titrimetric assay, up to 20% glucose could be oxidized during the 15 min assay time. Thus, there was even less glucose available to saturate the enzyme. The intermediate of glucose oxidation by the enzyme, δ-D-gluconolactone, has been reported to be an inhibitor of the enzyme (Nakamura and Ogura, 1962; Gibson *et al.*, 1964). As the hydrolysis of δ-D-gluconolactone to gluconic acid was slow (Whitaker, 1985), there might be accumulation of δ-D-gluconolactone and therefore inhibition of enzyme activity during the assay period. The production of gluconic

acid and the consumption of oxygen could also result in the shift of assay conditions away from the optimum.

4.1.3 Effect of pH on reaction velocity

The reaction velocities of the two commercial enzyme preparations at various pH values are shown in Figure 4.3. The pH-velocity curves for both enzyme preparations are bell-shaped, with the optimum pH around 5.0 for powder enzyme and 5.5 for liquid enzyme. Both enzyme preparations have quite a broad effective pH range (pH 4-7).

The effect of pH on the individual steps of the reaction has been extensively studied by Weibel and Bright (1971), Bright and Appleby (1969) and Gibson *et al.* (1964). It was suggested that the limiting step at low pH values was primarily the formation of E-S complex, while at high pH's the limiting step was the combination of reduced enzyme with O_2 (Bright and Appleby, 1969). Glucose oxidase enzyme was also found to be unstable at pH's greater than 8 (Boyer *et al.*, 1963).

4.1.4 Effect of temperature on reaction rate

The reaction rates of the two enzyme preparations at temperatures from 25 to 40°C are shown in Figure 4.4. The reaction velocity was found to be almost constant in the temperature range studied. Because of the limitation of the instrument, the effect of temperatures lower than 25°C or higher than 40°C could not be tested.

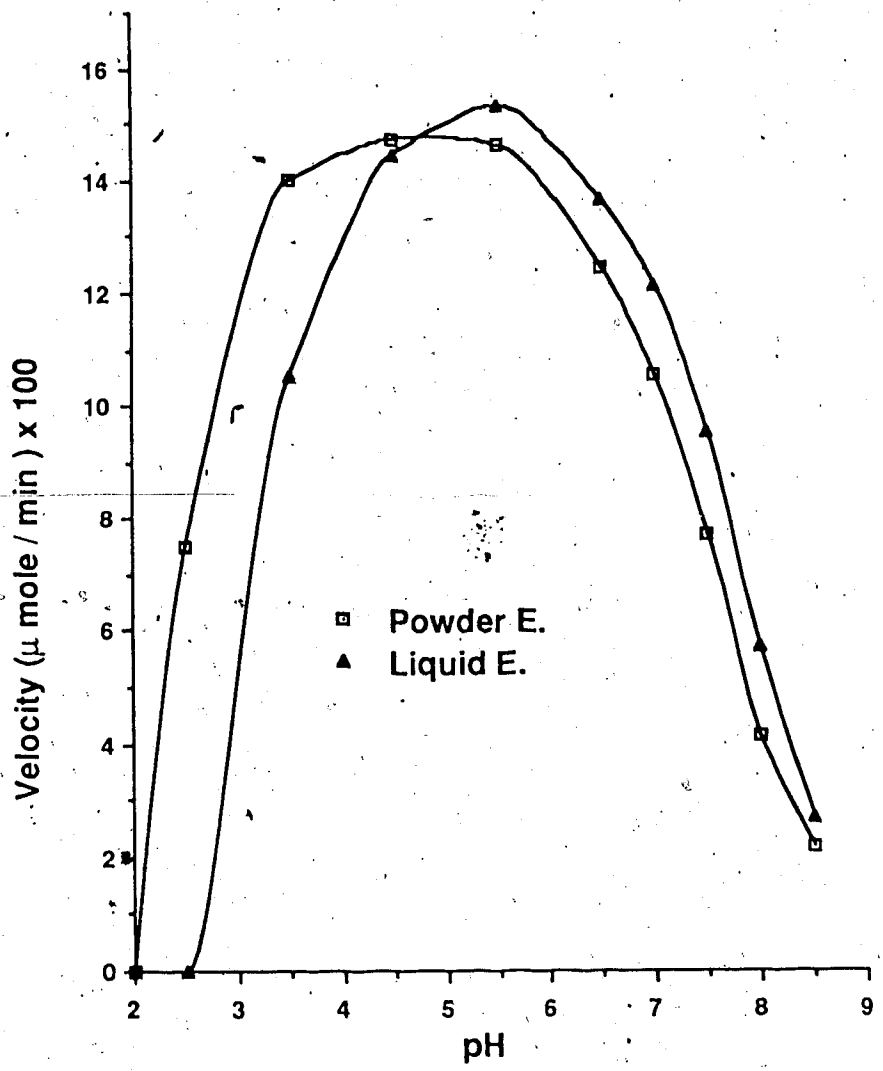


Figure 4.3 Effect of pH on reaction rate with 0.3 M glucose at 25°C.

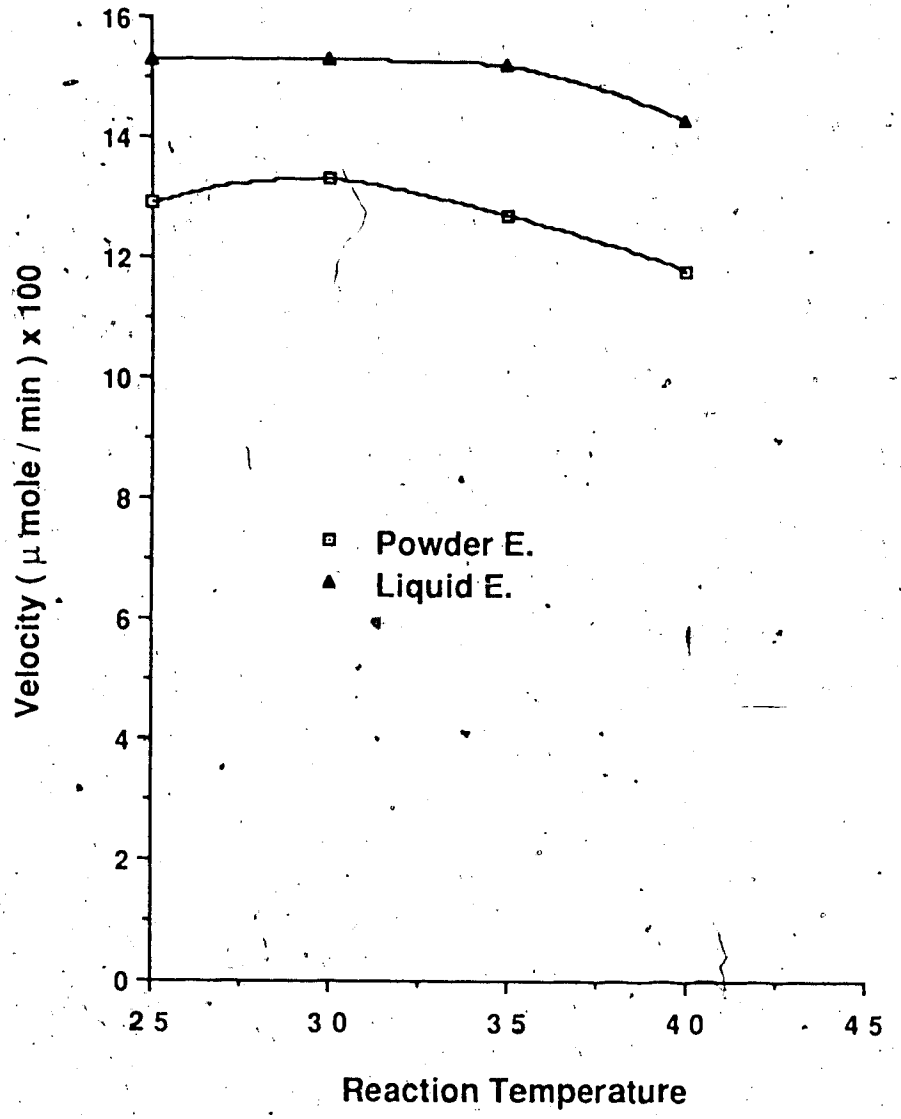


Figure 4.4 Effect of temperature on reaction rate with 0.3 M glucose at pH 5.5.

Usually, the reaction rate of an enzyme increases as the reaction temperature goes up, until heat denaturation of the enzyme begins. However, in the oxidation of glucose by glucose oxidase, the rise of temperature results in a drastic decrease of oxygen solubility in the solution (Figure 4.1). The activity limitation caused by the decrease in oxygen solubility was so severe that the enzyme reaction velocity was found to be substantially the same at 50°F (10°C) or 90°F (32°C) in desugaring process (Scott, 1975b).

4.1.5 Thermal stabilities of the two commercial preparations

The thermal stabilities of the two enzyme preparations are presented in Figures 4.5 and 4.6. Both preparations were quite stable at temperatures of 40 and 50°C. At 55°C, the powder enzyme lost 35% of its original activity after 20 min incubation, while the liquid enzyme lost only 7% under the same conditions. Both preparations lost their activity quickly at 60°C and above, with the powder enzyme losing its activity more quickly than the liquid form. In effect, the liquid enzyme was found to have a superior thermal stability to the powder enzyme. This phenomenon was also experienced by Scott (1975a).

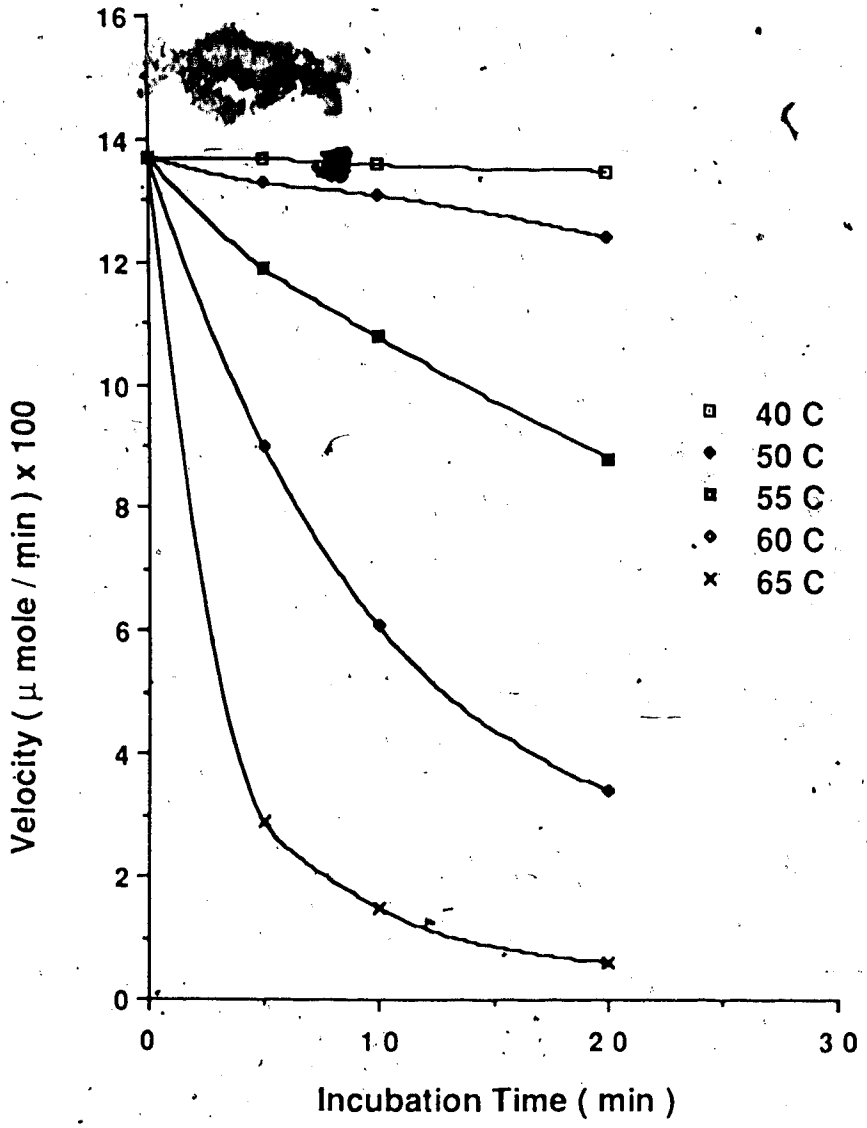


Figure 4.5 Thermal stability of powder enzyme. The remaining activity was assayed with 0.3 M glucose, pH 5.5 at 25°C.

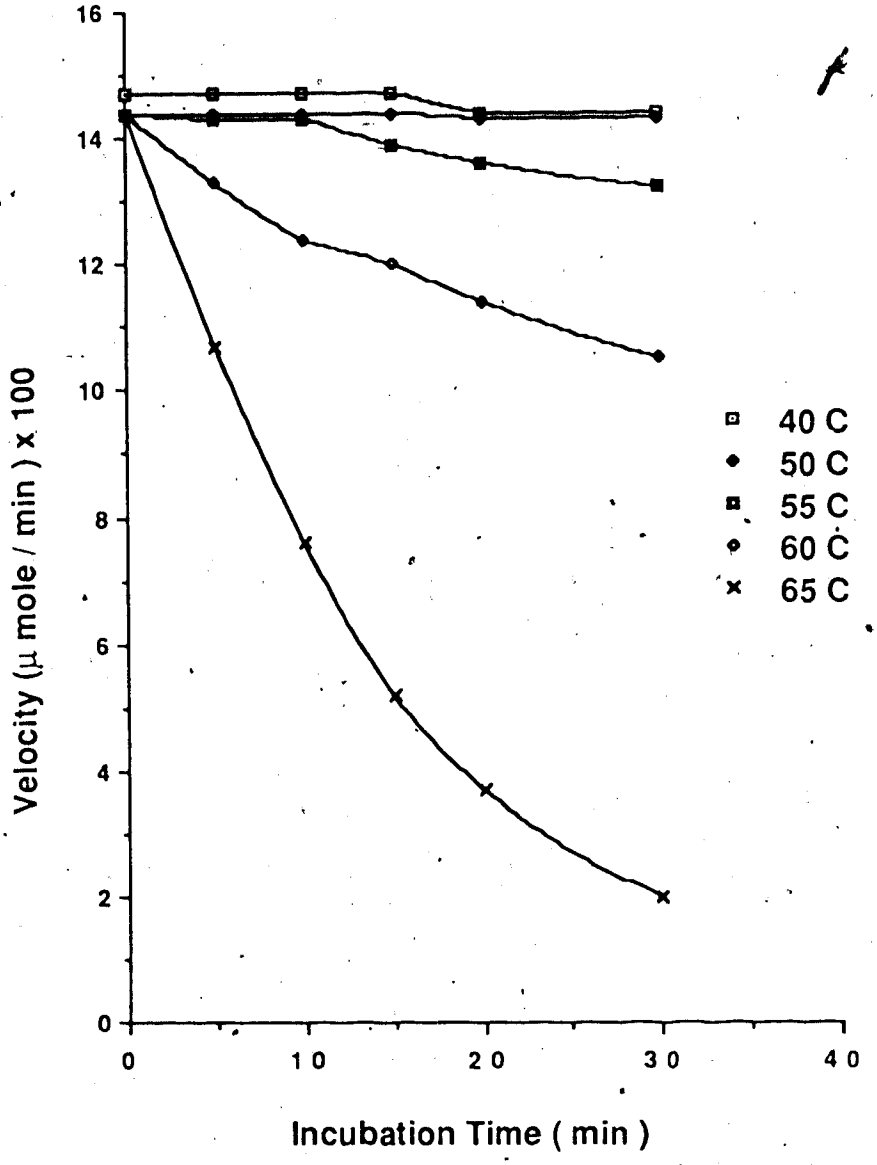


Figure 4.6 Thermal stability of liquid enzyme. The remaining activity was assayed with 0.3 M glucose, pH 5.5 at 25°C.

4.2 Studies on some Analytical Methods

4.2.1 Analysis of glucose by YSI analyzer

Various analytical methods have been used for the determination of glucose in potatoes. These include gas chromatography (GC) (Shaw, 1969), high performance liquid chromatography (HPLC) (Wilson *et al.*, 1981), and the immobilized glucose oxidase method (Valentova *et al.*, 1983). The immobilized glucose oxidase method with the YSI Model 27 Industrial Analyzer was used in this study because of its ease of operation and reasonable accuracy and precision when tested against the HPLC method, which is more complicated and time consuming.

During the preliminary studies of the analytical procedures, the glucose content was found to be unstable in the extracts of raw potato samples (Table 4.7). The glucose content in the extracts increased steadily with time. This increase has been attributed to the activity of some enzymes, such as invertase (Pressey and Shaw, 1966; Pressey, 1969). As the basal invertase activity varied among potatoes of different cultivars and post-harvest conditions (Pressey and Shaw, 1966), the rate of the increase of glucose might also be different in various potato samples (Table 4.7). The blending of the potato tissue also accelerated the invertase activity by bringing substrates closer to the enzymes.

In order to stabilize the extracts, the samples were heated after filtration in a water bath at 80°C for 10 min. The heated samples were refiltered through the Whatman No. 4 paper. Samples thus treated were stable in glucose

Table 4.7 Glucose content of potato extracts with and without heat treatment.

Time Between Sample Prep. and injection (min)	Sample 1 (% Glu.)		Sample 2 (% Glu.)	
	Without Heating	With Heating	Without Heating	With Heating
0	0.39	0.40	0.28	0.29
20	0.42	0.40	0.29	0.29
40	0.45	0.40	0.31	0.29

concentration (Table 4.7). The heat treatment probably destroyed the responsible enzymes, hence stabilizing the sugar content of potato extracts.

To check the reliability of the YSI analyzer, glucose content of the same sample was also determined by an HPLC method, which was kindly done by Dr. N. Low (1986). Results are shown in Table 4.8.

The correlation between the results from the two methods was highly significant ($r=0.986$ with d.f.=3). Therefore, the YSI method was considered adequately reliable for glucose content determination in potatoes.

4.2.2 Color measurement by HunterLab colorimeter

The color of potato chips can be evaluated both subjectively, by comparing the color of the samples with the standard color charts, and objectively, by employing various types of instruments, such as Agtron Reflectance Meter (Ewing *et al.*, 1981), Photovolt Photoelectric Reflectance Meter (Isleib, 1963), Hunter-Gardner Color Difference Meter (Clegg and Chapman, 1962), and HunterLab Color and Color Difference Meter (Habib and Brown, 1956; Maga, 1973).

The HunterLab Model D25M/L-2 Colorimeter was used in this study for objective color evaluation of potato chips and French fries. In order to determine the validity of the instrument, tests were carried out by comparing the results of the objective method with the subjective method. The results are presented in Table 4.9 for potato chips and

Table 4.8 Glucose content of potatoes determined by YSI and HPLC methods.

YSI Method[1] (% Glu.)	HPLC Method[2] (% Glu.)
0.40	0.38
0.64	0.66
0.59	0.59
0.55	0.59
0.50	0.51

1 average of triplicates with std. dev. ranging from 0.0% to 0.006%

2 average of two replicates

Table 4.10 for French fries.

In Table 4.9, samples 1 through 5 were commercial products purchased from local groceries, while samples 6 and 7 were prepared in this lab. Thus, it was possible to cover a wide range of potato chip color shades.

The simple coefficients correlating the subjective scores with objective L,a,b-values were 0.947, -0.967 and 0.862, respectively. The correlations between scores and L-values and between scores and a-values were highly significant (P=0.01), while that between scores and b-values was significant (P=0.05). Thus, the HunterLab L,a,b-values were reliable for measuring the color of potato chips. As L-values showed larger variations among samples than a- and b-values, the L-readings were used to represent the color of potato chips (Habib and Brown, 1956). However, the use of L-values was found to be complicated by the fat content of potato chips; this will be discussed in section 4.3.

The subjective scores and HunterLab L,a,b-values of some French fry samples are shown in Table 4.10. Samples were prepared from potatoes of varying sugar contents to obtain French fries of different shades of color. The samples of various color scores were chosen by comparing the color of the samples with the USDA Color Standards for Frozen French Fried Potatoes (USDA, 1972). As the highest score on the chart was "4", score "5" was used to represent those samples that were even darker than the darkest on the color standard.

Table 4.9 Results of subjective and objective evaluation of color of potato chips.

Sample No.	Subjective Scores[1]	HunterLab Readings[2]		
		L	a	b
1	80.0	61.6	3.3	25.0
2	65.0	58.4	7.0	26.7
3	62.0	56.6	7.2	25.3
4	68.0	56.4	6.1	24.6
5	76.0	62.0	4.4	26.2
6	40.5	36.5	11.5	17.6
7	49.3	38.2	12.3	18.3

1 average scores of five judges with std. dev. ranging from 4.5 to 7.1

2 average of two replicates

Table 4.10 Results of subjective and objective evaluation of color of French fries.

Sample No.	Subjective Scores	HunterLab Readings[1]		
		L	a	b
1	5[2]	23.8	3.3	4.0
2	4.0	24.7	4.4	4.7
3	3.5	26.1	3.0	5.6
4	3.0	27.0	3.1	5.9
5	2.5	27.8	3.2	6.4
6	2.0	29.2	2.9	7.2
7	1.5	30.2	2.5	7.5
8	1.0	31.3	2.4	8.2
9	0.5	32.5	1.9	8.3
10	0.0	32.8	1.5	8.0

1 average of triplicates with std. dev. ranging from 0.15 to 0.31 for L; from 0.12 to 0.38 for a; from 0.12 to 0.40 for b

2 indicating that the sample was even darker than the darkest on the color standard chart

The coefficients correlating the subjective scores with HunterLab L,a,b-readings were 0.994, -0.854 and 0.977, respectively. The correlations between the scores and L-, a- or b-values were all highly significant (P=0.01). Therefore, it was valid to employ the HunterLab to measure the color of French fries. For the same reason as with potato chips, the L-values were utilized to represent the color of French fries.

4.3 Enzyme Treatment of Potato Chips

The enzyme treatment of potato chips was carried out by dipping the potato slices into the enzyme solution at various combinations of temperature and time before frying. Factors that might affect the enzyme treatment, including enzyme level, treatment time and temperature, and the aeration of enzyme solution, were studied.

The HunterLab L-readings of potato chips with various pre-frying treatments are presented in Table 4.11.

4.3.1 Effect of fat content of chips on Hunter L-readings

During the color measurement, the Hunter L-readings of potato chips fried immediately without dipping treatment were often higher than those of chips with dipping treatment, although the dipping treated samples were much lighter in color than those without dipping treatment (Figure 4.7). In preparing the samples for HunterLab measurement, the dipping treated samples were found to be

Table 4.11 The Hunter L-readings of potato chips with various pre-frying treatments (average of three L-readings).

Dipping Temp.(°C)	Dipping Time(Min)	Without Dipping	Enzyme Conc. (Powder E.)			
			0.0% (Control)	0.005%	0.01%	0.02%
20	30	32.3	34.5	34.1	36.8	38.5
	60	33.7	33.7	35.3	35.8	35.6
	90	33.0	32.7	33.3	32.2	32.8
30	30	31.6	34.1	34.9	36.4	36.3
	60	31.8	34.5	34.6	36.5	37.2
	90	31.9	34.5	34.8	34.9	36.5
40	30	37.4	37.5	40.4	38.4	42.3
	60	34.6	35.9	35.3	36.2	36.1
	90	43.2	42.6	42.9	44.5	46.1

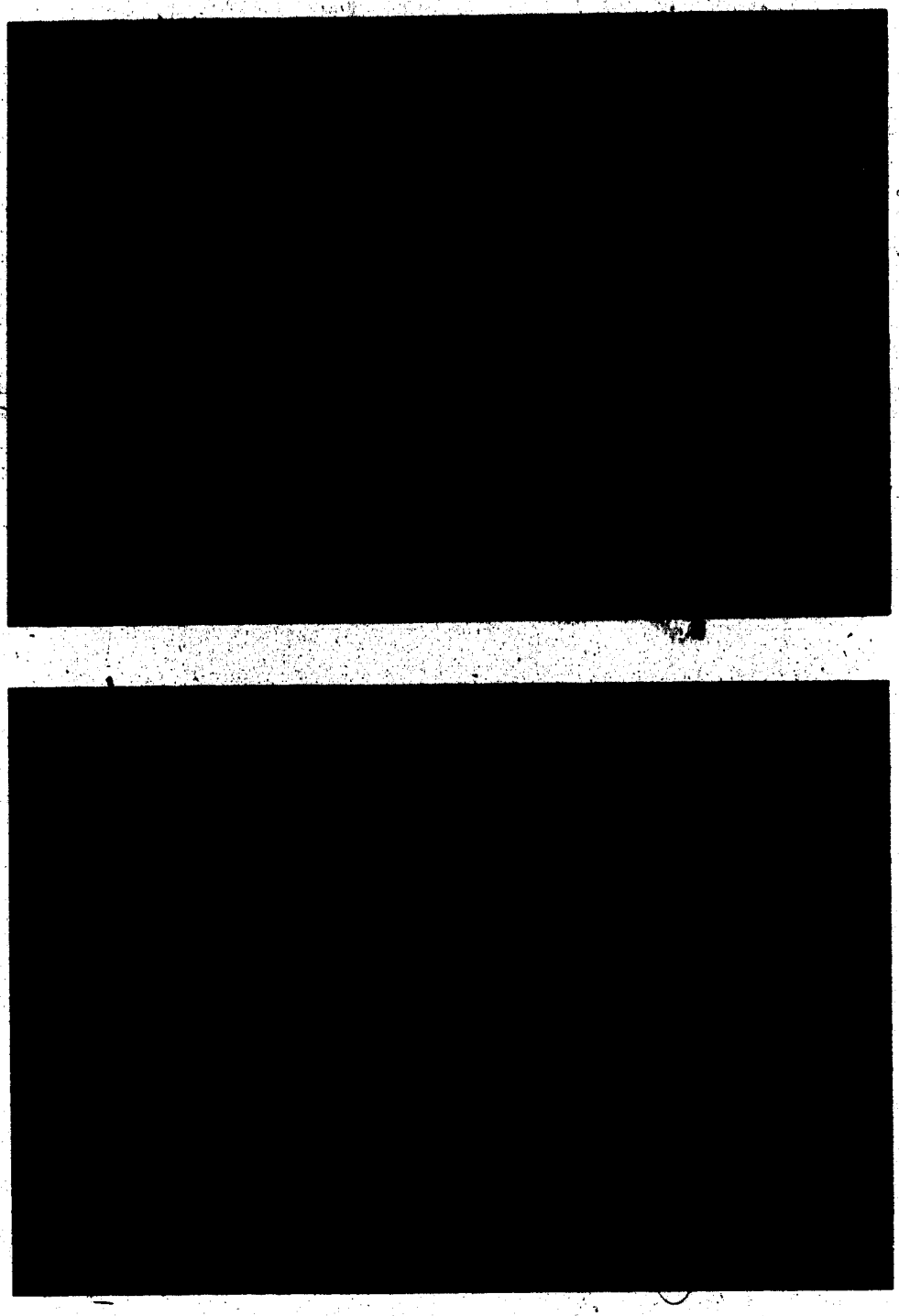


Figure 4.7 Potato chips produced by direct frying (no treatment), or dipping the slices in tap water (control), or in 0.1% liquid enzyme solution (0.1% L.E.). Dipping was at 40°C for 30 min (a) or 60 min (b).

more greasy, and more easily smoothened with a spatula than the samples with no dipping. The oil content of samples with and without dipping was determined and the results are shown in Table 4.12.

The oil content of chips without dipping was about 45%, while the average oil content of dipped samples, either in water or in enzyme solutions, was about 51%. A T-test indicated no significant difference in oil content between samples treated with either 0.1% liquid enzyme or 0.05% powder enzyme. Duncan's Multiple Range Test (DMRT) showed that the fat content of chips without dipping was highly significantly lower than those of dipped samples. However, among the dipped samples, whether in water or enzyme, no significant difference in oil content was detected.

It could be postulated that the higher oil content of dipped samples was responsible for their relatively lower Hunter L-readings. The high amount of oil in the samples would probably absorb more light, thus less light was reflected, giving a darker appearance to the surface than it actually was. Therefore, the Hunter L-readings could not be used to compare the color of the chips made with and without dipping treatment. However, since there was no significant difference between the oil content of potato chips with various dipping treatments, their color could be compared directly using Hunter L-values.

Table 4.12 Oil content (%) of potato chips prepared with or without dipping treatment (average of two replicates).

Enzyme Level	Without Dipping	Dipping			
		40°C, 30 min		40°C, 60 min	
		In Water	In Enzyme	In Water	In Enzyme
0.1 % Liquid E.	45.32	49.99	50.27	49.51	51.99
0.05 % Powder E.	45.35	51.17	51.14	49.99	51.22

4.3.2 Effect of enzyme concentration

The enzyme concentrations studied ranged from 0.005% to 0.02% for powder enzyme. The Hunter L-readings were shown to increase as the enzyme concentration increased (Table 4.11). Using factorial ANOVA (analysis of variance), the difference in the Hunter L-readings at various enzyme concentrations was found to be significant (P=0.05). The DMRT results of the Hunter L-readings at different enzyme concentrations are shown in Table 4.13.

The analysis of data presented in Table 4.11 shows that, although the color of chips treated with the enzyme at all three concentrations was lighter than the color of the control (Treatment I), only the color of the sample treated with 0.02% enzyme (Treatment IV) was significantly lighter (P=0.01) than that of the control (Table 4.13).

In order to test whether higher enzyme concentrations would further improve the color of potato chips, experiments were carried out at enzyme concentrations of up to 0.1% powder enzyme (0.2% liquid enzyme equivalent). The results are shown in Table 4.14.

The Hunter L-readings of potato chips increased as the enzyme concentration increased up to 0.05% powder enzyme (0.1% liquid enzyme), then decreased as the enzyme concentration was doubled to 0.1% (0.2% liquid enzyme). Therefore, the enzyme concentration of 0.05% (powder) or 0.1% (liquid) was considered to be the optimum level for treatment of potato chips. As the glucose content of potato

Table 4.13 Duncan's Multiple Range Test of color (Hunter L) of potato chips treated with enzyme at various concentrations.

Treatment	:	I	II	III	IV
Enzyme Conc. (%)	:	0.0	0.005	0.01	0.02
Means of L-readings	:	35.56	36.18	36.86	37.93
5% Level	:	——	——	——	—— *
1% Level	:	——	——	——	——

* indicating no significant difference among treatments on the same line

Table 4.14 The Hunter L-readings of samples treated with enzyme at various concentrations (average of two replicates).

Dipping Conditions	Control (0 % E.)	0.01 % E.	0.05 % E.	0.1 % E.
40°C,30 min (Powder Enzyme)	43.9	45.3	46.6	44.7
40°C, 60 min (Powder Enzyme)	43.1	43.3	47.3	45.6
40°C, 30 min (Liquid Enzyme)[1]	37.4	38.4	42.1	41.5

1 the concentrations for liquid enzyme were 0.02%, 0.10%, and 0.20%, v/v, respectively.

samples (about 0.3%) was very low, the limiting factor of the enzyme reaction would be the concentration of glucose. Hence, enzyme concentrations higher than 0.05% (powder) or 0.1% (liquid) did not result in additional glucose oxidation.

4.3.3 Effect of dipping time

Dipping time for the potato slices ranged from 30 to 90 min. Factorial ANOVA of the data in Table 4.11 showed that the interaction of dipping time and dipping temperature had highly significant effect on the chip color (Hunter L-readings). However, neither the dipping time nor temperature itself caused significant variation of the chip color.

Further study on the effect of dipping time on enzyme treatment was performed where enzyme concentration was kept constant (0.05% for powder and 0.1% for liquid) and the temperature was also kept constant at 40°C. The results (Table 4.15) show that Hunter L-readings of samples with 60 min dipping were higher than those with 30 min dipping. However, the lighter color of the 60 min dipped samples might be a result of the leaching of solutes into the dipping solution rather than of the enzyme reaction. In any case, the increase in L-values of the enzyme-treated samples over the control samples between 30 and 60 min dipping was not significantly different. This and the economic and practical reality in the industry would favor the shorter

Table 4.15 The Hunter L-readings of potato chips with different dipping time (average of two replicates).

	30 min Dipping		60 min Dipping	
	Control	0.05 % E. [1]	Control	0.05 % E. [1]
Powder Enzyme	44.0	46.1	44.0	47.4
Liquid Enzyme	46.1	48.1	47.7	50.9

1 enzyme concentrations were 0.05% (w/v) for powder enzyme and 0.10% for liquid enzyme

dipping time of 30 min.

4.3.4 Effect of dipping temperature

Like dipping time, different dipping temperatures were found to be not statistically significant in causing the variance of Hunter L-readings. However, the average Hunter L-values increased as the dipping temperature was raised from 20 to 40°C.

While the dipping temperature did not appear to significantly affect the enzyme reaction velocity, the leaching rate of the solutes from the surface of the potato slices might be affected by the temperature. Vukov (1977) stated that the diffusion coefficient of sugars from plant tissue, such as beet tissue, to the extraction solution is dependent on the temperature of the solution. He also stated, however, that the diffusion rate of solutes from living, sound and intact plant tissue was very low when compared with the damaged cells caused by heating or shearing stress. In the enzyme treatment of potato slices, the dipping temperature, as high as 40°C, was well below the cell-damaging temperature of 70-80°C. Therefore, the leaching rate of solutes in the dipping treatment would be slow, and the dipping temperature in the range of 20-40°C might not have a very significant effect on the color of potato chips. Hence, on the balance, the difference in the color between the control and the enzyme-treated products could be attributed mainly to the enzyme reaction to reduce the glucose content of

potato slices.

To take advantage of the slight improvement in chip color due to the increase in dipping temperature, 40°C dipping was used in subsequent experiments.

4.3.5 Effect of aeration

As dissolved oxygen in enzyme solution might also affect the rate of enzyme reaction, compressed air was bubbled through the enzyme solution during the dipping treatment in order to ensure adequate O_2 for the reaction. For the dipping length of both 30 and 60 min, the Hunter L-readings of the chips with aeration showed no increase over the samples without aeration (Table 4.16). This indicated that aeration, at least in this application, was not necessary for the oxidation reaction by glucose oxidase. This may be because, during the dipping treatment, the solution was stirred frequently, which might be adequate to provide O_2 required for the reaction. Also, with glucose concentration as low as 0.01% in the solution, the rate limiting factor of the enzyme reaction would be the glucose, rather than the dissolved oxygen.

4.3.6 Conditions and limitation of the glucose oxidase treatment

The preceding experimental results indicate the following optimum conditions for the enzyme treatment of potato chips to reduce browning: 0.05% solution of glucose

Table 4.16 The Hunter L-readings of chips with or without aeration (average of two replicates).

Dipping Time	Without Dipping	40°C Control	40°C 0.05% E.	40°C, 0.05% E. With Aeration
30 min	40.6	41.3	42.6	42.7
60 min	40.5	42.6	45.5	45.0

oxidase with the activity of 1500 GOU/g (powder form) or 0.1% of glucose oxidase with the activity of 750 GOU/mL (liquid form); temperature about 40°C; and dipping time of at least 30 min.

However, even under the optimum conditions, the enz[illegible] treatment was not always effective in improving the color of chips. It was noted that glucose content of potatoes played a crucial role in determining whether observable color improvement could be achieved by glucose oxidase treatment. When glucose content of potatoes was higher than 0.4%, the chips made from these tubers were too dark in color, and the effect of enzyme treatment could not be observed. Only with the potatoes of marginal quality with respect to chip production, i.e. with glucose contents between 0.2 and 0.4%, did the enzyme treatment appear to reduce the browning coloration noticeably.

When potatoes of marginal glucose contents were used for making chips, the improvement of the chip color, i.e. the increase of Hunter L-readings after enzyme treatment, was statistically significant. The increase in L-values was in the range of 2-5 units, which corresponded to about 3-8 units increase of color score on the Standard Color Chart. The treatment offered only limited improvement in the chip color because of the complicated nature of browning reactions in the product.

The browning discoloration of potato chips depends not only on the glucose content, but also on the content of

other chemical components, such as fructose, sucrose, ascorbic acid and amino acids (Wursch and Schaller, 1972; Shallenberger *et al.*, 1959). Glucose oxidase oxidizes glucose to gluconic acid, thus blocking it from entering the browning reaction. However, other reactants, such as fructose and sucrose, can not be oxidized by the enzyme, leaving them free to participate in the nonenzymatic browning reactions. The color of potato chips is known to be very sensitive to the reducing sugar content. As pointed out by Smith (1975b), potatoes containing more than 0.25% reducing sugars rarely produced acceptably colored potato chips. Thus, even if all the glucose in potato slices were oxidized by glucose oxidase, fructose and sucrose, which remain untouched, could still cause browning of the chips through Maillard reaction and caramelization.

Furthermore, it was impossible that all the glucose in the potato slices could be oxidized by glucose oxidase. While the glucose on the surface of the slices, or from damaged cells, was available for the enzyme reaction, the glucose in the undamaged inner part of slices could not be easily reached by the enzyme.

Glucose contents of potato slices with different treatments are presented in Table 4.15. The dipping treatment, either in water (control) or in enzyme solution, reduced the glucose content of potato slices by up to 42%. However, the reduction by the enzyme treatment did not appear to be significantly different from that by water

Table 4.17 Glucose content (%, w/w) of potato slices with different treatments (average of two replicates).

Dipping Temp.(^{o}C)	Dipping Time (min)	Glucose Content (% W/W)		
		Raw Potato Slices	Control (Tap Water)	0.02 % Enzyme
20	30	0.32	0.24	0.23
	60	0.27	0.19	0.20
	90	0.38	0.23	0.22
30	30	0.29	0.24	0.19
	60	0.32	0.18	0.18
	90	0.37	0.20	0.17
40	30	0.25	0.17	0.16
	60	0.31	0.18	0.16
	90	0.28	0.12	0.13

alone. The results clearly showed that not all glucose could be eliminated from the slices, either by leaching alone or by leaching plus glucose oxidase treatment. More than 50% of glucose, not to mention fructose and sucrose, remained, basically in the inner tissue of the thin slices. Since the slices were fried to near dryness, browning reaction did not occur only on the surface, but throughout the cross section of the slices. Hence, even if the browning of the surface was reduced, the intensity of the color developed in the inner portion of the slices would still be the controlling factor. This was in contrast with French fries, where color of inner tissue did not develop to the same extent, as will be discussed in the succeeding sections.

4.3.7 Color uniformity and fat content of enzyme treated chips

While the intensity of browning of potato chips could be measured in terms of Hunter L-values, the uniformity of the color on individual chips could not be so represented. Samples treated with glucose oxidase were usually more uniform in color than the control samples (Figure 4.7). The brown or dark spots and rings in potato chips are usually caused by disorders of potatoes, such as sugar end, which usually leads to higher reducing sugar content (Iritani *et al.*, 1973). When treated with glucose oxidase, the parts on the slices with high reducing sugar content might favor the enzyme reaction. Therefore, the enzyme treatment was able to

reduce the browning on such spots, thus improving the uniformity of color of the chips. However, the enzyme treatment had some disadvantages in the production of potato chips. Not only did it increase the cost of production due to this additional treatment, but it increased the oil content of the chips. As discussed previously, chips produced from the slices dipped in either water or enzyme solution absorbed more oil than those from slices not dipped (Table 4.12). High oil content of potato chips is undesirable to the processors because of the greater consumption of fairly costly oil, and to the consumers because of high caloric values from the oil.

4.4 Enzyme treatment of French fries

4.4.1 Pre-frying treatment of French fries

4.4.1.1 Various pre-frying treatments and color of final products

French fries could be made by either directly frying the potato strips in hot oil, or by first giving the strips various pre-frying treatments, e.g. blanching. The blanching process could be achieved by using live steam under atmospheric pressure, or with hot water at various temperatures. Pre-frying treatments affect the quality of French fries, especially the color of the products. Various pre-frying treatments were emplo[illegible]d in this experiment in order to determine their effect on the color of French

fries. The color scores of French fries with various pre-frying treatments are presented in Table 4.18. Steam blanching was carried out with live steam under atmospheric pressure for 7 min. The hot water blanching was the simulation of the blanching procedures employed by I & S Produce Ltd., as discussed in section 2.3.2. As the score for the darkest samples on the color standard was 4, with the lightest being 0, samples that were darker than 4 were given a score of >4. The commercially acceptable French fries usually score in the range of 1-2 (Weaver and Nonaka, 1976).

Using potatoes with glucose content of approx. 0.5%, French fries produced without blanching or with steam blanching were too dark in color, being scored higher than 2, while those produced with hot water blanching were too light, with scores <1. Even with potatoes containing as much as 1.0% glucose, the hot-water blanching treatment still enabled the production of French fries with acceptable color (score ≤2). It appeared that the color of the French fries was dependent on the extent of leaching of soluble solids from potato strips during the various pre-frying treatments. To this end, the hot water blanching was the most effective treatment to remove solutes, especially sugars. In steam blanching, a possible loss of solutes was through the initial condensation of steam on the strip surface, which subsequently dripped from it, and this appeared to be minimal since French fries subsequently made were still very

Table 4.18 Color scores of French fries with different pre-frying treatments (average of two replicates).

Glucose Content (%)	Without Blanching	Steam Blanching	Hot Water Blanching
0.41	> 4	> 4	0.5
0.52	> 4	> 4	0.5
1.0	—	—	1.5

dark. In fact, the leaching of solutes during the hot water blanching was so severe that the blanched strips had to be dipped in a glucose solution to achieve the desired French fry color. This is the common industrial practice, which results in higher production costs. Furthermore, excessive loss of solutes causes the loss of some desirable flavor, texture and taste in the product.

4.4.1.2 Effect of different blanching methods on flavor and texture of French fries

Because of the foregoing disadvantages of hot water blanching in the production of French fries, various attempts had been made to replace or eliminate hot water blanching. These included surface-freezing and warm water leaching of potato strips (Weaver *et al.*, 1972), and pre-frying and water leaching of potato strips (Weaver and Nonaka, 1976). In the present investigation, the merit of the steam blanching in retaining desirable flavor of the fries was recognized, and it was thought that it might be possible to improve the color of the fries with glucose oxidase treatment. Before proceeding with the treatment, however, it was necessary to determine whether, in fact, steam blanching produced French fries of superior sensory quality, except for color, to water blanching. To do this, potato strips were steam blanched for 7 min, followed by immersion in 35°C water for 15 min, to simulate the enzyme treatment before frying. The flavor and texture of the fried products were compared by 35 untrained panelists with those

water blanched at 80°C for 15 min, as described by Brown and Morales (1970), before frying. The sensory results are presented in Table 4.19.

The sensory test results revealed that 24 out of 35 panelists chose the product with steam blanching to have stronger flavor than that with water blanching, which was statistically significant at P=0.05. On the other hand, and of similar significance, 24 out of 35 judges indicated that French fries made with hot water blanching had a better texture than those with steam blanching. The superior texture of French fries with hot water blanching might be due to the activity of enzymes such as pectin methylesterase during the blanching. Pectin methylesterase was activated by low temperature blanching (70-82°C for 3-15 min) (Lindsay, 1985). The enzyme hydrolyzed pectic substances on potato cell wall and middle lamella to produce pectinic and pectic acids. Ca^{2+} released from the partially gelatinized starch would form bridges with carboxyl groups between adjacent pectin chains, causing the potato texture to become firmer (Haydar *et al.*, 1980). However, pectin methylesterase was inactivated if blanching was performed at 88-100°C for 3 min (Lindsay, 1985). Therefore, similar activity would not be possible during steam blanching. Nevertheless, this experiment confirmed that steam blanching deserved further investigation if color problems could be solved, and glucose oxidase might serve this purpose.

Table 4.19 Evaluation of flavor and texture of French fries produced with steam blanching and water blanching, by 35 untrained panelists.

	Number of Judges Voted	
	French Fries With Steam Blanching	French Fries With Hot Water Blanching
Stronger Flavor	24 *	11
Better Texture	11	24 *

* : pair comparison indicates siginificant difference at P = 0.05

4.4.2 Enzyme treatment of French fries

For glucose oxidase treatment of French fries which had been steam blanched, the effect of enzyme concentration, treatment time and temperature was studied using Hunter L-readings of the fries, glucose content of potato strips, and the pH of the dipping solution as the criteria.

4.4.2.1 Hunter L-readings

The Hunter L-readings of French fries treated with glucose oxidase solution at various concentrations, temperatures and times after steam blanching are shown in Table 4.20.

Duncan's Multiple Range Test revealed that in Run 1 the Hunter L-readings of all enzyme treated samples were significantly higher than those of the control (P=0.01). However, L-values of samples treated with enzyme at different concentrations did not differ significantly from one another, although the higher the concentration, the higher the L-value.

In Run 2, only Treatment IV (0.20% L.E.) produced French fries having significantly higher Hunter L-readings (P=0.01) than Treatment I (control), but Treatments II (0.05%) and III (0.10%) were not significantly different from the control. Nevertheless, the trend was that the higher the glucose oxidase concentration, the greater the color improvement.

The length of treatment time also affected the color significantly (P=0.01). The longer time produced a lighter

Table 4.20. Hunter L-readings of French fries treated with various enzyme levels (average of three L-readings).

Treatment Conditions			Hunter L Readings			
Run	Dipping Temp.(°C)	Dipping Time(min)	Control[1]	0.05 % L.E.[2]	0.10 % L.E.[2]	0.20 % L.E.[2]
Run 1	25	10	27.8	28.2	28.7	29.3
		15	28.7	29.9	29.6	30.0
	35	10	27.8	28.7	29.2	28.3
		15	29.0	30.3	30.2	30.2
Run 2	25	10	28.7	28.7	28.7	30.1
		15	29.5	29.3	29.9	30.9
	35	10	30.2	31.5	30.2	30.5
		15	31.2	32.5	31.8	32.8

1 in tap water

2 liquid enzyme

colored French fries, essentially, it was believed, due more to the leaching of solutes than further enzymatic reaction. This was because the rate of diffusion of solutes from potato strips structurally damaged by heat during steam blanching was much faster than from raw potato strips (Vukov, 1977). In fact, it was observed that dipping, either in water or enzyme solution, beyond 15 min resulted in French fries of unacceptably light color.

The effect of temperature was not significant in Run 1, but was highly significant in Run 2. Higher treatment temperatures usually produced French fries with higher Hunter L-readings, indicating a greater enzymatic activity and/or faster leaching of solutes at higher temperatures.

Generally, the glucose oxidase treatment resulted in as much as a 1.5 unit increase of Hunter L-readings. This was equivalent to about a 0.8 decrease of color score (i.e. lighter color). Also, the color uniformity was improved slightly by the enzyme treatment (Figure 4.8).

4.4.2.2 Glucose content of potato strips and pH values of dipping solutions

The glucose content (w/w, dry basis) of potato strips with various treatments is presented in Table 4.21.

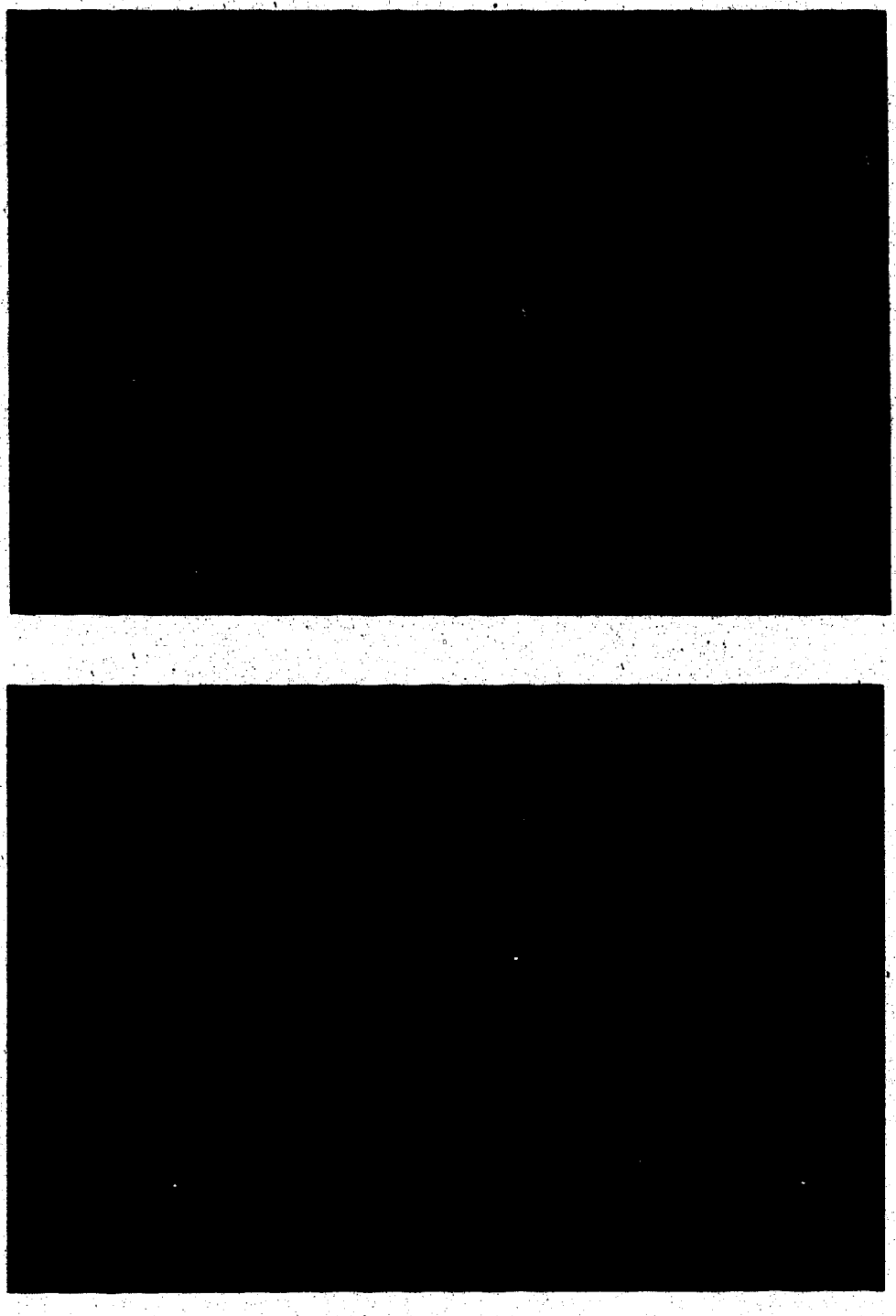
The decrease of glucose content in both runs, caused by the 0.1% liquid enzyme treatment and by dipping in water (control) after steam blanching, was statistically significant ($P=0.01$) when compared with raw potato strips. Glucose content in the enzyme treated samples was significantly

Table 4.21 Glucose content (% dry basis) of potato slices with various treatments (average of two replicates).

Run	Dipping Temp.(^{0}C)	Dipping Time (min)	Raw Potato	Control[1]	0.10 % L. E.[2]
Run 1	25	10	2.72	2.38	1.88
		15	2.72	2.05	1.85
	35	10	2.78	2.06	1.86
		15	2.78	1.95	1.82
Run 2	25	10	3.08	2.92	2.57
		15	3.08	2.42	2.26
	35	10	3.03	2.81	2.28
		15	3.03	2.11	2.05

1 in tap water

2 liquid enzyme



(a)

(b)

Figure 4.8 French fries produced with steam blanching followed by direct frying (no treatment), or dipping in tap water (control), or in liquid enzyme (L.E.). Dipping was at 25°C for 10 min (a) or 15 min (b).

lower ($P=0.01$) than that in the control. However, temperature and time of dipping had no significant effect on glucose content of the samples.

The reduction of glucose content in French fries by glucose oxidase treatment appeared to be much faster and color improvement more apparent than in potato chips. This difference might be due to: (1) higher glucose content in potato tubers for French fries (0.6%) than for potato chips (0.3%); and (2) faster diffusion rate of glucose in steam blanched French fry strips than in intact chip slices. Potatoes with lower glucose content had to be chosen for chips because chip color is much more sensitive to high glucose content. Therefore, the substrate concentration for enzyme reaction in the treatment of potato strips was almost [redacted]ce that in the enzyme treatment of potato slices. As the rate limiting factor for the enzyme reaction might be glucose concentration, as discussed previously, the reaction rate in the treatment of potato strips could be much faster than in the treatment of potato slices. Furthermore, the diffusion rate of glucose in steam blanched potato strips was most likely faster than that in intact potato slices (Vukov, 1977). Thus, the potato strips would be more likely to provide more glucose to the strip surface and to the dipping solution.

The production of gluconic acid by the enzyme could also be used as an indicator for the enzyme activity. The amount of the acid produced could be related to the change

in the pH of the dipping solution. The pH's of the dipping solution for various treatments are presented in Table 4.22.

While the pH of the control remained constant, the pH's of enzyme solutions decreased as the treatment progressed. The decrease of pH was greater in the solutions of higher enzyme concentration. This indicated that more glucose was oxidized when potatoes were dipped in the solution of higher enzyme concentration and for a longer time.

4.4.3 Oil content of French fries prepared with various treatments

As in potato chips, the oil content of French fries concerns both processors and consumers. Various pre-frying treatments might affect the oil content of the final products. The oil contents of French fries prepared with different procedures are presented in Table 4.23. The results of Duncan's Multiple Range Test of the oil contents are also presented (Table 4.24).

Factorial ANOVA indicated no significant difference between replicates or different dipping temperatures. However, the oil content of hot water blanched samples was significantly higher (P=0.01) than that of the other three treatments. Among the steam blanched samples, there was no significant difference (P=0.05) in oil contents of French fries with dipping treatment in water or in enzyme solution. However, the oil content of enzyme treated samples was significantly higher (P=0.05) than that of samples without

Table 4.22 pH of various dipping solutions (average of two replicates).

Dipping Temp.(^{0}C)	Enzyme Level	Begining Of Dipping	10 min Dipping	15 min Dipping
25	Control[1]	6.4	6.4	6.4
	0.05% L.E.[2]	6.4	6.3	6.2
	0.10% L.E.	6.4	6.2	6.1
	0.20% L.E.	6.4	6.1	6.0
35	Control	6.3	6.3	6.3
	0.05% L.E.	6.3	6.1	6.1
	0.10% L.E.	6.3	6.1	6.0
	0.20% L.E.	6.3	6.0	5.9

Table 4.23 Oil content of French fries prepared with various processing procedures (average of two replicates).

Dipping Conditions	Oil Content (% dry basis)			
	Hot Water[1] Blanched	Steam Blan.[2] No Dipping	Steam Blan. Control[3]	Steam Blan. 0.1% L.E.[4]
25°C 15 min	15.45	12.37	11.14	12.36
	16.99	11.81	12.76	13.38
35°C 15 min	16.46	11.96	13.10	14.77
	15.62	11.30	15.15	14.00

1 80° C, 15 min.

2 7 min under atmospheric pressure

3 dipped in tap water following steam blanching

4 liquid enzyme

Table 4.24 Duncan's Multiple Range Test of oil content of French fries prepared with various pre-treatments.

Treatment	Steam Bln., No Dipping	Steam Bln., Control	Steam Bln. 0.1% L.E.	Water Bln.
Means of Oil Content(%)	11.86	13.04	13.63	16.13
5% Level	_____	_____	_____	*
1% Level	_____	_____	_____	

* indicating no significant difference among treatments on the same line

dipping.

The oil content of French fries might be associated with the extent of leaching of solutes from French fry strips during the pre-frying treatments. It was the lowest in French fries pre-treated with steam blanching but without dipping, where virtually no leaching of solutes occurred. In the samples with hot water blanching, the leaching of solutes was severe, and the oil content was the highest among the four treatments. Thus, the leaching of solutes resulted not only in the loss of flavor components, but also in the increase of oil content of fried potato products. Glucose oxidase treatment not only improved color of the products by reduction of glucose content, but also minimized the loss of flavor by reducing the leaching of solutes.

4.5 Sensory Evaluation of Flavor of Enzyme Treated Potato Chips and French Fries

Sensory evaluation was carried out to determine whether the glucose oxidase treatment imparted any off-flavors on potato chips and French fries, using a descriptive test comparing two samples, enzyme treated and control.

Of 17 judges who participated in sensory evaluation, none reported any off-flavor on enzyme treated potato chips. Thirteen out of 17 judges found no difference in flavor between the control and enzyme treated samples. Three judges reported that the control sample was sweeter than the enzyme treated ones, while one reported the reverse.

Of 17 judges, none reported any off-flavor on enzyme treated French fries. Twelve out of 17 judges reported no difference in flavor of enzyme treated sample and the control. Four judges found that the control was sweeter than the enzyme treated sample, while one judge reported the reverse.

Therefore, glucose oxidase could be used to improve color of potato chips and French fries with no undesirable effect on the flavor of the products.

5. CONCLUSIONS AND SUGGESTIONS FOR FUTURE RESEARCH

5.1 Conclusions

The present study investigated the reaction properties of two commercial glucose oxidase preparations and the application of the enzyme in minimizing the undesirable non-enzymatic browning in potato chips and French fries. The conditions under which the enzyme treatments were carried out and the effects of these treatments on some physical and sensory properties of potato chips and French fries were also examined. The following are some conclusions that may be drawn from the results obtained.

5.1.1 Activity characteristics of glucose oxidase

1. The assayed activities of the two commercial glucose oxidase preparations (2870 IU/g for DeeO powder enzyme and 1560 IU/mL for Fermcozyme liquid enzyme) were much higher than the declared activities.

2. The Michaelis constants for the powder and liquid enzyme preparations were 0.027 M and 0.026 M glucose, respectively.

3. The optimum pH for enzyme reaction was about 5.0 for powder enzyme and 5.5 for liquid enzyme. Both enzyme preparations had a relatively broad effective pH range of 4 to 7.

4. Temperature, in the range of 25 to 40°C, had practically no effect on the reaction velocity of the

enzyme.

5. Glucose oxidase lost its activity at ≥55°C. Liquid preparation had a superior thermal stability to powder enzyme.

5.1.2 Application of the glucose oxidase treatment in the production of potato chips and French fries

1. The color of potato chips, in terms of lightness and uniformity, was improved by glucose oxidase treatment.

2. Improvement of color in potato chips was possible only when the potatoes used contained <0.3% glucose. The greatest benefit of the enzyme treatment was, therefore, obtained when the potatoes were of marginal quality for chip production with respect to glucose content.

3. To obtain optimal results, enzyme treatment could be carried out by dipping the potato slices in 0.05% (w/v) DeeO powder enzyme solution, or in 0.10% (v/v) Fermcozyme liquid enzyme at 40°C for at least 30 min, and preferably 60 min.

4. In the processing of French fries, steam blanching of potato strips avoided the severe loss of flavor components which was normally encountered in hot water blanching.

5. The discoloration of French fries prepared with steam blanching could be minimized by leaching the blanched potato strips in water at 35°C for up to 15 min before frying.

6. The glucose oxidase treatment of steam-blanched potato strips reduced the browning of the French fries and made the color more uniform in comparison with the water leaching treatment.

7. The enzyme treatment could be carried out by dipping the blanched strips in 0.20% (v/v) Fermcozyme liquid enzyme solution or in 0.10% (w/v) DeeO powder enzyme solution at ≤35°C for up to 15 min.

8. Potatoes containing as much as 0.6% (w/v, wet basis) glucose could be used to produce French fries with acceptable color by steam blanching followed by glucose oxidase treatment.

9. The glucose oxidase treatment had no adverse effect on the flavor of potato chips or French fries.

10. The dipping treatment, either in water or enzyme solution, resulted in higher oil content in potato chips than that in the chips produced without dipping. However, steam blanching followed by dipping in water or enzyme solution produced French fries of lower oil content than those produced through hot water blanching.

5.2 Suggestions for Future Research

1. The activity assay method for glucose oxidase needs further investigation. Various assay methods should be compared so that the confusions caused by some assay methods can be clarified.

2. The present project in this lab focuses on the application of glucose oxidase enzyme in the processing of potato granules, potato chips and French fries. The application of the enzyme could be extended to prevent or minimize the undesirable nonenzymatic browning in other dehydrated potato products, such as potato flour, diced potatoes, shoestring potatoes and potato flakes, in which sulfites have often been used as anti-browning agents (Willard, 1975; Kueneman, 1975). The study on the application of this enzyme could also be extended to other food commodities which suffer from a similar undesirable nonenzymatic reaction.

3. The steam-blanching and water-leaching process for French fry production needs a more thorough investigation. The surface cellular structural damage described in two U.S. patents, i.e. the surface-freezing process (Weaver and Hautala, 1972) and the pre-frying water-leaching process (Weaver and Nonaka, 1976), could be achieved instead with steam blanching. If the steam blanching of potato strips was carried out at high temperature (high pressure) for a short period of time, the structural damage could be confined to only the surface layer of potato strips. Thus, the subsequent water leaching would remove solutes from the surface layer only. By steam blanching the strips again after water leaching to heat them more completely or frying the strips directly, the severe loss of flavor components associated with water blanching could be avoided. Comparing

this to the patented surface treatments, the steam-blanching process has advantages such as ease of operation, low cost, and no chemical substances are introduced to the products.

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