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RESEARCH NOTE:

HEAT FLUX SENSORS TO MEASURE EFFECTIVE THERMAL CONDUCTIVITY OF MULTILAYERED PLASTIC CONTAINERS

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ABSTRACT

A technique was developed to determine effective thermal conductivity of a retortable multilayered plastic container. The technique involved measuring the heat flux through the plastic container wall using a heat flux sensor under a known temperature difference when heat transfer coefficients on both sides of the container were very large. Effective thermal conductivity of the container was found to be independent of temperature in the range 20–80C and had an average value of 0.254 ± 0.008 W/mK.

INTRODUCTION

Shelf stable food products in retortable multilayered plastic containers, which can be reheated in a commercial counter-top microwave oven, are increasingly popular in the market. In addition to microwaveable characteristics, other consumer preference features of plastic containers over metal cans are their attractive appearance, light weight, flexibility, stackability, and capabilities to serve and store foods. the plastic container has at least 5 layers one of which is either ethylene vinyl alcohol polymer (EVOH) or polyvinyldiene chloride polymer (PVDC) as an oxygen barrier.

[1]Paper number D-10209-1-92 of the New Jersey Agricultural Experiment Station.

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Shelf stability of microwaveable products in plastic containers is achieved by conventional steam/hot water sterilization. In steam sterilization of semisolid and solid foods (heat transfer is by conduction only) in metal cans, the resistance for heat flow through can walls is normally negligible, and the heat transfer coefficient for condensing steam is large enough so the Biot number can be assumed to be infinite. In other words, the rate of heat transfer is only limited by heat conduction in the food. However, for plastic cans, the assumption of negligible resistance to surface heat transfer is not necessarily valid since plastic materials have much lower thermal conductivities than metals (Lebowitz and Bhowmik 1990; Sheen *et al.* 1991). In order to accurately predict thermal processing of foods in plastic containers, effective thermal conductivity or the heat flux through the plastic wall must be known.

Thermal conductivity measurement techniques have been reviewed by Reidy and Rippen (1971), Choi and Okos (1986), Wallapapan *et al.* (1986), and Murakami and Okos (1988). However, most methods are unsuitable for thin materials with multilayered construction. Therefore, the objective of this study was to develop a technique to measure effective thermal conductivity of a retortable multilayered plastic container.

MATERIALS AND METHODS

Experimental Setup

The experimental setup for measuring effective thermal conductivity of the multilayered plastic container is shown in Fig. 1. The bowl-shaped container, purchased locally, has five layers, an average thickness of 1.1 mm, and a volume of 250 mL. A heat flux sensor (Micro-Foil Heat Flow Sensor, RdF Corp., Hudson, NH) was bonded to the inner surface of the plastic wall with a very thin layer, high thermal conductivity adhesive (Omegatherm 201, Omega Engineering, Inc., Stamford, CT) to assure good contact between the plastic wall and the heat flux sensor. Millivolt signals generated by the heat flux sensor were amplified 50 times by a D.C. millivolt amplifier (Model OMNI IIA, Omega Engineering, Inc., Stamford, CT) and then recorded with a strip chart recorder (Model L6512-2, Linseis, Inc., Princeton Junction, NJ).

The plastic bowl was immersed up to 85% of its height in a constant temperature ($T_2 \pm 0.1$C) water bath (Model MW-1120A-1, Blue M Electric Co., Blue Island, IL). the container was filled with water, and a 1/8 in. O.D. copper coil connected to a constant temperature circulating water bath (Model 9000, Fisher Scientific, Springfield, NJ) was used to control water temperature, T_1. In this study T_1 was higher than T_2. Unlike T_2, which was constant during the measurement, T_1 was dependent upon the temperature of the circulating water bath, heat transfer rate

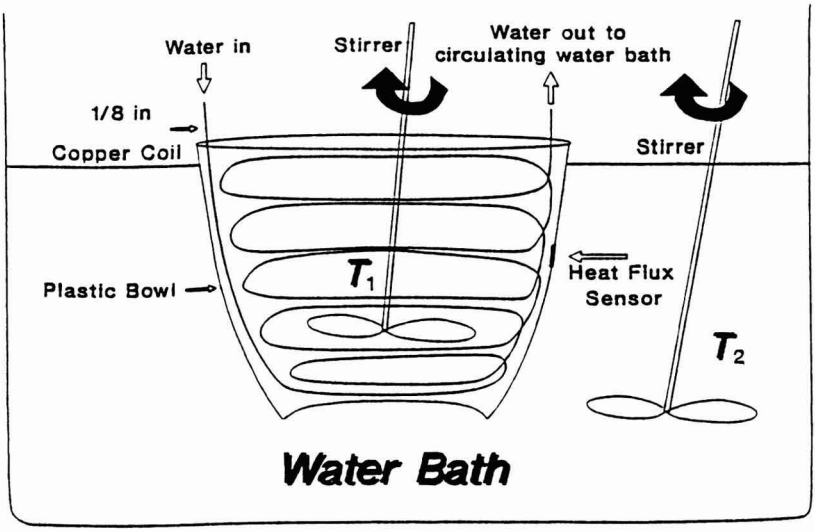

FIG. 1. EXPERIMENTAL SETUP FOR MEASURING EFFECTIVE THERMAL CONDUCTIVITY OF A MULTILAYERED PLASTIC CONTAINER

between water and copper coil heat exchanger, and the heat flux through the plastic container. Mechanical stirrers were used to agitate the water both inside and outside the container to reduce internal and external film resistances. At steady state, the temperature difference ($\Delta T = T_2 - T_1$) and the heat flux were recorded. Effective thermal conductivity of the plastic container was evaluated at $(T_2 + T)/2$.

Mathematical Analyses

At steady state, the heat flux through a multilayered container can be expressed as:

$$q = U(T_2 - T_1) \tag{1}$$

where q = heat flux (W/m^2)
U = overall heat transfer coefficient (W/m^2K)

$$1/U = 1/h_i + 1/h_e + \Sigma(x_i/k_i) \tag{2}$$

where h_i = internal heat transfer coefficient (W/m^2K)
h_e = external heat transfer coefficient (W/m^2K)
x_i = thickness of each layer (m)
k_i = thermal conductivity of each layer (W/mK)

When h_i and h_e are very large, in other words, $(1/h_i + 1/h_e) << \Sigma(x_i/k_i)$, Eq. (2), can be reduced to:

$$1/U = \Sigma(x_i/k_i) \tag{3}$$

Since it is not possible to measure thermal conductivity for each layer, an effective thermal conductivity can be used so that:

$$\Sigma(x_i/k_i) = x_p/k_{eff} \tag{4}$$

where x_p = thickness of the plastic container (m)
k_{eff} = effective thermal conductivity of the plastic container (W/mK)

Combining Eq. (1), (3), and (4) yield:

$$q = (k_{eff}/x_p)\ (T_2 - T_1) \tag{5}$$

Therefore, Eq. (5) can be used to calculate effective thermal conductivity of the plastic container.

RESULTS AND DISCUSSION

In order to measure effective thermal conductivity accurately, it was necessary to assure that the internal and external heat transfer coefficients were sufficiently large so that the only resistance for heat transfer was in the plastic container. As expected, the heat flux through the plastic layer increased significantly as mechanical agitation increased on both sides of the container. However, at a certain agitation rate, further increases in heat flux were not observed even though agitation speed was increased, indicating negligible convective thermal resistance.

A temperature difference in the range of 5–7C was always obtained in this study by controlling the temperature of the circulating water bath. Effective thermal conductivity of the plastic container was found to be independent of temperature in the range of 20–80C, as shown in Fig. 2. An average value of 0.254 ± 0.008 W/mk was obtained. This number is in the range for many plastic materials (Perry *et al.* 1984).

Recently, Sheen *et al.* (1991) developed a computer program using a finite difference technique to predict temperature distributions in a bowl-shaped plastic container as used in this study during sterilization using 7.5% bentonite paste and apple sauce as model food systems. Predicted temperatures agreed well with

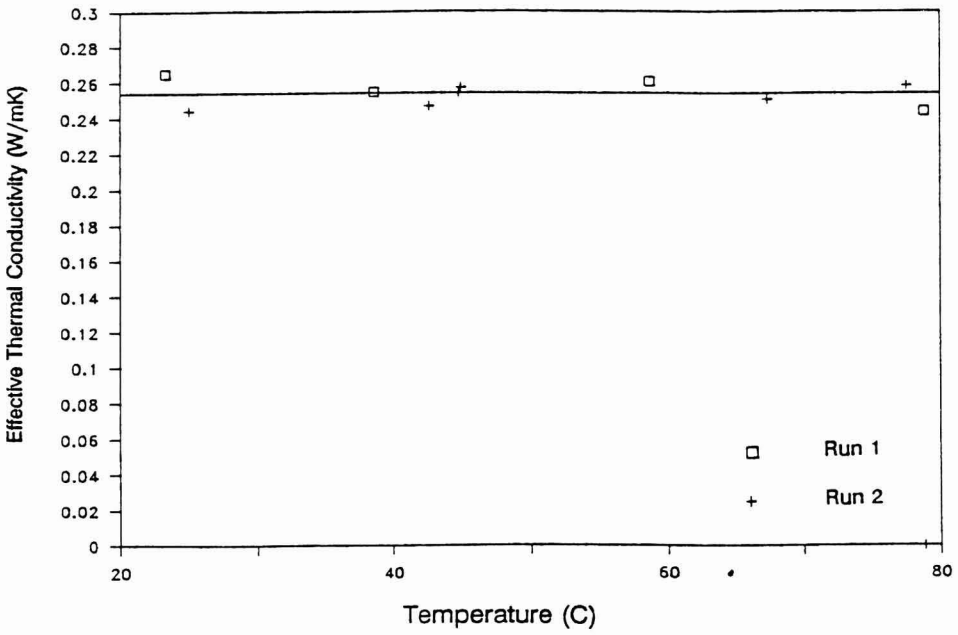

FIG. 2. EFFECTIVE THERMAL CONDUCTIVITIES OF A MULTILAYERED PLASTIC CONTAINER AT DIFFERENT TEMPERATURES

experimental data when an effective thermal conductivity of 0.254 W/mK was used for the plastic container. Their simulation data also showed there was a large thermal gradient across the plastic container (up to 20–30C), suggesting that thermal resistance for the plastic container cannot be neglected. Therefore, reliable data on effective thermal conductivity of retortable multilayered plastic containers are important and essential for modeling thermal processing of these microwaveable food products.

CONCLUSIONS

A technique based on the use of a heat flux sensor was developed to measure effective thermal conductivity of a multilayered plastic container. Effective thermal conductivity of the container was found to be independent of temperature in the range 20–80C and had an average value of 0.254 $\pm$ 0.008 W/mK. Effective thermal conductivity data are required for retortable plastic containers in designing thermal processes for food products in this type of container.

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PHYSICOCHEMICAL PROPERTIES OF RAW AND BLANCHED TARO FLOURS

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ABSTRACT

The physicochemical properties of raw and blanched flours from five promising varieties of taro were determined and compared with those of wheat flour. All taro flours studied had lower protein, fat and starch contents and higher sugar and fiber contents than wheat flour. Water and fat absorption capacities of raw and blanched taro flours were higher, whereas the foam capacity, foam stability, whippability and nitrogen solubility were generally inferior compared to wheat flour. Addition of salt up to 2% concentration in the flour suspension increased the foam capacities of all flours studied. The least concentration end point (LCE) for gelation of both raw and blanched taro flours was comparable to that of wheat flour.

While inferior to wheat flour in composition and most functional tests, the utilization of taro flour may be enhanced by incorporation of protein supplements, thereby improving its nutritional value and functional properties.

INTRODUCTION

The processing capability of taro greatly depends on its composition and physical properties. In the Philippines, very minimal processing of taro is practiced (Villanueva 1979). In fact, most of the produce is boiled as vegetable or sliced thin and fried to produce chips (Knott and Deanon 1967). In Hawaii, taro has been experimentally processed into many consumable products however, commercial processing is limited mainly to the manufacture of poi (taro paste) and deep fried chips (Plucknett 1979). The potential of taro is indeed promising, and one way to maximize and make feasible the conversion of taro into selected pro-

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cessed product is first to look into its physicochemical properties. Information on the chemical composition of taro corms has been reported (Bradbury and Hallway 1988; Lambert 1982). However, information on the physicochemical properties of taro flour is lacking. The present investigation was therefore undertaken in order to determine the physicochemical properties of taro flour from five promising Philippine varieties of taro and to compare these properties with those of wheat flour.

MATERIALS AND METHODS

Taro Samples

Taro samples studies included five promising varieties, namely: PRG-062, PRG-066, PRG-092, PRG-337 and PRG-068. Because of its high yield high dry matter, very low acridity and good eating quality, PRG-068 was recommended for national release by the Philippine Seedboard.

Flour Preparation

Most varieties of taro have the tendency to darken upon peeling, thus blanching was done to eliminate browning and to attain the required color of the resulting flour.

Three types of taro flours — raw, blanched at 60C for 8.3 min and blanched at 100C for 1 min — were prepared.

Newly harvested roots of selected taro varieties were washed carefully to remove soil and adhering debris. They were manually peeled and made into semicylindrical, 50 mm × 5 mm (length × thickness) chips using a designed chipper (Philippine Root Crop Research and Training Center, Visayas State College of Agriculture, Baybay, Leyte). In making the blanched treatments, the chips were placed in nylon screen bags and dipped in water at the specified temperature and duration (chips-to-water ratio was 1:4 weight/volume). The raw and blanched chips were sun dried up to 10–12% moisture, ground in a Wiley mill to a 60-mesh flour, packed in plastic pouches and stored at 4–6C until use.

Proximate Analysis of Flour

Representative samples of raw and blanched taro flours and wheat flour (control) were analyzed for protein, fat, fiber, starch, sugar and ash contents using standard methods of the AOAC. Crude protein was expressed as %N × 6.25. All results were expressed on a dry weight basis.

Physical Characteristics of Flour

Water absorption capacity was determined. Whippability, foam capacity and foam stability were determined by the methods of Coffman and Garcia (1977) and Lin *et al.* (1974) as cited by del Rosario and Flores (1981). The least gelling concentration of the coagulum was done using the method of Coffman and Garcia (1977), while nitrogen solubility was analyzed following the method of Narayama and Rao (1982). The capacity of the flour to bind fat was characterized by the method of Lin *et al.* (1974) as cited by del Rosario and Flores (1981). Sensory evaluation of the flour is covered by a separate study and is still under investigation.

Experimental Design and Layout

A 5 × 3 factorial experiment (CRD) was used for statistical analysis of the data to compare the chemical composition of the three flour preparations made from the five varieties with that of the wheat flour. Appropriate statistical analysis was also used in interpreting the physical characteristics of the three kinds of flour preparations.

Each of the treatments under study was replicated three times.

RESULTS AND DISCUSSION

Proximate Composition of Flour from Different Taro Varieties

All varieties evaluated were inferior in protein, starch and fat contents, but higher in fiber and ash, compared to wheat flour (Table 1). PRG-068, a recommended variety, had the highest protein content at 3.52%, while PRG-066 had the lowest at 2.29%. Starch and sugar ranged from 53.71 to 59.33% and 1.02 to 1.79%, respectively; whereas, fiber was 4.77 or less in all test flours. Fat content ranged from 1.66 to 3.12%, while ash ranged from 1.51 to 2.54%.

Effect of Blanching Taro on Proximate Composition of Flours

The specific effect of water may include chemical changes produced by chemical reactions with water (hydrolysis reaction) starch hydration, and as well as its effect as solvent in simply dissolving water soluble nutrients from food (Bradbury and Hallway 1988). Results of the present study confirmed these effects (Table 2). The sugar and ash contents were decreased significantly by blanching at 60C, indicating that these nutrients were dissolved in the cooking water; however, protein was significantly decreased at 100C, brought about by it irreversible denatura-

TABLE 1.
PROXIMATE COMPOSITION OF FLOUR
FROM FIVE VARIETIES OF TARO[1]

Variety	Starch	Sugar	Ash	Protein	Fat	Crude Fiber
PRG - 062	58.58^{b}	1.79^{a}	1.51^{c}	2.57^{d}	2.35^{b}	3.80^{c}
PRG - 066	59.33^{b}	1.33^{b}	1.98^{c}	2.29^{e}	3.12^{a}	4.12^{ab}
PRG - 068	56.43^{bc}	1.02^{c}	2.09^{b}	3.52^{b}	2.36^{b}	4.53^{ab}
PRG - 092	53.71^{c}	1.42^{b}	1.99^{b}	2.50^{d}	1.66^{c}	4.77^{a}
PRG - 337	54.55^{c}	1.26^{b}	2.54^{a}	3.11^{c}	1.86^{c}	4.04^{ab}
Wheat Flour	74.93^{a}	1.28^{b}	0.68^{d}	12.90^{a}	3.23^{a}	1.02^{d}

[1]All values are expressed on dry basis. Means of triplicate determinations

Means with the same letter do not significantly differ at 5% level Duncan's Multiple Range Test

TABLE 2.
EFFECT OF BLANCHING TARO CHIPS
ON THE PROXIMATE COMPOSITION OF FLOUR[1]

Flour Preparation	Starch	Sugar	Ash	Protein	Fat	Crude Fiber
Raw	55.01^{b}	1.62^{a}	2.28^{a}	2.98^{b}	1.54^{b}	4.24^{a}
Blanched at 60 C	56.06^{b}	1.19^{b}	1.79^{b}	2.94^{b}	2.29^{b}	4.41^{a}
Blanched at 100 C	58.49^{b}	1.28^{b}	2.01^{b}	2.47^{c}	1.97^{b}	4.09^{a}
Wheat Flour	74.93^{a}	1.28^{b}	0.68^{c}	12.90^{a}	3.49^{a}	1.02^{b}

[1]All values are expressed on dry basis. Means of triplicate determinations

Means with the same letter do not significantly differ at 5% level Duncan's Multiple Range Test

tion (Bradbury and Hallway 1988). In every blanching treatment, the carbohydrate and fiber contents were slightly increased, but the fat slightly decreased. The findings of the present study agreed with the reports of Pawar and Ingle (1988) and Meiners *et al.* (1976) on moth bean and dry legumes, respectively.

Physical Properties of Flour from Different Taro Varieties

Fat and Water Absorption Capacity, Foam Capacity, Gelation Capacity and Whippability. The fat and water absorption capacity, foam and gelation capacity and whippability were evaluated and compared with wheat flour (Table 3). Results revealed that only variety PRG-337 performs better in functionality tests for whippability and foam capacity. This suggests that its potential for bakery and other food products is promising, especially if the flour is made from raw/uncooked chips, since the functional properties of its raw form are comparable with that of wheat flour (Fig. 1 and 2). The rest of the taro flours have whippability and foam capacity values in the range of 2.55–4.42% and 5.56–8.36 mL, respectively. The water and fat absorption capacity of all taro flours was, however, higher, while the gelation capacity was comparable with that of wheat flour.

TABLE 3.
PHYSICAL PROPERTIES OF FLOUR FROM FIVE VARIETIES OF TARO

Variety	Fat Abs. Cap. gram fat/ gram flour	Foam Cap. (mL)	Gelation Cap. (%)	Water Abs. Cap. gram water/ gram flour	Whippability (%)
PRG - 062	1.09^{b}	6.41^{d}	1.07^{b}	1.51^{c}	4.42^{c}
PRG - 066	0.96^{c}	5.56^{d}	1.31^{a}	1.37^{d}	2.55^{d}
PRG - 068	1.07^{b}	8.36^{c}	0.74^{e}	1.44^{c}	3.95^{c}
PRG - 092	1.26^{a}	6.21^{d}	0.73^{f}	1.66^{b}	2.67^{d}
PRG - 337	1.12^{b}	19.21^{b}	1.07^{b}	1.74^{a}	14.27^{b}
Wheat flour	0.56d	40.17^{a}	0.78^{d}	0.68^{e}	23.61^{a}

Means with the same letter do not differ significantly at 5% level Duncan's Multiple Range test

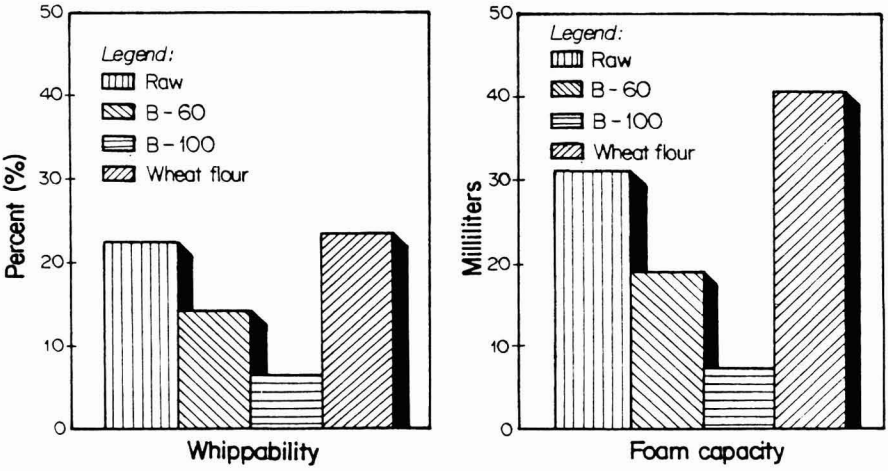

FIG. 1. WHIPPABILITY AND FOAM CAPACITY OF PRG-337 FLOUR AS COMPARED TO WHEAT FLOUR

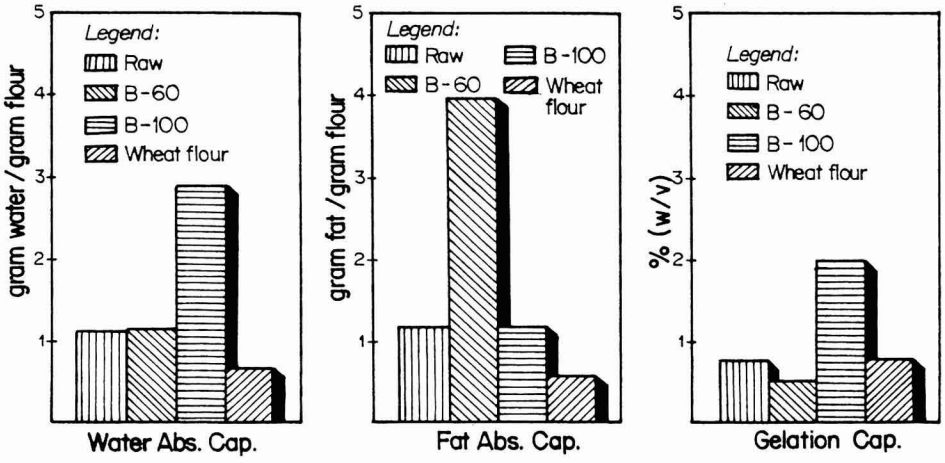

FIG. 2. WATER AND FAT ABSORPTION AND GELATION CAPACITY OF PRG 337 FLOUR AS COMPARED TO WHEAT FLOUR

Foam Stability. Foam stability was measured by volume decrease of foam with time. Of all varieties tested, only PRG-337 showed values that were better than wheat flour (Fig. 3). After 120 min, the volume decrease of raw PRG-337 flour was about 67.7%, but was 80% for wheat flour. Other taro flours evaluated have a foam volume of about 0–2.0 mL after min.

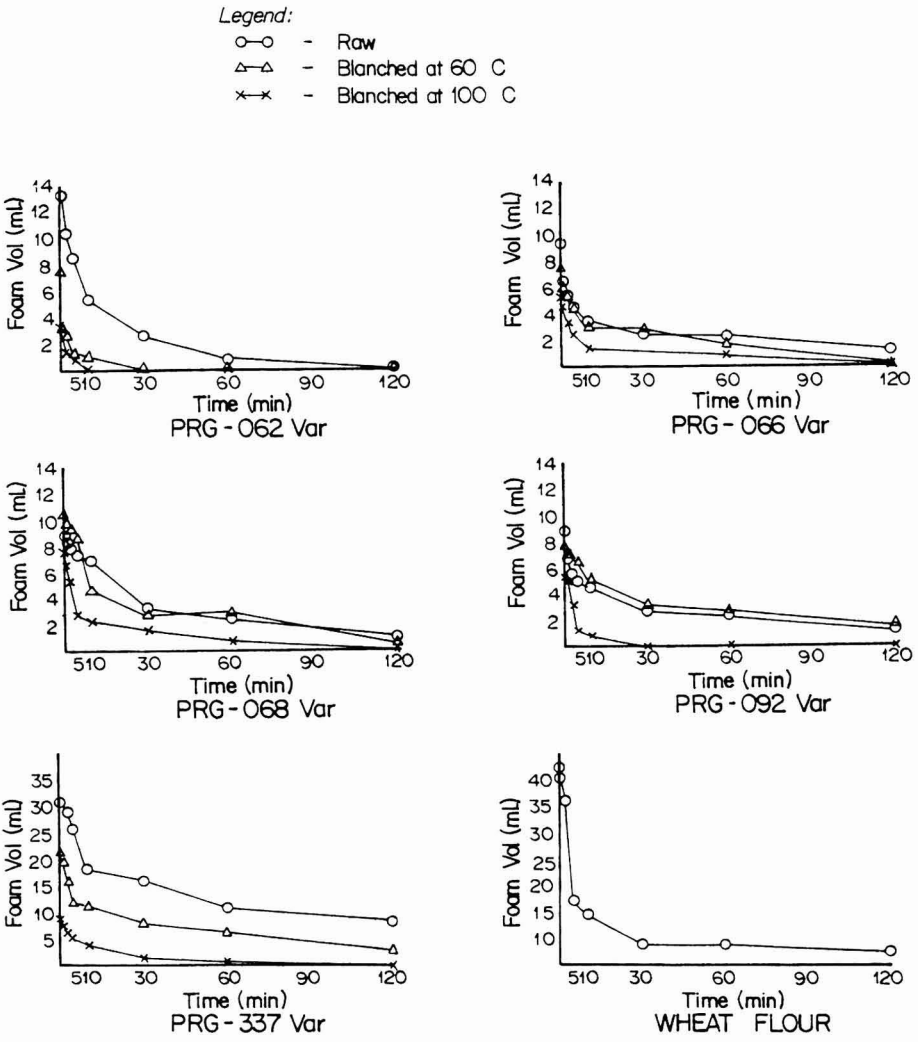

FIG. 3. FOAM STABILITY OF DIFFERENT TARO FLOUR PREPARATIONS

Nitrogen Solubility. The nitrogen solubility profile of taro flour studied is illustrated in Fig. 4. Except for flour from variety PRG-068 blanched at 100C, where minimum nitrogen solubility was observed from pH 4–8, all other taro flours exhibited minimum nitrogen solubility at pH 6.0. Minimum nitrogen solubilities ranging from 10–30% were obtained for varieties PRG-337, 092, 062, 066 and 068.

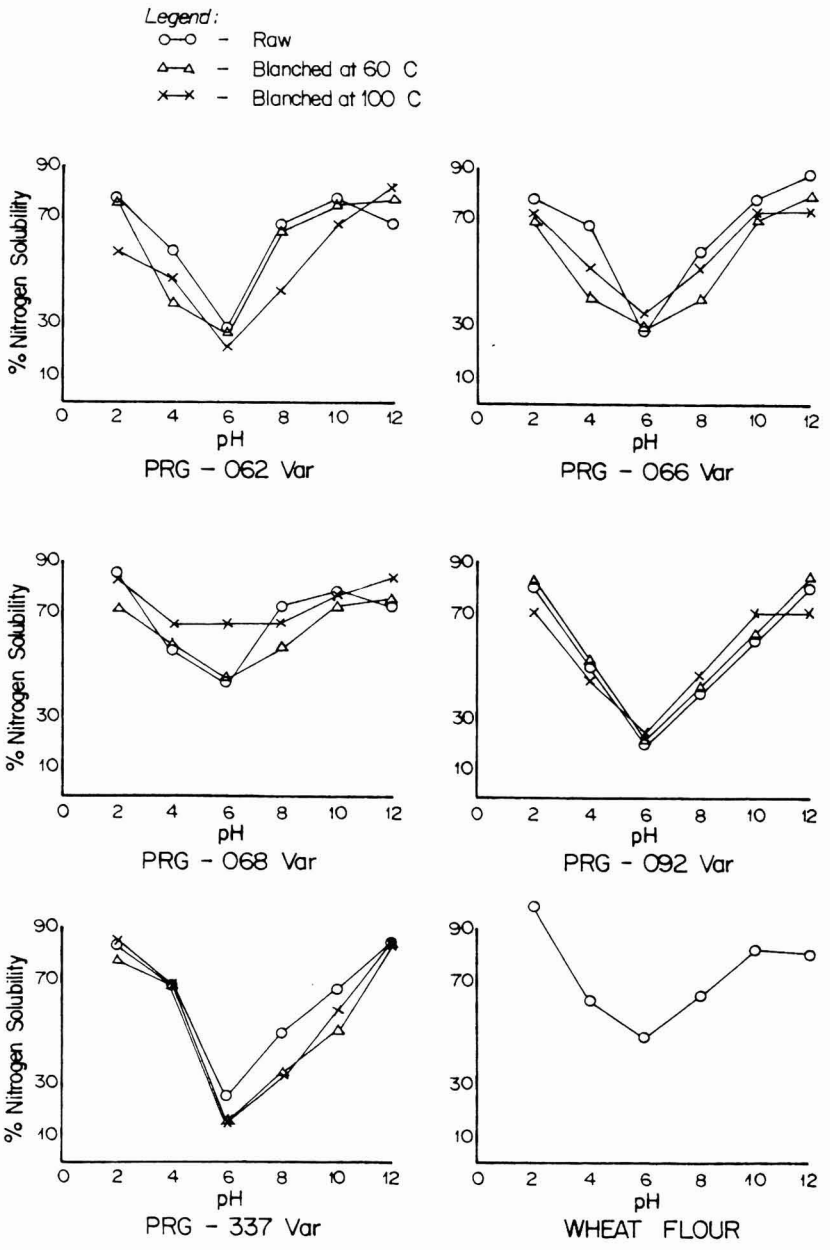

FIG. 4. NITROGEN SOLUBILITY OF DIFFERENT TARO FLOUR PREPARATIONS

Effect of Blanching Taro on the Physical Properties of Flours

Water Absorption Capacity. There was no significant difference in water absorption capacity between raw flour and flour blanched at 60C. This may be due to the fact that the gelatinization starting temperature of raw taro flour is between 83–84C. However, flour blanched at 100C had higher water absorption capacity than flour blanched at 60C (Table 4). Wu and Inglet (1974) had reported that water absorption capacity of soy flour increased due to heat processing. The same observation was also reported by del Rosario and Flores (1981) on blanched mung bean flour and by Narayama and Rao (1982) on autoclaved winged bean flour. Pawar and Ingle (1988) have mentioned that carbohydrates may also play a role in water absorption. During cooking, gelatinization of the carbohydrates and swelling of the crude fiber may occur, which could lead to increased water absorption. Water absorption capacity of wheat flour was two to three times lower than raw and blanched taro flours.

Fat Absorption Capacity. The ability of protein to bind fat is important, since fats act as flavor retainer and enhance the mouthfeel of foods (Kinsella 1976) as cited by Rahma and Mastafa (1988). Raw taro flour had a fat absorption capacity of 1.21 g/g flour, and this value decreased to 1.08 g/g flour after blanching for 8.3 min at 60C (Table 4). Rahma and Mastafa (1988) reported that the fat absorption capacity of peanut flour decreased slightly at 100C and 120C mild heat treatment for 15–30 min. No difference was observed, however, between flours blanched at 60C and 100C. The fat absorption capacity of wheat flour was two times lower than that of raw taro flour.

Gelation Capacity. Raw taro flour had a gelation capacity that nearly approximates the value obtained for wheat flour (Table 4). Blanching the chips up to 60C lowered the gelation capacity to as low as 0.44%. This result suggests that gelation is not only a function of protein quality and quantity but also of nonprotein components, such as starch, as well. Taro flour is high in starch so that even at very low concentration, it would exhibit gelation. Further increase in temperature to 100C significantly raised the gelation capacity to more than twice that of the raw preparation. Ledward (1979) and Mauron (1981) as cited by Enwere and Ngoddy (1986) reported that the increase in the least gelling concentration is attributed to denaturation, aggregation and precipitation of protein and pregelatinization and thermal degradation of starch.

Foam Capacity: Whippability and Foam Stability. The results of the foam capacity, whippability and foam stability measurements are shown in Table 4 and Fig. 5. Significant reduction in these properties due to blanching treatments were

TABLE 4.
EFFECT OF BLANCHING TARO CHIPS
ON THE PHYSICAL PROPERTIES OF FLOUR

Flour Preparation	Fat Abs. Cap. gram fat/ gram flour	Whippa-bility (%)	Foam Cap. (mL)	Water Abs. Cap gram water/ gram flour	Gelation Capacity (%)
Raw	1.21[a]	8.15[b]	13.24[b]	1.14[b]	0.79[b]
Blanched at 60 C	1.08[b]	5.50[c]	8.94[c]	1.25[b]	0.44[c]
Blanched at 100 C	1.02[b]	3.05[d]	5.28[d]	2.23[a]	1.72[a]
Wheat flour	0.56[c]	23.61[a]	40.17[a]	0.68[c]	0.72[b]

Means with the same letter do not significantly differ at 5% level Duncan's Multiple Range Test

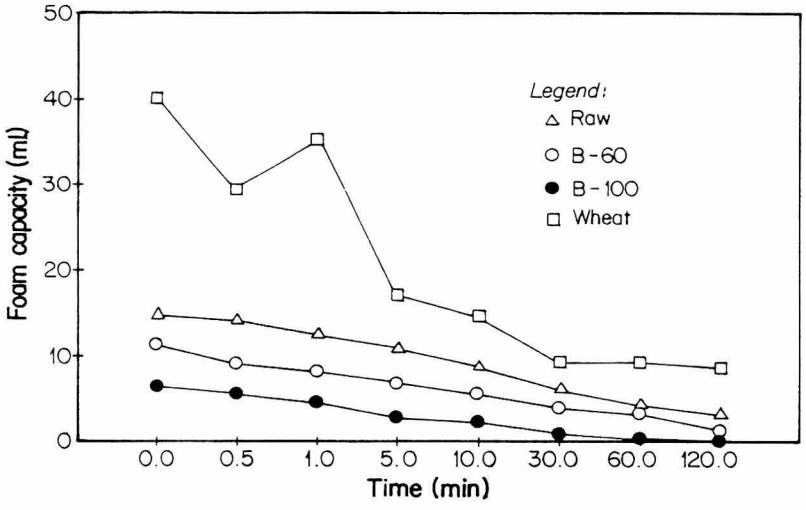

FIG. 5. EFFECT OF BLANCHING ON THE FOAM STABILITY OF TARO AND WHEAT FLOUR

noted with the maximum decrease at 100C blanching treatment. The foam capacity and whippability of raw taro flour decreased from 13.24 to 5.28% and 8.15 to 3.05%, respectively, as compared to flour blanched at 100C. On the other hand,

considerable reduction in the foam stability of blanched taro flours was noted, especially with flours subjected to a 100C heat treatment (Fig. 5). Decrease in whippability and foaming property is correlated to the amount of protein present. During blanching, proteins are denatured so that these properties are correspondingly reduced. Similar observations were reported for winged and moth bean flours and also peanut flour (Narayama and Rao 1982; Pawar and Ingle 1988; Rahma and Mastafa 1988).

Wheat flour had high whippability, foam capacity and foam stability. This is expected, since wheat flour is high in protein content, and this property reduces surface tension of the water and aids in the formation of food foams.

Effects of Salt Concentration on the Foam Capacity of Taro Flour. The addition of sodium chloride up to 2 concentration in the flour suspension increased the foam capacity for all flours studied. Narayama and Rao (1982) reported that the addition of salt at a low concentration enhanced protein solubility, whereas high concentration decreased it. Since foam capacity appears to be due to solubilized protein, the differing effect of salt concentration can be explained on this basis.

Blanching the chips, however, decreased the foam capacity at all levels of salt concentration with considerably lower values obtained in chips blanched at 100C (Fig. 6).

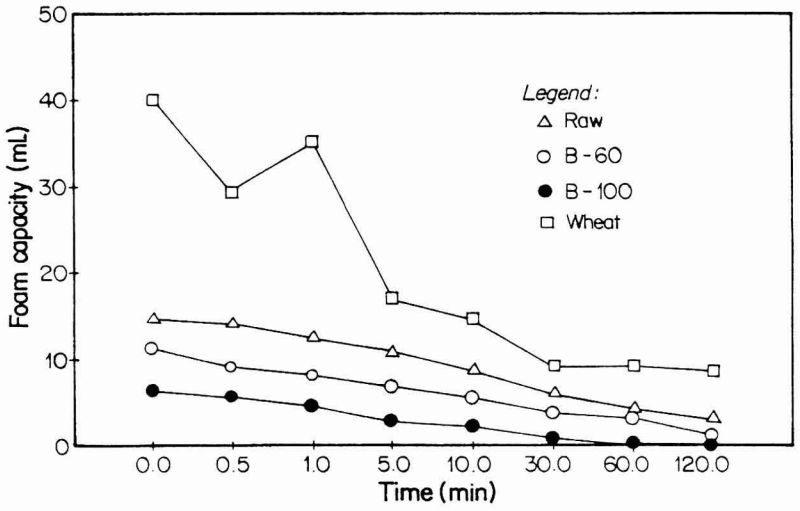

FIG. 6. EFFECT OF SALT CONCENTRATION ON THE FOAM CAPACITY OF RAW AND BLANCHED TARO FLOURS AND WHEAT FLOUR

Nitrogen Solubility. The effect of blanching on the nitrogen solubility of taro and wheat flour is shown in Fig. 7. All flours studied showed minimum nitrogen solubility at pH 6.0 with flour blanched at 60C having the lowest solubility. On the other hand, greatest solubility occurred at pH 2.0 and 10.0, with wheat flour nearly 100% soluble at pH 2.0, followed by raw taro flour at 85%. Blanching results in protein denaturation; therefore, nitrogen solubility decreased in blanched taro flour in all pH levels studied. The decrease was more conspicuous in flour blanched at 60C. Reduction in nitrogen solubility due to heat processing has also been reported in the case of winged, mung and moth beans (Narayama and Rao 1982; del Rosario and Flores 1981; Pawar and Ingle 1988).

The nitrogen solubility of wheat flour was very much higher than any of the other flours studied.

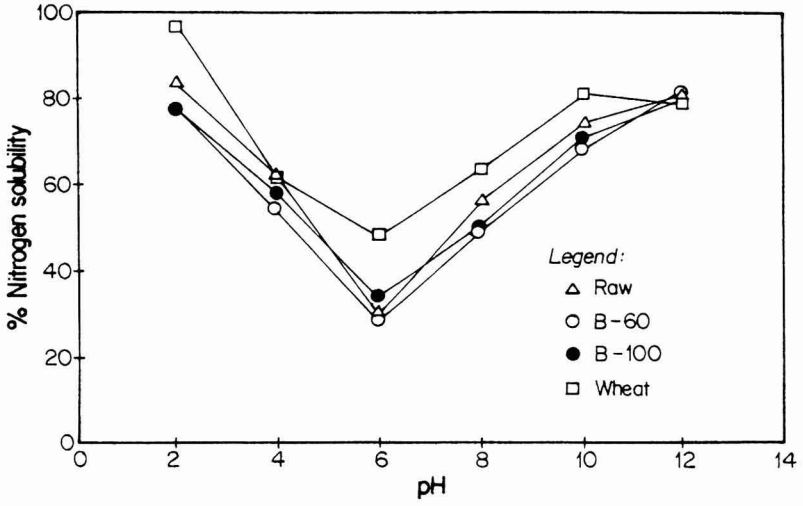

FIG. 7. NITROGEN SOLUBILITY VERSUS pH PROFILE OF RAW AND BLANCHED TARO FLOURS AND WHEAT FLOUR

SUMMARY AND CONCLUSIONS

Three types of taro flours were prepared and their proximate composition and functional properties were evaluated and compared with that of wheat flour. Results showed that taro flour cannot fulfill the nutritional expectations of wheat flour. It is high only in fiber, sugar and ash contents and falls short of protein, fat and starch. In terms of functional properties, it is superior only in water and fat absorption capacities.

Blanching the chips prior to flour preparation caused losses of protein, sugar and other minerals to different degrees, with blanching at 100C giving the greatest reduction in protein content. The water and fat absorption capacities were, however, increased by the heat treatment.

The study provides information that taro flour can function as a very good binder for food formulations containing high water and fat. Its use in processed products like bread, cake, and noodles, etc., is limited due to its inherent low protein content. Thus, a protein supplement may be incorporated to make taro flour comparable in protein and energy level to conventional wheat products, as well as improve some its functional properties.

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DRY ROASTING FOR POOR QUALITY CHICKPEAS (*CICER ARIETINUM*) CV. SURUTATO-77

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ABSTRACT

*Low commercial quality chickpeas (*Cicer arietinum *cv. Surutato-77) were dry roasted at two sets of temperature-time combinations: 140C, 24 min; and 160C, 17 min. Flours produced from the dry roasted grains were investigated for chemical, functional and nutritional characteristics. Dry roasted chickpea flours were not different in chemical composition, but exhibited significantly different functional characteristics. Based on protein digestibility, lysine content and protein quality, the chickpea flours were nutritionally adequate and may be considered as an ingredient for the fortification of cereal-based products.*

INTRODUCTION

Legume grains are considered an important source of protein, certain vitamins and minerals for people in underdeveloped areas of the world (Singh 1985). Among the various food legume grains, chickpeas are highly nutritive due to protein content and low incidence of antinutritional factors (Singh 1985). Mexico is classified within the five major producers of chickpeas worldwide (Salunkhe 1985) and due to the quality of the grain, especially that produced in the Northwest part of the country, is highly priced in the international market.

Most of the chickpeas are exported, leaving 20% of total production for the domestic market, mainly used for animal feeding. Chickpeas cannot be considered

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a basic ingredient in the Mexican diet, perhaps due to the restricted alternatives for human consumption.

The purpose of this research is to study an experimental dry roasting process of low commercial grade chickpeas and to produce chickpea flours with acceptable nutritive value for utilization as an ingredient for the fortification of highly consumed, cereal based products.

MATERIALS AND METHODS

Chickpeas and Treatments

Chickpea (*Cicer arietinum* cv. Surutato-77) classified as low commercial grade #3 was used for this study. The grain was harvested in the Costa de Hermosillo agricultural zone in the summer of 1988. Chickpeas were cleaned, sorted, selected and packed in 5 kg plastic bags and kept at 5C throughout the experimental period.

Dry Roasting Device

An experimental type of dry roaster, designed and built locally was used (Fig. 1). The main features of the dry roaster are: a Stainless steel cylindrical drum of 30 × 60 cm as a heating device; a motor adapted to a set of specially design pulleys, to provide a rotatory movement at 50 rpm; a thermocouple adjusted to record the interior ambient temperature; and a gas input meter to control the heating intensity.

Preliminary Tests

Several tests were conducted to establish the most significant parameters for the dry roasting of chickpeas, such as: the optimal sample size, which was set at 3.0 kg, and the drum velocity, which was set at 50 rpm. Preliminary tests also provided various combinations of heating temperatures and chickpeas residence times. The dependent variable against which the dry roasted grain was sanctioned were chickpea final moisture and color. A Surface Response Methodology program (Walker and Parkhurst 1984) was used to select the best combinations of temperature and time necessary to produce acceptable dry roasted chickpeas. Dry roasted chickpeas were milled (Laboratory Mill md. 3100) to a 100 mesh (Tyler) particle size. Dry roasted chickpea flours were further evaluated in their functional and nutritional characteristics.

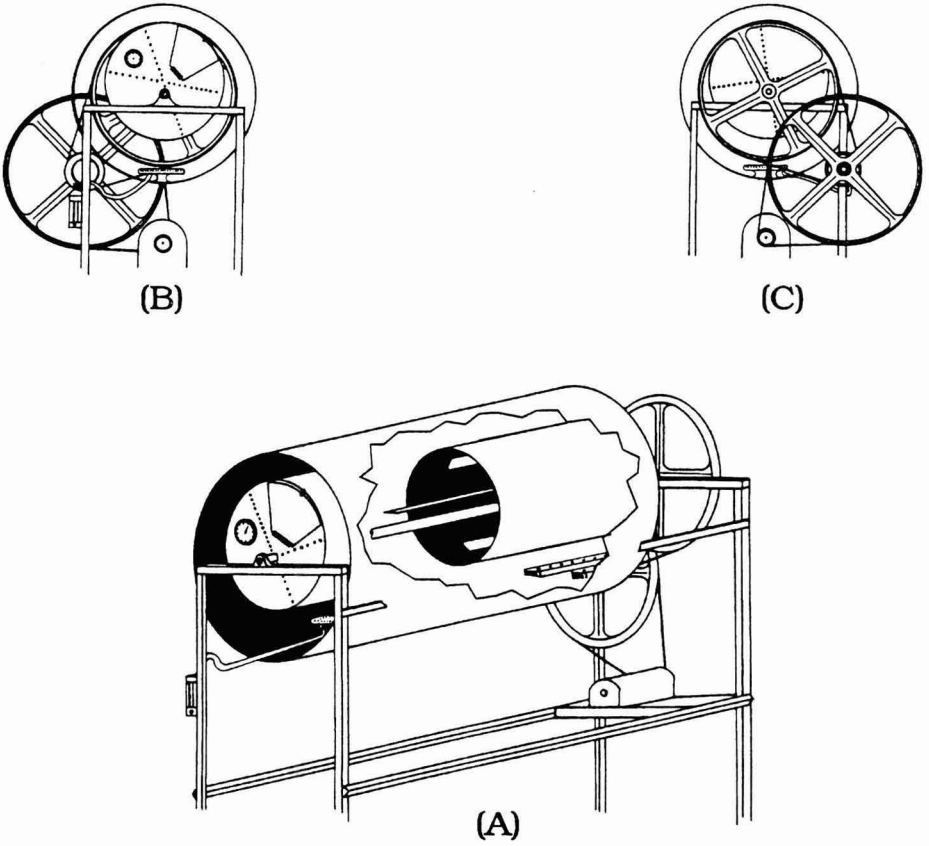

FIG. 1. SCHEMATIC DRAWING OF THE EXPERIMENTAL DRY ROASTER USED IN THIS STUDY
(A) General perspective. (B) Lateral view from the left side. (C) Lateral view from the right side.

Dry Roasted Chickpea Flour Evaluations

Chickpea flours were evaluated in proximate chemical composition by recommended procedures (AACC 1984), with raw chickpea flour as a control. Differences in color were determined by measuring the Relative Reflectance Index (AGTRON) of chickpea flours. Water absorption capacity of chickpea flours was determined through the Relative Absorption Index (Anderson *et al.* 1969). Viscosity was measured using the Brabender Viscoamylograph (Method 22-10, AACC 1984) combining dry roasted chickpea flours with wheat flour (50:50 by weight, dry basis) to determine viscoamylographic properties.

Nutritional evaluation of dry roasted chickpea flours included assays of *in vitro* indicators of protein quality, such as nonreactive lysine, analyzed with a dye binding procedure (DBP) proposed by Hurrell *et al.* 1979; and percent N digestibility, measured by the multienzymatic technique suggested by Satterlee *et al.* 1982. The presence of trypsin inhibitors in the chickpea flours was analyzed with a spectrophotometric procedure originally reported by Kakade *et al.* 1974, and reported as trypsin units inhibited (TUI/mg) per milligram.

Protein quality evaluations of the dry roasted chickpea flours were conducted using rat bioassays. The apparent and true nitrogen digestibilities were assayed using recommended procedures (AOAC 1984). The net protein ratio (NPR) as described by Bender and Doell (1957) was also determined. Rat feeding experiments were conducted in the Center's Animal Experimental Unit, using a Sprague-Dawley rat colony. A duplicate was run for each chickpea flour diet analyzed.

Data Analysis

Analysis of variance (ANOVA) and Duncan's significance test were used to establish differences among dry roasted flours, in the various parameters evaluated.

RESULTS AND DISCUSSION

By means of preliminary tests, and using the SRM program, two dry roasted chickpea flours were selected in terms of color and final moisture characteristics. The temperature-time combinations for the dry roasting of chickpeas were established at 140C for 24 min and 160C for 17 min. Dry roasting conditions were considered optimal for the experimental equipment designed for this investigation. Dry roasted at the selected temperature-time combinations, chickpea flours gave a final moisture content in the range of 4.0–4.5 and a desirable light cream color.

As expected, proximate analysis of dry roasted chickpea flours were not different in composition. Thus, dry roasting conditions had no influence on the content of major nutrients from chickpeas. On the other hand, characteristics such as color and water absorption index were significantly affected by dry roasting conditions (Table 1). A considerable reduction in the relative reflectance index (blue spectrum) were obtained for dry roasted chickpea flours, when compared to control chickpea flour. Processed chickpea flours were darker in color, and this effect was more pronounced at severe processing conditions (160C; 17 min). Water absorption capacity (WAC) of chickpea flours was significantly increased by both dry roasting conditions. Again, the more severe condition for dry roasting resulted

TABLE 1.

COLOR AND WATER ABSORPTION CAPACITY OF DRY ROASTED CHICKPEA FLOURS

Flours	AGTRON Blue Spectrum	WAC (g gel/g dry flour)
Flour A	31.9	1.65[b]
Flour B	19.5	1.51[c]
Control	60.1	1.35[a]

Values with different letter in the same column are statistically different($P<0.05$)
Values are means of triplicates
Flour A = dry roasted at 140 C; 24 min
Flour B = dry roasted at 160 C; 17 min
Control = raw chickpea flour

in a greater WAC. In this respect, the hydrophilic nature of chickpea proteins, which is reported to be high in other legumes (Paredes-Lopez *et al.* 1991; Uebersax and Ruengsakulrash 1989), was increased by dry roasting processing conditions. The increase in WAC represents an important commercial factor for the application of chickpea flours, when incorporated to cereal based products.

Dry roasted chickpea flours present a significantly lower viscosity when compared to wheat flour (Table 2). Raw chickpea flour had a much lower viscosity than wheat flour (30%) and processing conditions for dry roasting caused a further decrease in the viscosity of chickpea flours. This effect was greater at more severe processing conditions. Apparently, these chickpea flours have poor stability in starch components; even when total carbohydrate content was considerably high. Results may define, to a certain extent, the future application of dry roasted chickpea flours.

From the nutritional standpoint, chickpea flours had a considerably high lysine content as determined by the dye binding capacity procedure, and the lysine is in the available state. Even though the dry roasting conditions reduced the nonreactive lysine content of dry roasted chickpea flours (Table 3), the remaining lysine is quite acceptable to consider chickpea flours an important source of the amino

TABLE 2.

MAXIMUM VISCOSITY VALUES OF DRY ROASTED CHICKPEA FLOURS WITH WHEAT FLOUR

Flours	Viscosity (BU)	T (C)
Flour A + 50 % Wheat Flour	695[a]	86.1
Flour B + 50 % Wheat Flour	620[b]	87.0
Control + 50 % Wheat Flour	865[c]	86.0
Wheat Flour 100 %	2 500	88.5

Values with different letter in the same column are statistically different ($P<0.05$)
Values are means of duplicates
Flour A = Dry roasted at 140 C;24 min
Flour B = Dry roasted at 160 C;17 min
Control = Raw chickpea flour

acid, and thus, a valuable fortifying ingredient for cereal based products. Trypsin inhibitor (TI) content of chickpeas was significantly decreased by dry roasting (Table 4). This confirms the heat-labile nature of the TI present in chickpeas (Smirnoff *et al.* 1976; Belew 1977).

Dry roasting did not have a significant effect on the digestibility of chickpea flours (Table 4). Both temperature-time combinations gave an increase in digestibility, as compared to the raw chickpea flour control. The increase in digestibility, though slight, was observed in both *in vitro* and *in vivo* methodologies used for the determination of percent N digestibility. Most probably, the higher temperature (160C) employed for dry roasting did inactivate the major antinutritional factors present in raw chickpeas, with the consequent improvement on digestibility. As for percent N digestibility, protein quality of chickpea flours was quite acceptable (Table 4). The NPR values for chickpea flours was high when compared to NPR values obtained for other legume grains heat treated under

TABLE 3.

NONREACTIVE LYSINE AND TRYPSIN INHIBITOR CONTENTS OF DRY ROASTED CHICKPEA FLOURS

Flours	Nonreactive Lysine[1] (g/16 gN)	Trypsin Inhibitor[2] (TUI/mg Protein)
Flour A	6.94[b]	2.94[b]
Flour B	6.48[b]	2.46[b]
Control	7.87[a]	36.36[a]

Values with different letter in the same column are statistically different ($P<0.05$)
Values are means of triplicates
[1]Hurrell et al. 1979
[2]Kakade et al. 1974
Flour A = Dry roasted at 140 C;24 min
Flour B = Dry roasted at 160 C;17 min
Control = Raw chickpea flour

similar conditions (Wolzak *et al.* 1981). Based on percent N digestibility and NPR values, dry roasted chickpea flours exhibit an acceptable protein quality, containing enough lysine for complementing protein in cereal-based food products.

CONCLUSIONS

Experimental conditions for the application of the dry roasting process to low-commercial grade chickpea grains were established. Two sets of temperature-time combinations were chosen in terms of the grain final moisture and color. Dry roasted chickpea flours were similar in chemical composition, and exhibited differences in water absorption capacity and color. The heat treatment reduced chickpeas trypsin inhibitor considerably. From the nutritional standpoint, dry roasted chickpea flours had an acceptable digestibility, a high lysine content and a fairly high protein quality, as measured by the NPR bioassay. The 140C for

TABLE 4.

DIGESTIBILITY AND PROTEIN QUALITY OF DRY ROASTED CHICKPEA FLOURS

Flours	Digestibility (%)			NPR_c
	In vitro	In vivo		
		Apparent	True	
Flour A	86.7[a]	80.5	83.3[b]	3.9[b]
Flour B	84.5[a]	79.6	82.1[b]	3.5[b]
Control	76.8[b]	80.3	82.6[b]	3.8[b]
Casein	90.0[c]	90.3	92.8[a]	4.3[a]

Values with different letter in the same column are statistically different ($P<0.05$)
Values are means of duplicates
Flour A = Dry roasted at 140 C;24 min
Flour B = Dry roasted at 169 C;17 min
Control = Raw chickpea flour

24 min dry roasting is recommended for the preparation of dry roasted chickpeas in the experimental dry roaster. Based mainly on nutritive value, dry roasted chickpea flours may be considered for the fortification of widely consumed, cereal-based food products.

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THE SYNERGISTIC ANTIOXIDANT EFFECT OF ROSEMARY EXTRACT AND α-TOCOPHEROL IN SARDINE OIL MODEL SYSTEM AND FROZEN-CRUSHED FISH MEAT

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ABSTRACT

The treatment of α-tocopherol-rosemary mixture (0.05% + 0.02%) had the strongest antioxidant activity among the antioxidants tested in sardine oil model system and frozen-crushed fish meat. In sardine oil, it delayed the onset of oxidation 5 days longer than either α-tocopherol or rosemary extract alone, and its antioxidant activity was comparable to that of BHA. It was found that the α-tocopherol in sample of mixture treatment remained 5 days longer than in sample of α-tocopherol. In frozen-crushed bonito meat, the thiobarbituric acid value (TBA) in sample of mixture treatment was the lowest among antioxidant treatments. The analysis of the ratio of EPA+DHA/16:0 during the storage also showed that the ratio in sample of mixture treatment was about 10% higher than that in sample of α-tocopherol. This suggested that oxidation of polyunsaturated fatty acid was inhibited. The treatment of α-tocopherol-rosemary mixture also caused less triglyceride hydrolysis than either α-tocopherol or rosemary extract alone. A synergistic effect between α-tocopherol and rosemary extract was observed.

INTRODUCTION

Fish is a valuable natural resource because of its highprotein content and its long-chain, highly unsaturated n-3 fatty acid contents. Recently, a new product,

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frozen-crushed fish meat, was developed and is made by crushing of frozen fish previously eviscerated under the N_2 atmosphere. This procedure results in the avoidance of oxidation and nutrient loss during manufacturing. Frozen-crushed fish meat is becoming a highly desirable new ingredient because of its nutritive value (Fujita 1984). With crushing, however, the surface area of products becomes large and lipids are easily oxidized. Control of lipid oxidation during transportation and preservation is a basic problem in the utilization of frozen-crushed fish meat. α-Tocopherol has often been suggested as an antioxidant for fish oil, because it is an effective peroxy radical scavenger (Niki 1988). As early as 1956, Dugan had shown that tocopherol had a good antioxidant effect in lard as well as other food products such as crackers, cake and potato chips used at concentration of 0.05% (Dugan and Kraybill 1956). Loury *et al.* (1966) also suggested that tocopherol is an effective antioxidant when used at a concentration below 0.05% in butter and in a mixture of fatty acid methyl esters made from soybean oil. However, a number of reports have revealed that the antioxidant capability of tocopherol is limited (Cort 1974; Kanematsu *et al.* 1972), and it can also act as a prooxidant when high concentrations are used (Loury *et al.* 1966; Bazin *et al.* 1984). Therefore, it is necessary to find a synergist to improve the antioxidant effect of tocopherol. Some researchers have shown that ascorbic acid, phosphatidylethanolamine, phosphatidylcholine, phosphatidylinositol, DNA, RNA, some amino acids such as L-histidine, L-tryptophan, L-methionine, L-lysine, and several organic acids such as citric, malic and succinic are effective synergists in improving the antioxidant activity of tocopherol, even though individually they have no antioxidant activity (Aoyama *et al.* 1985a,b; Vatassery *et al.* 1989; Kanematsu *et al.* 1984; Hildeberand *et al.* 1984; Kwon *et al.* 1984; Aoyama *et al.* 1987a,b; Kanematsu *et al.* 1983a,b). Ascorbic acid, a poor antioxidant, is often used as a synergist, and its effect was seen as early as 1941 by Columbic and Mattill (1941), but since it is water soluble, it is difficult to use with oil and lipid containing foods.

In this paper, we selected rosemary extract (RM) as a synergist to enhance the antioxidant capability of α-tocopherol. Previously, rosemary extract was prepared as a antioxidant from Rosemary (*Rosmarinus officinalis L.*) using an organic solvent. Some of the active compounds in this extract have been identified (Chipault *et al.* 1952; Nakatani and Inatani 1983, 1984; Nozaki 1989). Rosemary extract has a high antioxidant activity, which increases with increased concentrations. The study of the antioxidant effect of rosemary extract by Chang *et al.* (1977) showed that rosemary extract, used at a concentration of 0.02%, not only effectively inhibited the oxidation of soybean oil, but also improved its flavor stability. However, they also indicated that rosemary extract imparts a strong and undesirable taste to products when it was used in high concentration. For those reasons, we used rosemary extract as a synergist at a low concentration

of 0.02%. The rosemary (0.02%)/α-tocopherol (0.05%) mixture was added to both sardine oil as a model system and frozen-crushed bonito meat. The synergistic antioxidant effects between α-tocopherol and rosemary extract were investigated.

MATERIALS AND METHOD

Antioxidant Effect for a Sardine Oil Model System

Sardine oil was extracted from fresh sardine (bones and internal organs previously removed) by the Bligh-Dyer (1959) method. Antioxidants were added to the sardine oil as the following five treatments. The final concentrations of antioxidants in samples were (1) control containing no antioxidants, (2) mixture treatment containing 0.05% of α-tocopherol and 0.02% of rosemary extract, (3) 0.05% of α-tocopherol, (4) 0.02% of rosemary extract, (5) 0.02% of butylated hydroxyanisole (BHA), a synthetic antioxidant. Samples of (1), (2), (3), (4), and (5) are designated on the tables and figures using these abbreviated symbols: control, Toc+RM, Toc, RM, and BHA, respectively. Each of the antioxidant treatments was used in 20 g of sardine oil, which was poured into dishes (10 cm i.d.), stored at 30C, and stirred twice a day.

Peroxide value (PV), and α-tocopherol concentration were tested after 0, 1, 3, 5, 7, 10, and 15 days of storage. The same experiment was done for storage at 60C.

Preparation of Frozen-Crushed Bonito Meat with Antioxidants

The same concentrations of antioxidants were also used in frozen-crushed fish sample. Frozen-crushed meat was prepared with the method described elsewhere (Fujita 1984). Each of the antioxidants was dissolved in 10 mL of hxane, then was added to the frozen-crushed meat. After stirring, solvent was removed by evaporator at 40C. A control was also prepared without an antioxidant. Each of treatments was divided into 4 dishes (20 g per dish) and stored at 30C.

Assessment of Lipid Oxidation

PVs were determined by Official Method 2. 4. 12-71 of the Japan Oil Chemists' Society (1977).

Thiobarbituric Acid Value (TBA). Samples of 0.1–0.3 g were put into test tubes and accurately weighed. TBA numbers were determined according to the method described by Sinnhuber and Yu (1977).

Analysis of α-Tocopherol

α-Tocopherol was analyzed by high performance liquid chromatography (HPLC). HPLC conditions for analysis of α-tocopherol were performed as follows with a Shimadzu 6A HPLC system. A nucleosil column (50-5, 4.6 × 250 mm) was used for the separation of α-tocopherol. The mobile phase was hexane: tetrahydro furan:methanol (92.5:25:2.5, v/v) pumped at a flow rate of 1 mL/min. α-Tocopherol was detected by a fluorescent detector using excitation and emission wavelengths of 298 nm and 325 nm.

Analysis of Lipid Class

Thin layer chromatography (TLC) was used to determine lipid class composition in frozen-crushed bonito meat using Kieselgel 60 plates. The plate was developed with petroleum ether:diethyl ether:acetic acid (80:20:1, v/v). The plate used was obtained from E. Merck AG and thickness of the coating was 0.25 mm. After the sample was spotted and developed, a liquid of saturated potassium dichromate in 75% sulfuric acid was sprayed on the TLC plate and was activated at 130C for 5 min. The spot sample was analyzed by using a SHIMADZU high speed TLC scanner (CS-9200) to determine the lipid class composition quantitatively.

Fatty Acid Composition of Bonito Lipid

Bonito lipids were converted to their fatty acid methyl esters by heating the lipids in a mixture of benzene and boron trifluoride methanol complex solution at 85C for 30 min. Methyl esters were analyzed by gas liquid chromatography (GLC) on a glass column (2 m × 3 mm i.d.) packed with Unisole 3000 (Gasukuro Kogyo Inc., Japan). The chromatographic conditions were as follows: oven temperature 215C, injection port temperature 250C, flame ionization detector at temperature of 250C. Nitrogen was used as carrier gas with a flow rate of 50 mL/min.

RESULTS AND DISCUSSION

Synergistic Antioxidant Effect in a Model System

Antioxidants and their concentrations added are listed in Table 1. Since lipid oxidation in frozen-crushed fish meat is easily influenced by biochemical and bacterial changes that occur during storage, the initial experiment was performed in sardine oil as model system.

TABLE.1

CONCENTRATION OF ANTIOXIDANTS USED IN SARDINE OIL MODEL SYSTEM AND FROZEN-CRUSHED FISH MEAT

Additives	Abbreviation	Concentration(%)
Control	Control	0.00
Mixture	Toc+RM	0.05+0.02
α-Tocopherol	Toc	0.05
Rosemary	RM	0.02
Butylated hydroxyanisole	BHA	0.02

Additives of Toc(purity,above 95%), RM(alcohol extract), and BHA(purity,above 98%) were obtained from Sigma chem. (MO,USA), Asama kasei(Tokyo,Japan), and Wako pure chem. (Tokyo,Japan), respectively.

Figure 1 shows the changes of PV in sardine oil during the storage at 30C. Compared to the control, samples of Toc treatment and RM treatment showed slower formation of peroxides. During storage, PV rapidly increased after 10 days. The induction period in samples of Toc treatment and RM treatment were 5 days longer than that of control. The antioxidant activity of rosemary extract was supposed to be higher than that of α-tocopherol, since the PV of RM treatment was lower compared to the Toc treatment during the storage for 10 days, even though the concentration of rosemary extract was lower than α-tocopherol. However, the Toc+RM treatment had the strongest antioxidant ability among the all of natural antioxidant treatments. With no sharp increase in PV seen during the storage of 15 days, the antioxidant effect of the Toc+RM treatment was comparable with that of the synthetic antioxidant of BHA.

Results of the analysis of α-tocopherol concentration are shown in Fig. 2. Control contained about 10 mg/100 g of α-tocopherol, which had oxidized after storage for 7 days. The α-tocopherol content of Toc treatment, which began with 53 mg/100 g was almost unchanged during first storage of 5 days, but declined to about 50% fo the original content after storage for 7 days, and finally became undetectable after 10 days. However, the α-tocopherol content of Toc+RM treatment, which began with about 53 mg/100 g, the same concentration as Toc treatment, changed a little during the first 7 days of storage, and only about half of the original content oxidized after 10 days, and then became undetectable after

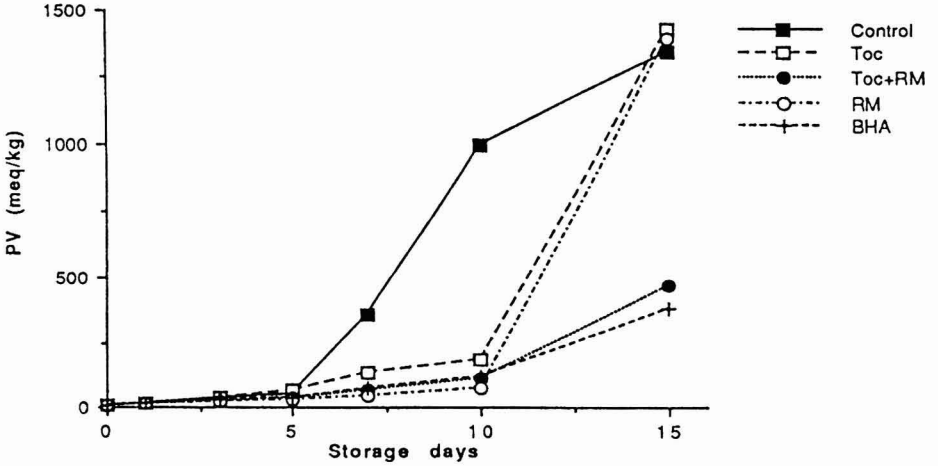

FIG. 1. CHANGES IN PV OF SARDINE OIL WITH ANTIOXIDANT ADDITIVES DURING STORAGE AT 30C

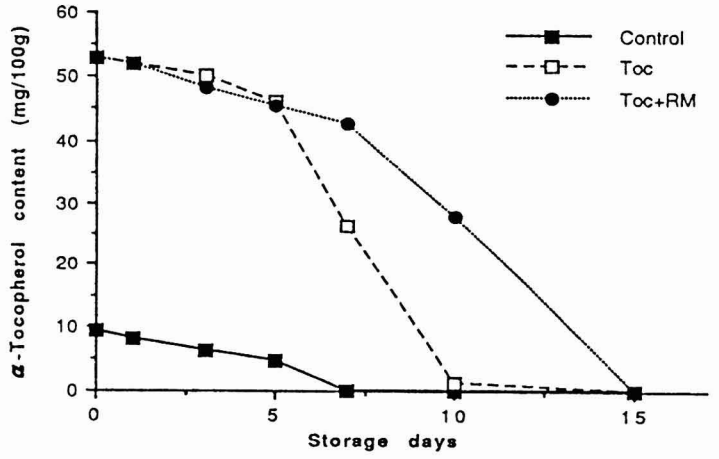

FIG. 2. CHANGES IN α-TOCOPHEROL CONTENT OF SARDINE OIL TREATED BY ANTIOXIDANT DURING STORAGE AT 30C

15 days. The lifetime of α-tocopherol in Toc+RM treatment was 5 days longer than that in Toc treatment. This shows a synergistic effect between α-tocopherol and rosemary extract, in which the rosemary extract acted as a synergist to regenerate α-tocopherols by providing them with hydrogen atoms or preventing their inactivation by combining with substances such as metal ions.

In comparing the changes of PV and α-tocopherol content, both Toc treatment and Toc+RM treatment, oxidized quickly once the α-tocopherol level became zero. From this, the mechanism of the antioxidant synergistic effect of α-tocopherol and rosemary extract could be theorized such that α-tocopherol behaves as a radical scavenger, which combines with radicals and stabilizes them, generating tocopheroxy radicals, which are regenerated by the rosemary extract providing the H-atoms. When the regenerator was oxidized completely, α-tocopherol itself begins to be oxidized. When the α-tocopherol is completely reacted, the lipid oxidation advances quickly.

Synergistic effects of tocopherol and rosemary were also investigated during storage at 60C. The changes in PV and α-tocopherol content are shown by Fig. 3 and Fig. 4. Higher storage temperature caused rapid oxidation compared to storage at 30C. For control, BHA and RM treatments, PV rapidly increased after storage for 2 days. However, for Toc treatment and Toc+RM treatment, PV had a sharp increase after storage for 3 days, the induction period being one day longer than other treatments. Especially, the PV of Toc+RM treatment was significantly lower compared with Toc treatment. This confirmed the synergistic effect between α-tocopherol and rosemary extract. But, rosemary extract itself showed no antioxidant activity during the storage at 60C, since its induction period was same as control. This perhaps due to poor temperature stability of rosemary extract or due to reaction with a greater amount of secondary products of lipid oxidation. This could also explain the weakened synergistic effect of rosemary extract at high temperature, which was seen when the α-tocopherol content of

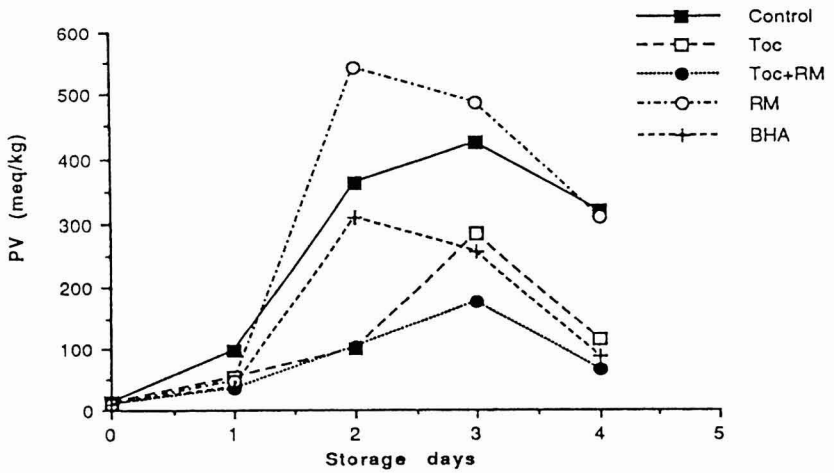

FIG. 3. CHANGES IN PV OF SARDINE OIL WITH ANTIOXIDANT ADDITIVES DURING STORAGE AT 60C

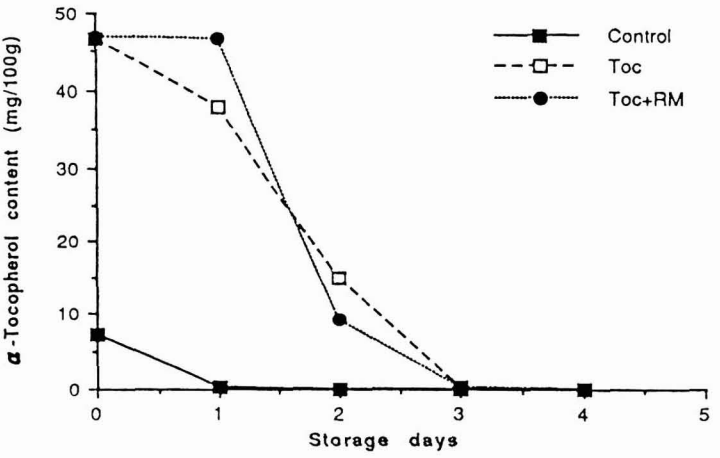

FIG. 4. CHANGES IN α-TOCOPHEROL CONTENT OF SARDINE OIL TREATED BY ANTIOXIDANT DURING STORAGE AT 60C

Toc+RM treatment and Toc treatment became zero on the same day. The temperature stability of rosemary extract remains to be determined.

Synergistic Antioxidant Effect in Frozen-Crushed Fish Meat

The mixture of α-tocopherol and rosemary extract showed strong synergistic antioxidant activity in the model system (sardine oil). This activity was also seen in the inhibitor of oxidation of lipid in frozen-crushed fish meat.

Figure 5 showed the changes of TBA in frozen-crushed bonito meat during the storage at 30C. Since the control had the consistently highest TBA value among the five treatments during storage (and other treatments in order of the size of TBA number were BHA > RM > Toc > Toc+RM), the antioxidant effects of each treatment was considered in order as follows: Toc+RM > Toc > RM > BHA > control. Significantly, the Toc+RM treatment had the strongest effect of all of the treatments, because its TBA was the lowest, and no sharp increase was seen during 6 days of storage. Efficient synergism between α-tocopherol and rosemary extract was affirmed in bonito samples.

The next experiment tested the effectiveness of Toc treatment and Toc+RM treatment in preventing the oxidation of polyunsaturated fatty acid. Fish muscle contains characteristic high amounts of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Sicne EPA and DHA are very easily oxidized because of their high unsaturation, a decline in the ratio of EPA+DHA/16:0 fatty acid was measured to elucidate oxidative deterioration of polyunsaturated fatty

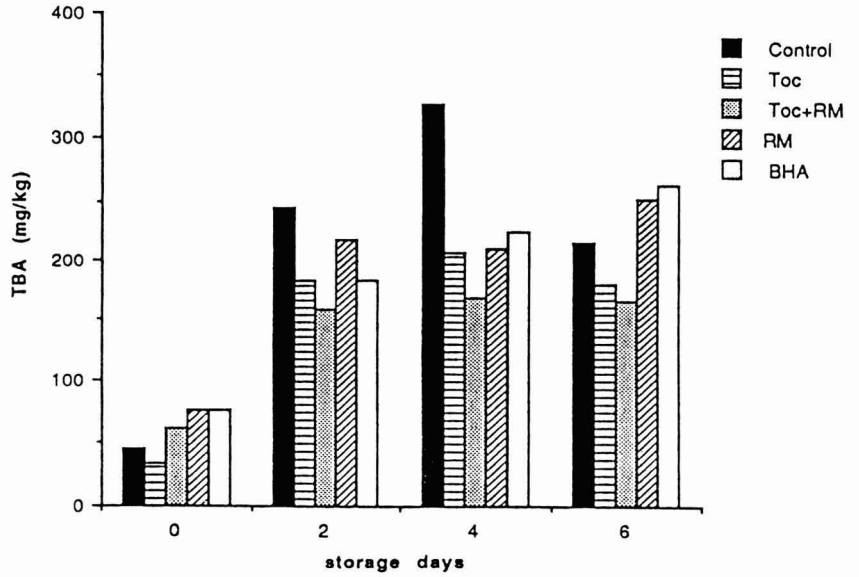

FIG. 5. CHANGES IN TBA OF FROZEN-CRUSHED BONITO MEAT DURING STORAGE AT 30C

acid in fish oil. The results shown in Fig. 6 indicate that Toc+RM treatment in bonito fine meat was more effective in inhibiting the oxidation of polyunsaturated fatty acid than the Toc treatment. The ratio of EPA+DHA/16:0 in sample of

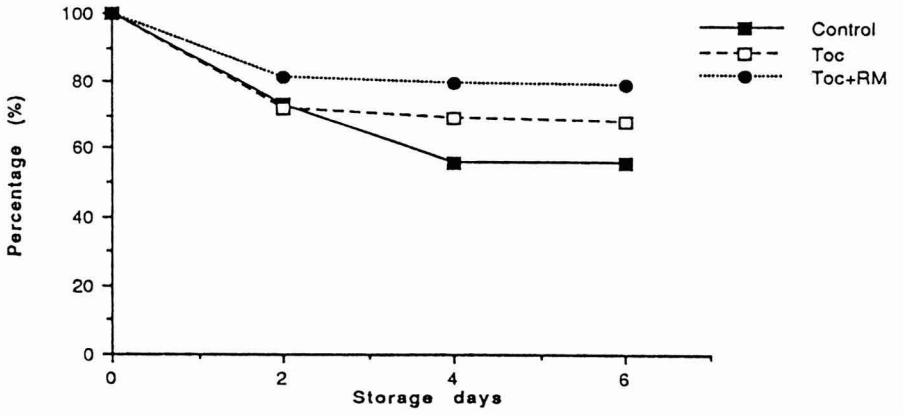

FIG. 6. CHANGES IN THE (EPA+DHA)/16:0 RATIO OF FROZEN-CRUSHED BONITO MEAT DURING STORAGE AT 30C

Toc+RM treatment was about 10% higher than that in sample of Toc treatment, when stored at 30C for 6 days.

The changes in lipid composition were also investigated. Table 2 lists the changes in composition of triglyceride (TG) and free fatty acid (FFA) in frozen-crushed bonito meat stored at 30C. Toc+RM treatment had the lowest TG decomposition rate among the antioxidant treatments; its TG composition was about 10% higher than that of Toc treatment during storage. Correspondingly, Toc+RM treatment had also the lowest increase in FFA. Its FFA composition was always

TABLE.2

CHANGES IN TG AND FFA COMPOSITIONS OF FROZEN-CRUSHED BONITO MEAT DURING STORAGE AT 30 C[a]

Composition	Sample	Storage days			
		0	2	4	6
Triglyceride X±SD (%)	Control	66.0±1.1	52.3±0.8	36.3±0.6	15.1±0.4
	Toc	66.4±1.4	43.8±0.9	40.9±1.2	27.1±1.1
	Toc+RM	67.7±0.9	59.5±0.9	51.5±0.5	36.3±0.8
	RM	66.3±0.8	56.6±0.7	55.2±0.9	16.9±0.5
	BHA	66.2±1.2	46.6±0.5	43.7±0.7	15.2±0.9
Free Fatty Acid X±SD (%)	Control	11.6±0.1	19.5±0.6	30.0±0.9	56.5±1.0
	Toc	12.2±0.8	23.4±0.8	30.5±0.2	41.3±1.2
	Toc+RM	11.6±0.9	18.1±0.1	26.1±0.2	38.3±0.7
	RM	11.7±0.8	23.4±0.5	28.2±0.1	63.8±1.7
	BHA	11.7±0.7	21.5±0.8	31.0±0.1	50.8±1.9

a. Means of triplicate values

lower than other treatments throughout storage of 6 days. The composition was always lower than other treatments throughout storage of 6 days. The composition of triglycerides and further oxidation were restrained efficiently through the synergistic effect of α-tocopherol and rosemary extract.

From those results, the synergistic effects of rosemary extract could be seen as follows: it not only restrained the oxidation of α-tocopherol, but also inhibited catalytic lipid oxidation caused by hemoprotein or metal ion in fish meat, as well as inactivated the enzyme to reduce decomposition of triglycerides in fish lipid. The synergistic effect of rosemary extract and α-tocopherol had a stronger antioxidant activity than that of BHA.

To summarize, the synergistic effect of α-tocopherol and rosemary extract was clearly observed both in the sardine oil model system and in frozen-crushed meat.

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SEPARATION OF EGG YOLK PROTEINS AND LIPIDS WITH CARBOXYMETHYL CELLULOSE[1]

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ABSTRACT

A process was developed to separate proteins and lipids from egg yolk using carboxymethyl cellulose (CMC) solutions with the intent of maintaining the proteins near their native state. The process produced pure globulins (γ-livetins), concentrated total lipids present in yolk plasma into a semi-solid fraction, and yielded a subnatant containing about 68% proteins with a yield of 111 mg/g egg yolk. The method required no pH adjustment and took less centrifugation time as compared to other methods for egg yolk globulin separation. Total lipids of yolk plasma were recovered from the supernatant fraction by hexane-isopropanol (HIP) extraction. The protein residue obtained after HIP extraction of fresh egg yolk retained all the natural proteins of the yolk.

INTRODUCTION

Egg yolk is a complex mixture of microscopic sized granules and a soluble phase called plasma (Powrie and Nakai 1990). When yolk is centrifuged, granules are sedimented and a clear yellow supernatant (plasma) is formed (Schmidt *et al.* 1956). Furthermore, dilution of yolk with water or a dilute solution of NaCl reduces the centrifugation time for sedimentation of granules into a compact mass (Alderton and Fevold 1945; Burley and Cook 1961).

The plasma in the fresh egg yolk represents about 78% of the total liquid yolk, which consists of 77–81% lipid, 2.2% ash and 18% protein (Saari *et al.* 1964).

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The plasma proteins are composed of livetins, which are lipid-free globular proteins and low-density proteins (McCully *et al.* 1962), and represent about 10.6% and 66% of the yolk solids, respectively.

Several procedures for fractionation of egg yolk proteins have been published in the literature (Evans and Bandemer 1957; Joubert and Cook 1958; Bernardi and Cook 1960; Seideman *et al.* 1969; Hatta *et al.* 1988; Kwan *et al.* 1991). Procedures for the production of egg yolk oils have also been published (Anon. 1966; Larsen and Froning 1981; Tokarska and Clandinin 1985). Advantages of protein precipitation by polyelectrolytes over nonionic polymers, isoelectric precipitation or salting-out methods are: low polyelectrolyte dosage and high protein removal. The success of fractionation using polyelectrolytes depends on increased ionic strength, pH, charge density, molecular weight of polyelectrolyte and its dosage (Hansen *et al.* 1971; Hill and Zadow 1978). Clark and Glatz (19877) reported the specific dominance of electrostatic forces in precipitation of egg white proteins by carboxymethyl cellulose (CMC). Furthermore, Hansen *et al.* (1971) recovered more than 90% of proteins present in cheese whey with CMC.

Purification methods related to isolation of egg yolk globulins/immunoglobulins have also been described in the literature (Polson *et al.* 1980; Jensenius *et al.* 1981; Bade and Stegemann 1984; Itoh *et al.* 1986; Hatta *et al.* 1988; Hassl and Aspock 1988; McCannel and Nakai 1989, 1990). Schimizu *et al.* (1988) described an anti-*E. coli* immunoglobulin Y, isolated from egg yolk of immunized chickens, as a potential food ingredient.

The published methods for fractionation of egg yolk have certain limitations for routine processing of the yolk. These include extraction with organic solvents, long ultracentrifugation time, repeated precipitation with salts or use of ion exchange chromatography and gel filtration. Therefore, the objective of this research was to develop simple method(s) to separate proteins without excessive exposure to organic solvents, especially the egg yolk globulins and the lipids, and characterize the fractions produced in the separation process.

MATERIALS AND METHODS

Hen eggs in batches of 12 were obtained within a day of laying from the Purdue University Poultry Farm. Food grade sodium CMC (high viscosity) and reagent grade isopropanol were from Sigma Chemical Co. (St. Louis, MO); and reagent grade hexane (boiling point: 65.8–69.6C) was from Fisher Scientific Co. (Pittsburgh, PA). Each egg was broken manually, the white portion was discarded and the yolk was rolled over on paper towels to remove the chalaza. Yolks from several eggs were combined to make a batch for separation experiments. Three or more trials were performed for each method of separation. CMC solutions

of 0.05, 0.15 and 0.25% (w/w) were prepared by dissolving the respective amounts of CMC powder in deionized distilled water at 60C with rapid agitation. The solutions were cooled and kept at 4C prior to use. The process alternatives to achieve separation of the yolk constituents are shown in Fig. 1.

Process Alternatives

Method 1. The yolks were diluted with an equal amount of distilled water and then centrifuged in a Beckman J2-21 centrifuge at 17,400 × g and room temperature (≈ 25C) for 30 min. The precipitate (granular material) was separated from the supernatant (diluted plasma) by decanting. Each supernatant was divided into two portions. One portion and the granules were freeze-dried. The second

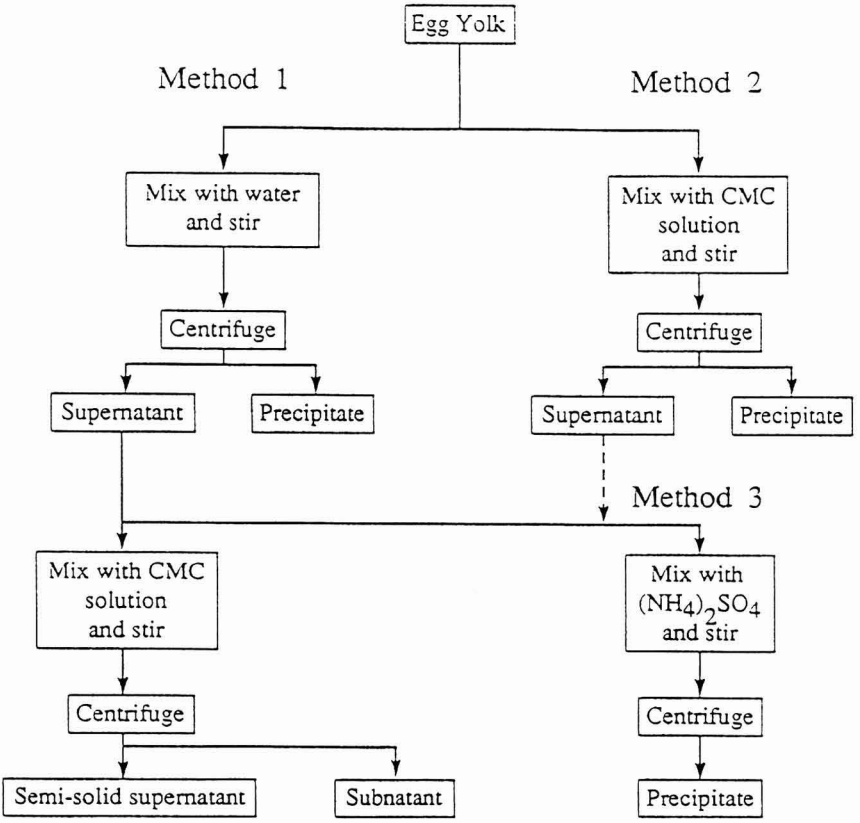

FIG. 1. GENERAL SCHEME FOR SEPARATION OF EGG YOLK INTO LIPOPROTEIN-CMC-LIPID COMPLEX, WATER-SOLUBLE PROTEINS AND PROTEIN PRECIPITATION BY $(NH_4)_2SO_4$

portion of diluted plasma (DP) was mixed with the CMC solution at a ratio of 1:1 (w/w) and then centrifuged as mentioned earlier. A semisolid yellowish supernatant was removed with a spatula and the subnatant liquid was decanted from a small portion of precipitate settled at the bottom of the centrifuge tube. The liquid and the semisolid supernatant were freeze-dried, and in some cases a portion of the semisolid supernatant was vacuum-dried at 40C for 14 h.

Method 2. The yolks were homogenized with a Polytron homogenizer (Brinkmann Instruments, Westbury, NY) for 3–5 min at 900 rpm and room temperature. The CMC solution was then added at a ratio of yolk to CMC solution 1:1 or 1:1.5 (w/w). The mixture was then centrifuged as mentioned in Method 1. The supernatant was decanted from the precipitate. The precipitate and the supernatant were freeze-dried.

Method 3. In separate experiments, DP of Method 1 and supernatant of Method 2 were mixed with ammonium sulfate (18.5 g/100 mL), then centrifuged in a clinical centrifuge at 2700 × g for 5 min. Supernatants were discarded and the protein precipitates were freeze-dried.

Analytical Procedures

Electrophoresis. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out according to Weber and Osborn (1969) using 8–18% (gradient) gel. The protein was stained overnight with Coomassie Brilliant Blue R-250 (0.05%, w/v) in a mixture (1:1) of 30% (v/v) methanol in 10% (v/v) acetic acid and photographed. The standard used for molecular weight was Dalton Mark VII-L (Sigma Chemical Co.), which is a mixture of bovine and egg albumins, glyceraldehyde-3-phosphate dehydrogenase, carbonic anhydrase, tripsinogen, trypsin inhibitor, and α-lactalbumin.

Lipid. Two (2) g yolk or yolk fraction was defatted by extraction with 2 × 20 mL of hexane-isopropanol (HIP) and the volume was made to 50 mL in a volumetric flask. Duplicate 10-mL aliquots were removed and the organic solvent was evaporated by passing nitrogen gas through the sample flask. The residual syrup-like material was further dried under 685 mm Hg vacuum at 40C for 4–6 h and weighed. Total lipids were determined using the following formula (Fletcher *et al.* 1984):

$$\% \text{ lipid} = (\text{weight of fat in } 10 \text{ mL}) \times (5) \times (100)/\text{sample weight}.$$

Protein. Protein (nitrogen × 6.25) was determined by the micro-Kjeldahl method (Method 925.31; AOAC 1990).

Thin-Layer Chromatography (TLC). TLC was performed with commercially coated aluminum sheets with silica gel 60 (No. 5553, 0.2 mm, E. Merck) (EM Science, Gibbstown, NJ) using chloroform and chloroform-methanol-water (65:25:4, v) solvent systems for qualitative analysis of neutral lipids and phospholipids, respectively. The spots on the chromatogram were visualized by charring at 120C for 20 min after spraying with 50% (w/v) sulfuric acid containing 0.06% (w/v) potassium dichromate (Hatta *et al.* 1988). Standards used for comparison with samples on the TLC spots were purchased from Sigma Chemical Co.

RESULTS AND DISCUSSION

Lipid Separation

Table 1 shows the total lipid content in egg yolk, granule and some representative samples of egg yolk-CMC fractions when defatted by HIP (3:2, v/v) ex-

TABLE 1.

LIPID CONTENTS IN REPRESENTATIVE SAMPLES OF EGG YOLK FRACTIONS[a]

	Sample description	Total lipids (%)	Non-extractable residue[b] (%)
1.	Yolk liquid	33.0 (0.13) wwb	15.5 (0.05) wwb
2.	Yolk granule	24.2 (0.18) dwb	69.9 (1.5) dwb
3.	Protein-CMC-lipid complex (Precipitate)[c]	53.6 (0.18) dwb	41.9 (0.15) dwb
4.	Yolk plasma-CMC (semisolid supernatant)[d]	78.9 (2.2) dwb	16.1 (0.06) dwb

[a] Values are mean of triplicate determinations with standard deviation in parenthesis.
[b] Mostly protein residue after lipid extraction by hexane-isopropanol (3:2, v/v).
[c] Method 2 (Fig. 1)
[d] Method 1 (Fig. 1)
wwb = wet weight basis
dwb = dry weight basis

traction. The total lipid content of egg yolk was 33.0% (wet weight basis). This was quite comparable to the reported value of total lipid content of 30.7–36.1% by HIP extraction method (Fletcher *et al.* 1984). However, the egg granular material under similar treatment with HIP yielded ≈24% (dry weight basis) total lipids (Table 1), and molecular mass of polypeptide in the residual protein-rich material was unaffected by the extraction and drying (see Protein Separation section). Similarly, the egg yolk residue obtained after HIP extraction and vacuum-drying was also unaffected and the proteins were in their original state in comparison to those of egg yolk proteins (see discussion on protein separation). Extraction of a protein-CMC-lipid complex (precipitate) of Method 2 with HIP showed a total lipid content of about ≈54% (dry weight basis). Similarly, the semisolid supernatant (Method 1) gave a total lipid content of about 79% (dry weight basis). According to Saari *et al.* (1964), the plasma consists of 77–81% (dry weight basis) lipid; therefore, about 79% lipid content in the semisolid complex indicates that the total lipid of the yolk plasma was in this fraction. Conceivably, addition of CMC and subsequent centrifugation should make the subnatant fraction totally lipid-free. Therefore, lipid percentages in this extract and the supernatant of Method 2 were not determined. The residual protein-CMC complexes of the last two fractions, however, were slightly tinged with yellow color after HIP extraction and subsequent vacuum-drying. It seems that the HIP method could be used for extraction of total lipids from fresh egg yolk and from the semisolid fraction obtained in Method 1 and to a slightly lesser extent from the yolk granule (lipid content 34%, dry weight basis , Saari *et al.* 1964) and the precipitate of Method 2.

Thin-layer chromatrographic analysis of the lipids extracted by HIP (Sample 1-4, Table 1) indicated the presence of a minor fast-moving constituent (unidentified), triglycerides, cholesterol and phospholipids on the base line (in order of their decreasing Rf) when chloroform was used as a developing solvent (Fig. 2A). In chloroform-methanol-water (65:25:4), these extracts contained phosphatidylethanolamine (PE) and phosphatidylcholine (PC) in high proportions in addition to the triglycerides and cholesterol, and a negligible amount of phospholipids, such as lyso PE and lyso PC (Fig. 2B). These results of TLC analysis were similar to those reported by Hatta *et al.* (1988). Hatta *et al.* purified phospholipids from the precipitated lipoproteins by 95% ethanol extraction followed by $ZnCl_2$ precipitation, and subsequent washing with acetone. Phospholipid isolation from egg yolk by the HIP method seemed to be a more convenient process than that described by Hatta *et al.* in which the presence of $ZnCl_2$ and acetone cannot be excluded. If the product prepared by the method of Hatta *et al.* were to be used in foods, the residual acetone would have to be removed and the presence of $ZnCl_2$ would be labeled as an additive.

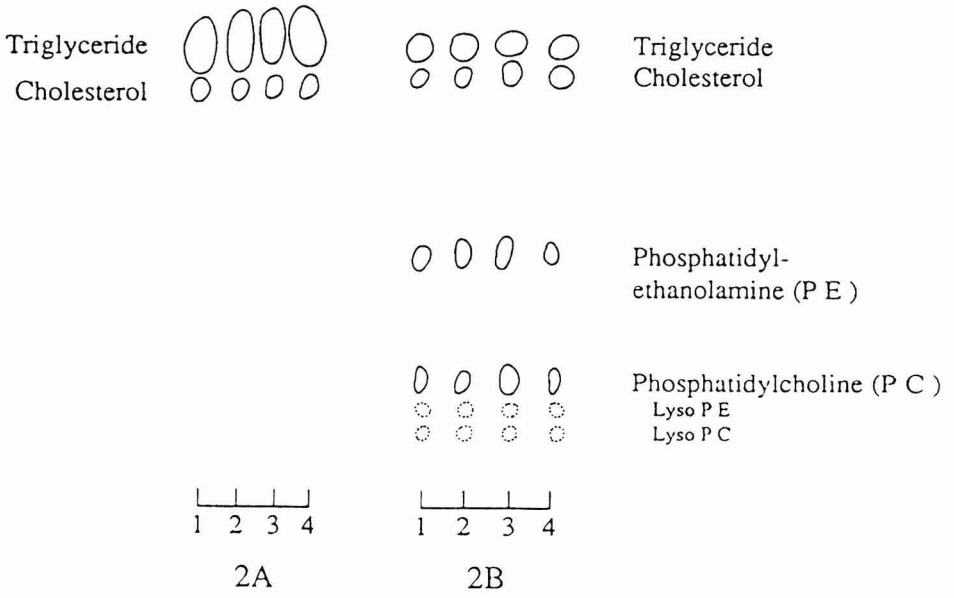

FIG. 2. THIN-LAYER CHROMATOGRAPHY PATTERNS OF NEUTRAL AND PHOSPHOLIPIDS OF SAMPLES 1-4 IN TABLE 1

Protein Separation

Table 2 shows the protein content of various fractions of egg yolk separated with water or CMC solution. When the yolk was diluted with equal weight of water, the plasma contained about 16% protein, whereas the granular material had ≈65% protein. Yolk lipid extraction with HIP (3:2) resulted in an increase in the protein content of the residual mass to about 85% (Sample 2). The protein content in the supernatant was higher when yolk was mixed with 0.25% CMC solution at a ratio of 1:1.5 (w/w) than at 1:1 (w/w) (Sample 9 vs 5, Table 2) and required no pH adjustment, as pH about 5.9 was suitable to cause egg yolk protein precipitation. Larsen and Froning (1981) have extracted various components of egg yolk at pH 6 using mixtures of organic solvents. The protein precipitation with CMC remained static upon increasing the volume of CMC solution into the egg yolk diluted with water (Sample 19 vs 20). The protein content of supernatants at yolk to CMC ratio of 1:1.5 (w/w) increased with increasing CMC concentration from 0.05 to 0.25% (samples 7–9). This was further supported by the higher protein content (≈40%) in Sample 10 in the precipitate (protein-CMC-lipid complex) at yolk to CMC ratio of 1:1.5 (w/w) than that with higher CMC concentration, i.e., 0.15% or 0.25% CMC. When a 0.25% CMC

solution was mixed with plasma and centrifuged, a nonsticky semisolid yellowish supernatant was formed, and a clear colorless liquid subnatant was obtained. The separation of a packed semisolid complex at the upper portion of the centrifuge tube and a clear liquid subnatant at the lower portion in Method 1 was just opposite to that obtained by a direct mixing of CMC with egg yolk (Method 2), in which a precipitate settled at the bottom. The subnatant contained more protein (Samples 16–18) than the supernatant (Samples 13–15). Again, the protein content in subnatant liquid was more in the fraction obtained with 0.15% CMC solution than using either 0.05% or 0.25% CMC solution.

Figures 3–5 show the compositional relationship between apoproteins present in various yolk fractions obtained through Methods 1–3. Whole egg yolk showed an SDS-PAGE pattern, which contained polypeptides in the molecular weight range of 20 kD to 215 kD (Lane 6, Fig. 3), which was similar to that reported by Jensenius *et al.* (1981) and Itoh *et al.* (1986). The major proteins with molecular weight 38, 58, 64, 74 and 109 kD in the protein-CMC-lipid complex (precipitate) obtained by Method 2 using either 0.05, 0.15 or 0.25% CMC concentration were

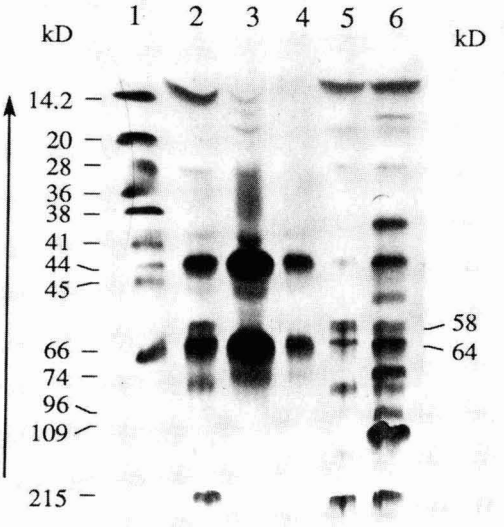

FIG. 3. SDS-PAGE PATTERNS OF APOPROTEINS FROM FRACTIONATED EGG YOLK AND THEIR MOLECULAR WEIGHT
(1) Standard molecular weight marker (Dalton Mark VII-L, Sigma Chem. Co.); (2) subnatant (method 1, 0.05% CMC); (3) subnatant (method 1, 0.15% CMC); (4) subnatant (method 1, 0.25% CMC); (5) semi-solid supernatant (0.25% CMC); (6) whole egg yolk.

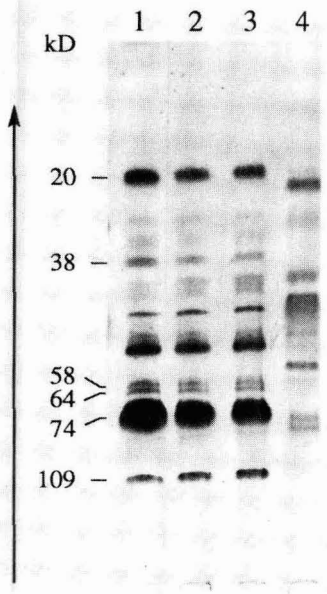

FIG. 4. SDS-PAGE PATTERNS OF APOPROTEINS FROM FRACTIONATED EGG YOLK
(1) Precipitate (method 2, 0.05% CMC); (2) precipitate (method 2, 0.15% CMC); (3) precipitate (method 2, 0.25% CMC); (4) whole egg yolk.

almost identical (Lanes 1–3, Fig. 4). The supernatants of this method contained similar polypeptides in the molecular weight range of 38–74 kD, which were more concentrated in the CMC (0.25%) fraction (data not shown).

The proteins of the yellowish semisolid supernatant (Method 1) consisted of four major bands at molecular weight of 58, 64, 74 and 215 kD (Lane 5, Fig. 3), which were similar for all the CMC concentrations used. Note that these fractions were very rich in total lipid content (Table 2). The subnatants produced in this process, however, contained two major polypeptides with molecular weights of 44 and 64 kD, respectively (Lanes 2–4, Fig. 3). Separation of these two proteins with a 0.15% CMC solution showed denser bands (Lane 3, Fig. 3) than that by the other two CMC solutions because equal volume or unequal protein loads were used. However, using unequal protein loads does not affect the molecular mass of peptides seen on the SDS-PAGE gels. The 0 .25% CMC solution caused better fractionation than the other two solutions because only polypeptides with molecular weight of 44 and 64 kD appear on the gel (Lane 4, Fig. 3). The 44 kD band corresponded well with the 42–45 kD molecular weight of β-livetin (Powrie and Nakai 1985). The higher molecular weight polypeptide (64

TABLE 2.

PROTEIN CONTENTS OF EGG YOLK FRACTIONS SEPARATED WITH WATER OR CMC SOLUTIONS[a]

	Sample description	CMC solution (%, w/w)	Fraction	Protein (%)	Color
1.	Egg yolk[b]			15.5 (0.5)	Yellow
2.	Egg yolk + HIP		Residue	86.1 (1.1)	Colorless
3.	Yolk + water (1:1, w/w)		Plasma	16.2 (0.42)	Yellow
4.	" "		Granule	65.5 (1.0)	Light Yellow
5.	Yolk + CMC (1:1, w/w)	0.25	Supernatant	20.8 (0.57)	Colorless
6.	" "	0.25	Precipitate	32.7 (0.63)	Yellow
7.	Yolk + CMC (1:1.5, w/w)	0.05	Supernatant	21.6 (0.53)	Yellow
8.	" "	0.15	"	30.2 (0.7)	Light Yellow
9.	" "	0.25	"	68.3 (0.97)	Colorless
10.	" "	0.05	Precipitate	40.1 (0.72)	Yellow
11.	" "	0.15	"	29.0 (0.58)	Yellow
12.	" "	0.25	"	28.7 (0.6)	Yellow
13.	Plasma + CMC (1:1, w/w)	0.05	Semisolid Supernatant	8.98 (0.33)	Yellow
14.	" "	0.15	"	6.69 (0.27)	Yellow
15.	" "	0.25	"	14.1 (0.35)	Yellow
16.	" "	0.05	Subnatant	26.9 (0.56)	Light Yellow
17.	" "	0.15	"	68.3 (0.99)	Colorless
18.	" "	0.25	"	28.1 (0.22)	Colorless
19.	Yolk + water (1:1, w/w) + CMC (25 mL)	0.25	Precipitate	33.6 (0.84)	Yellow
20.	" (50 mL)	0.25	"	28.2 (0.71)	Yellow

[a] Values are means of triplicate determinations with standard deviations in parenthesis.
[b] % yolk solids.
HIP = Hexane-isopropanol (3:2, v/v).
CMC = Carboxymethyl cellulose.

kD), as visualized on SDS-PAGE (Fig. 3), corresponded well with the location of a major polypeptide from γ-livetin (heavy chain, molecular weight of 62–64 kD) as also shown by Hatta *et al.* (1988). Furthermore, the livetin bands were relatively purer than those obtained with polyethylene glycol (PEG) precipita-

tion method and those using DEAE-cellulose column, as reported by Carroll and Stollar (1983). It also showed only two bands of β- and γ-livetins, respectively, as compared to three bands shown by Hatta *et al.* (1988). The purity of these two globular proteins was quite comparable and their combined yield of 111 mg/g egg yolk (on dry weight basis) was much higher than that obtained by other reported methods of egg globulins or IgG purification, either using alginate (Hatta *et al.* 1988) or by improved PEG or hydrophobic interaction chromatography plus gel filtration (3 mg/mL egg yolk) (Hassl and Aspock 1988) or metal chelate interaction chromatography (2 mg/mL egg yolk) (McCannel and Nakai 1989) or DEAE-cellulose column (1 mg/mL egg yolk) (McCannel and Nakai 1990). The recovery of about 11.1% globular proteins was again comparable to that of 10.6% reported by Saari *et al.* (1964).

An ammonium sulfate precipitation technique, when applied to the plasma (Method 1) and/or the supernatant of Method 2, resulted in livetin fractions containing γ-livetin (heavy chain) and β-livetin as major components. These also

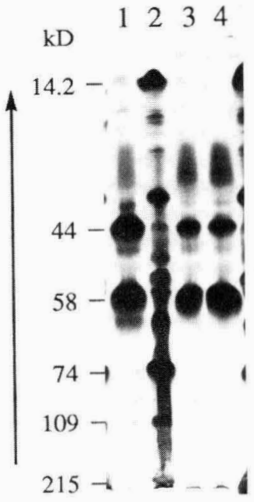

FIG. 5. SDS-PAGE PATTERNS OF APOPROTEINS FROM FRACTIONATED EGG YOLK

(1) Protein residue of whole egg yolk after HIP extraction and 0.25% CMC treatment; (2) protein residue of whole egg yolk after HIP extraction; (3) $(NH_4)_2SO_4$-precipitated proteins from subnatant of method 1; (4) $(NH_4)_2SO_4$-precipitated proteins from supernatant of method 2.

contained minute amounts of γ-livetin (light chain) (Lane 3 and 4, Fig. 5). These protein profiles were similar in their major livetin contents when compared with those fractionated from DP using CMC solutions. The residue of egg yolk obtained after purification with HIP extraction procedure contained almost all the major protein constituents similar to that detected in a freeze-dried yolk sample (Lane 2, Fig. 5). This also indicated that the molecular mass of polypeptides from egg yolk proteins is minimally affected in the HIP extraction process in contrast to the other processes of lipid extraction, e.g., chloroform-methanol or ether extraction. A suspension of this protein residue in water (1 g/10 mL water) treated with 10 mL CMC (0.25%), upon centrifugation, yielded a precipitate. The SDS-PAGE profile of such precipitate was rich in γ-globulin content (Lane 1, Fig. 5) as discussed earlier.

CONCLUSIONS

A process for separation of egg yolk lipid and proteins was developed. It produced value-added fractions, while maintaining the molecular mass of proteins near that of their native state. The protein fractions or CMC-protein complex can be used as food ingredients depending on their functionality. The lipids can be further fractionated into triglycerides and lecithin, which can also be used as food ingredients.

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EVALUATION AND COMPARISON OF SIMPLE METHODS FOR pH MEASUREMENT OF REDUCED-MOISTURE SOLID SYSTEMS

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ABSTRACT

It is desirable to quickly and accurately determine the pH of reduced-moisture solid food systems. Several techniques of pH determination were evaluated on such systems. Methods involving the addition of water yielded pH values higher than actually existed based on pH-dependent chemical reactions. The Ultra M® micro electrode had problems with reproducibility and stability in the reduced-moisture systems as well as the buffer solutions used for calibration. The Horiba Cardy pH meter and the surface pH electrode used directly on the surface of the reduced-moisture solid system yielded pH values similar to those determined using pH-dependent chemical reaction markers. The ability to measure the actual pH of reduced-moisture solid systems would enable better understanding of potential modifications in product formulation needed to retard microbial growth and limit chemical reactions responsible for loss of shelf-life.

INTRODUCTION

The importance of pH on food product stability has been well-documented. For example, pH influences the degradation rate of vitamins, such as ascorbic acid (Lee 1974), folacin (Paince-Wilson and Chen 1979), and thiamine (Mauri *et al.* 1989). The pH-dependence of the Maillard reaction, which decreases the nutritional availability of amino acids, has also been documented (Kaanane and Labuza 1989). The effect of pH on product stability is not limited to high moisture

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systems ($a_w > 0.99$) and solutions, but is also important at significantly reduced water activities. Bell and Labuza (1991a) showed that pH was an important factor in influencing the rates of aspartame degradation in a solid model system at water activities of 0.34 and 0.56. It is thus important to be able to measure the pH of these reduced-moisture solid systems to better understand how to control or retard deleterious reactions and extend shelf-life.

The pH of reduced-moisture solid systems is often ignored or assumed to be similar to that of the hydrated system. Bell and Labuza (1991b) used pH-dependent chemical reaction markers to show that the actual pH of the aqueous phase in reduced-moisture solid model systems ($a_w > 0.34$) was not the same as the initial "wet" pH of the system $a_w > 0.99$). They showed that the degradation product patterns of aspartame changed as the water activity of an agar/microcrystalline cellulose gel system was lowered. The results implied that there was a decrease in the pH of the reduced-moisture solid system as the water activity was lowered. For example, a hydrated system containing 0.1 molal phosphate buffer initially at pH 6.0 had an estimated pH value of 3.0–3.5 at a water activity of 0.34 based on the pH-dependent degradation mechanism of aspartame (Bell and Labuza 1991b). Bell (1992) found similar results using the pattern of ampicillin degradation product formation. Therefore, it cannot be assumed that the final pH of a reduced-moisture product is the same as that of the high-moisture mixture prior to processing. Unfortunately, it takes days to months to determine the pH of reduced-moisture systems using a chemical reaction marker. It would be desirable to have available a quick (i.e., seconds to minutes) method of pH determination for reduced-moisture solid systems which gives a pH value similar to that determined by a chemical reaction marker. Thus, the objective of this research was to accurately measure the pH of reduced-moisture solid systems using a variety of quick techniques and compare them to each other as well as to the pH indicated by a pH-dependent chemical reaction marker. Two newer pH electrodes were also evaluated as part of the comparison.

MATERIALS AND METHODS

Model System

The model system consisted of freeze-dried agar/microcrystalline cellulose gels prepared by the method of Biquet and Labuza (1988) as modified by Bell and Labuza (1991a). Prior to freeze-drying, 0.1 molal phosphate buffer (Fisher Scientific, Fair Lawn, NJ) was incorporated into the gel to yield wet pH values between 2.65 and 7.0. These dehydrated gels were equilibrated for 3 weeks to water activities of 0.34 over saturated $MgCl_2$ and 0.75 and over saturated NaCl. These

had dry basis moisture contents of 6 and 20 g water/100 g solids, respectively. "Wet" gels ($a_w > 0.99$) were also utilized as a part of this study.

pH Techniques

Two devices that may be able to directly measure the pH of reduced-moisture solid systems were evaluated. The first of these was the Horiba Cardy pH meter (Markson, Phoenix, AZ), which has a large flat sensor designed for surface pH measurement. Six to eleven slices of intact gels at different a_w values were placed on the sensor while applying a constant pressure (i.e., a 200-g weight) to ensure adequate contact. The other device was the Ultra M® micro combination pH electrode (Lazar Research Labs, Inc., Los Angeles, CA), which can, according to the literature provided, measure the pH in 10 μL volumes (i.e., a cube of 2.15 mm per side, which is still quite large). Assuming water exists in small connected pockets within the equilibrated gel, inserting this electrode into the gel might produce a detectable pH. Thus, this electrode was inserted into 10–13 different locations of representative gels at the two a_w values, and the pH values were recorded. Manufacturer directions were followed with both of these devices.

Gels initially at a "wet" pH of 5 and 7 and equilibrated to an a_w of 0.34 were pulverized into a fine powder using a Regal® Coffee and Spice Mill. A 1 g portion of this powder was combined with either 99 g or 9 g of Milli-Q purified water (Millipore Corporation, Milford, MA) in duplicate. After shaking or stirring for a minimum of 5 min, the pH of the mixture was measured with a glass combination electrode and Orion model 811 pH meter (Orion Research Inc., Boston, MA). This method of utilizing the addition of water to a portion of dried material to enable pH measurement is mentioned in the pH textbook by Westcott (1978) and has become rather traditional in the food industry.

Another method involved the addition of various small volumes of purified water to 1 g of the low moisture gel powder prepared initially at pH values of 5 and 7. These volumes ranged from 1 to 4 mL. The mixture was sitrred thoroughly with a glass rod to hydrate all the powder. This mixture was shaped into a flat cake on which the pH was measured with a surface combination electrode (Fisher Scientific, Pittsburgh, PA). The pH was plotted against the volume of water added using 100% volume-corrected Gran plot paper (originally supplied by Orion Research Inc., Boston, MA, but no longer available commercially). Extrapolating to no-water-added provides an approximate pH value for the low-moisture system. This method was used by Labuza (1974) in studies of the pH of freeze-dried foods and is a modification and application of equivalence point determination in potentiometric titrations (Gran 1950, 1952).

The final method tested used intact dehydrated gels, again prepared initially at pH 5 and 7, which were dried and equilibrated to a_w 0.34. To these low moisture

gels, 5 drops of water (0.15 mL) were added to their surfaces. The pH was measured using a surface combination electrode at three different locations. This technique was also mentioned by Westcott (1978) and is another traditional approach of pH measurement. Despite the fact that this electrode is designed for hydrated surfaces, an attempt was also made to measure the surface pH of the gel using the same electrode without the addition of the water.

RESULTS AND DISCUSSION

Table 1 lists the pH values of the dried and rehumidified gels as determined by direct measurement without the addition of water. As seen in this table, reproducibility is a problem with the Cardy pH meter. The reproducibility of pH values using the Cardy pH meter is better at a_w 0.75 ($\pm$0.09, $\pm$0.10, and $\pm$0.13) and 0.99 ($\pm$0.07) than at a_w 0.34 ($\pm$0.18, $\pm$.039, $\pm$0.45). For the system at a water activity of 0.34, the pH values for any initial pH differed over a range of at least 1.1 pH units. Periodically the Cardy pH meter gave an extreme pH value as shown for the systems at pH 6.5 and a_w 0.75 as well as at pH 5.0 and a_w 0.34. In all cases, the pH of the reduced-moisture systems was less than that of the wet system. For example, with the gel initially at pH 6.5, the Cardy meter indicated a pH decline of 1 unit at a_w 0.75 and 2.8 units at a_w 0.34. This can be explained by the precipitation of dibasic phosphate buffer accompanied by a shift in equilibria. At an initial wet pH of 4.0, the decrease in pH was 0.9 units at both a_w 0.34 and 0.73. Buffer composition at pH 4.0 is 99% monobasic phosphate buffer, which means that even as buffer salts precipitate, the ability to shift the equilibrium is limited. The small difference in dry basis moisture contents (6% and 20%) accompanied by the limited shift in equilibrium resulted in both a_w values reaching a pH near 3.

Table 1 also shows the pH values measured using the Ultra M® micro pH electrode. Reproducibility was poorer ($\pm$0.23 to $\pm$.059) than for the Cardy meter ($\pm$0.09 to $\pm$0.45). Stability was also a problem as indicated by the drifting of pH values, shown in this table. If pockets of moisture do exist, variability may be attributed to inserting the electrode tip into these pockets sometimes and missing the pockets other times. However, it was difficult to standardize the pH meter with the buffer solutions due to the drifting of pH values. At a_w 0.73 and an initial "wet" pH of 4.4, the measured pH was less than that of the hydrated system. As seen, this electrode indicated that the system imitially at a "wet" pH of 6 had a measured pH of 5.4 at a_w 0.76.

Table 1 also lists the pH values of a 0.34 a_w system measured using the surface electrode without the addition of water. The pH values also drifted as noted. The measured pH values were again less than the initial pH values, being 3.1 to 3.6

TABLE 1.
pH MEASUREMENT BY DIRECT METHODS (NO MOISTURE ADDITION)

Initial Wet pH	Water Activity	Measured pH Values	Average with S.D.[1]
Cardy pH Meter			
4.0	0.34	2.47, 2.97, 3.06, 3.14, 3.50, 3.50, 3.57	3.17±0.39
5.0	0.34	1.9[2], 3.43, 3.43, 3.48, 3.65, 3.85	3.57±0.18
6.5	0.34	3.0, 3.2, 3.3, 3.4, 3.4, 3.6, 3.6, 4.0, 4.2, 4.2, 4.3,	3.65±0.45
4.0	0.73	2.90, 2.91, 2.98, 3.02, 3.11, 3.13, 3.15, 3.17, 3.30	3.07±0.13
5.5	0.76	3.57, 3.67, 3.67, 3.71, 3.76, 3.79, 3.81, 3.87	3.73±0.10
6.5	0.75	1.5[2], 5.30, 5.37, 5.38, 5.41, 5.44, 5.51, 5.56	5.42±0.09
2.65	0.99	2.43, 2.43, 2.43, 2.43, 2.44, 2.45, 2.45, 2.46, 2.50, 2.50, 2.68	2.47±0.07
Micro pH Electrode			
5.0	0.34	4.0, 4.0, 4.1, 4.2, 4.3, 4.3, 4.5, 4.5 4.6, 5.1, 5.5, 5.8, 5.0-6.3[2,3]	4.58±0.59
4.4	0.73	3.92, 3.93, 4.05, 4.12, 4.13, 4.13, 4.20 4.24, 4.41, 4.51, 4.67, 4.54→4.23[2,4]	4.21±0.23
6.0	0.76	5.01, 5.1, 5.40, 5.47, 5.63, 5.67, 5.1-5.2[2,3], 5.0-5.3[2,3], 5.65-5.75[2,3], 5.5→5.2[2,4]	5.38±0.27
Surface Electrode Without Addition of Water			
5.0	0.34	3.1-3.2[3], 3.3-3.6[3]	N.D.
7.0	0.34	5.21, 5.1-5.5[3]	N.D.

[1]S.D. = standard deviation; [2]Not used in average;
[3]Unstable reading over the specified range; [4]Slow drifting.
N.D. = not determined

at a_w 0.34 for a system prepared initially to a wet pH of 5.0. The reduced-moisture pH range of 3.1 to 3.6 is similar to that measured with the Cardy meter (pH 3.6), both of which were lower than that measured with the micro pH electrode (pH 4.7).

Table 2 lists the results of the pH methods involving the addition of water to the solid model system humidified to a water activity of 0.34. It is generally ac-

TABLE 2.
pH MEASUREMENT BY METHODS UTILIZING ADDITION OF WATER TO THE MODEL SYSTEM AT a_w 0.34

pH Method	Initial Wet pH	pH Values
1 g gel + 9 g H_2O[1]	5.0	4.91, 4.91
	7.0	6.97, 7.00
1 g gel + 99 g H_2O[1]	5.0	4.88, 4.88
	7.0	7.21, 7.20
Wet Surface Electrode	5.0	4.50, 4.63, 4.75
	7.0	6.59, 6.73, 6.83
Gran Plot[2]	5.0	4.0
	7.0	6.4

[1]Glass combination electrode; [2]Surface electrode

cepted that pH techniques involving moisture addition are reproducible (Orion Research Inc., 1988), so this aspect was not investigated. As shown in this table, the methods involving the addition of 9 g water or 99 g water had pH values similar to that of the initial gel prior to dehydration. The method utilizing the surface electrode with water addition provided pH values slightly less than those techniques using larger amounts of water. The Gran plot technique gave the lowest pH value of methods using moisture addition.

By comparing the pH data at a_w 0.34 for the system initially at a wet pH of 5.0 (Tables 1 and 2), it can be seen that each method yields a different pH value, ranging from 3.1 to 5.8. The same trend was true for the gels at an a_w of 0.34 having an initial pH of 7, with values ranging from 5.1 to 7.2. Methods utilizing the addition of water provided a higher pH value than with direct pH measurement on the reduced-moisture systems. However, methods involving direct pH measurement without the addition of water have the limitations of being less reproducible and stable; these methods may possibly be used to get an approximate range within which the true pH value probably exists. The Gran plot method gave values somewhat between those of the moisture addition methods and direct pH measurement of the reduced-moisture system. Orion Research Inc. (1988) mentioned that each pH method produced a different pH value, which is supported by the data in Tables 1 and 2. The question thus arises as to which method gives a better estimate of the true pH.

Using aspartame as a chemical reaction marker, Bell and Labuza (1991b) found that the actual pH of the same a_w 0.34 gels initially at a wet pH of 5 was 2.6–3.0, while those initially at pH 7 ranged from 5.5 to 6.5 (Table 3). This table also

TABLE 3.
COMPARISON OF METHODS USED TO ESTIMATE THE ACTUAL pH OF THE MODEL SYSTEM AT a_w 0.34

Initial Wet pH	pH of Saturated Solution[1]	pH by Aspartame Loss[2]	pH by Ampicillin Loss[1]	Cardy Meter	Dry Surface Electrode	Estimated Overall pH Range
4.0	3.4	2.2	2.5	2.8-3.6	N.D.	2.2-3.6
5.0	3.7	2.6-3.0	3.0-3.5	3.4-3.8	3.1-3.6	2.6-3.8
7.0	6.3	5.5-6.0	5.5-6.5	N.D.	5.1-5.5	5.5-6.5

[1]From Bell (1992); [2]From Bell and Labuza (1991b); N.D. = not determined

lists the measured pH values of the reduced-moisture model system (a_w 0.34, 6% dry basis moisture) using the Cardy meter and dry surface electrode in comparison to the measured pH of a saturated buffer solution. The pH values of the saturated buffer solutions and those predicted by Gran plots (Table 2) were higher than those suggested by chemical reaction markers and those measured with dry techniques. The lower pH values, compared to the pH of saturated buffer solutions, suggest the possibility of buffer supersaturation in the reduced-moisture solid systems. Supersaturation of buffer salts in these systems also was indicated by an evaluation of aspartame degradation kinetics as a function of buffer concentration (Bell 1992). The other four techniques listed in Table 3 yielded pH values of the same magnitude and the results were combined to give an estimated overall pH range of the reduced-moisture solid system.

In all cases, the inital high-moisture pH value was highest, while that predicted by use of chemical reaction markers and the two surface electrodes was lowest; the pH of the saturated solutions and that predicted by the Gran plot method fell between the other values. It appears that the Gran plot method, which utilized the addition of small quantities of water, predicted pH values to the buffer saturation point, but cannot predict the pH beyond that (i.e., for supersaturated buffer systems). The Cardy pH meter and surface electrode used without the addition of water appear to be able to estimate the pH of reduced-moisture solid systems and produce values similar to those based on predictions using a chemical reaction marker in the same system.

Results shown here and in the chemical marker studies indicate that the pH of systems containing phosphate buffer salts decreased with decreasing water activity, which could result in changes in reaction rates and certainly would account for part of the synergistic effect of pH and a_w on microbial growth, termed a "hurdle" effect by Leistner (1985). The ability to measure or estimate the actual pH of the reduced-moisture solid system would allow for the understanding of how to modify product formulation so as to retard microbial growth and reduce

chemical reactions responsible for loss of shelf-life. This is beneficial and important to know during product development and for rapid on-line pH determination in food processing facilities.

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BOOK REVIEW

PLASTICS IN FOOD PACKAGING. William E. Brown. 1992. Marcel Dekker, Inc., 270 Madison Avenue, New York, NY 10016, 560 pages, $150.00.

This is a well-organized reference book containing thirteen chapters, an index, and appendices. The emphasis of the book is on properties, design, and fabrication of plastic containers, bottles and films, although several chapters are also devoted to other related topics such as regulations, manufacturing cost, trends and developments, and package tests.

The first three chapters review some of the basic principles and technologies of food packaging: the physical, chemical, optical, transport, and thermal properties of packaging materials; the uses of glass, metal, paper, plastics, and composites as packaging materials; the deterioration modes of foods, the methods of food preservation, and their relationship with food packaging. Although the materials in these chapters can be found in general food science textbooks, they serve well in the book as an introduction and a quick reference.

Chapter 4 summarizes the properties, chemical structures, and applications of the principal types of food packaging plastics — polyolefins, styrenics, vinyls, polyesters, as well as high oxygen barrier plastics such as PVDC, EVOH, and nylon MXD6. Chapters 5 and 6 (108 pages) discuss the principal fabrication processes for plastic packages, with emphasis on processes such as coextrusion, thermoforming, blow molding, injection molding, coating, and laminating.

Perhaps the most useful section of the book is Chapters 7 through 9 (150 pages), which focus on design and shelf-life testing, because the subject is so inadequately covered in other books. Chapter 7 discusses the design considerations for strength, stiffness, impact loading, stress concentration, etc. Chapter 8 discusses the design considerations for barrier properties of plastics, including the principles of sorption, diffusion, and permeation; measurements and calculations gas and water vapor transmission rates; and recent developments in barrier plastics. Chapter 9 discusses the general considerations of shelf-life testing, elucidating the relationship among intrinsic and extrinsic stability of foods, testing condition, and package requirement. Many useful equations, tables, figures, and examples are provided in these chapters. The readers should, however, exercise care when applying these equations because the limitations of some of these equations are not well-explained in the book.

The remaining chapters provide a summary of safety and regulations of food packaging, package manufacturing cost, trends and developments, and package

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tests, which are also important aspects of food package design. The chapter on trends and developments is particularly enlightening, where interesting trends such as active packaging, modified atmosphere packaging, aseptic packaging, and waste disposal are discussed.

In general, *Plastics in Food Packaging* is one of the very few food packaging references that treat the subject with adequate depth. The book should be a useful reference for packaging engineers and designers and quality assurance personnel, in which the readers can find many time-saving figures, tables, equations, and references. Because the book focuses on general food packaging principles, it provides little information on packaging of specific food commodities. Finally, students will greatly benefit from the book if problem sets and more examples are to be incorporated in the second edition.

KIT L. YAM, Ph.D

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