er processors in the fish and fisheries industry. The visual approach may be useful for rapid screening of fish for freshness or spoilage, but it is highly subjective.

The procedure presented here for quantitating hypoxanthine in channel catfish offers a relatively inexpensive, objective, and accurate approach if performed in exact accordance with the directions. Temperatures and concentrations of the reactants must be constant. Just as the rate of hypoxanthine formation in fish tissue is increased at elevated temperatures, so is its rate of oxidation in the test tube. Hypoxanthine concentrations exceeding 10 μg were observed to completely decolorize the 40 μg of dye in the assay mixture after 5 min. Proportionate concentrations of reactants as they are outlined result in a standard curve with a straight line at concentrations ranging from 2 to 7 μg of hypoxanthine in the reaction mixture. These concentrations can be read in Klett units as approximately 92 to 28, respectively, at the end of the 5-min reaction time. All hypoxanthine levels calculated for chilled channel catfish were based on a reading of 60 Klett units, a point approximately midway on the straight-line portion of the standard curve.

The apparent accumulation of hypoxanthine in chill-stored catfish is shown in Figure 2. Values plotted are means of 24 determinations for each evaluation date. Standard deviations are shown. Increased concentrations were demonstrated to occur in a linear fashion, reaching a maximum of 2.7 μmol per g of fish muscle during the first 17 days of storage. Levels subsided somewhat at 22 days, perhaps due to leaching into the drip. The data of greatest interest are those obtained before Phase 2 (7-9 days) of spoilage. As reflected by aroma and total sensory scores (Figure 1), catfish were rated acceptable during a period of up to 6 days *postmortem*. Hypoxanthine analyses resulting in concentrations nearing 1.3 μmol per g in catfish muscle would indicate that the chill-stored catfish are borderline with respect to acceptance based on sensory ratings. Therefore, a catfish processor might employ the objective method of hypoxanthine analysis in combination with sensory ratings (especially aroma) in an attempt to more accurately assess the freshness of catfish which are delivered to his plant.

Little variation in hypoxanthine concentrations among fish analyzed at specific storage times was observed in this study. However, the rates of accumulation may differ in catfish stored at other temperatures. It should also be kept in mind that various conditions of culturing, harvesting, and handling could effect the rate of hypoxanthine accumulation in the stored catfish. Whether or not concentrations will reach approximately 1.3 μmol per g of fish muscle within the same length of time *postmortem* required for rejection of the fish based on sensory evaluation remains to be demonstrated. Nevertheless, no other chemical test holds as much promise as the hypoxanthine test as an index of freshness for freshwater fish. Data presented in this study indicate that measurement of hypoxanthine in chilled channel catfish may be a useful, inexpensive, objective method to assess the degree of freshness prior to incipient spoilage.

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Effects of Coatings on Weight Loss and Ethanol Buildup in Juice of Oranges

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Oranges coated with commercial solvent-type wax lost less weight than those with comparable amounts of water wax or polyethylene coatings. Ethanol buildup in juice and off-flavors occurred after multiple coatings of water wax or solvent-type wax; less ethanol accumulation and no off-flavors were noted in polyethylene-coated fruits. Citrus fruits lose weight at rates not necessarily related to original weight or surface area. Individual fruits lose weight at a constant rate over short time periods and this should be considered in determining the amount of coating by weighing procedures. A method is presented to determine the weight of coating applied.

The use of coatings for citrus fruits to impart gloss and prevent weight loss is well established. Rind disorders, such as "pitting," sunken areas usually associated with cold injury, and "aging," rind tissue collapse near the stem end, may be alleviated with proper coatings. For example, pitting of grapefruit was reduced substantially by polyethylene emulsion coatings (Davis and Harding, 1960) which, in addition, were compatible with fungicides for decay control (Davis and Smoot, 1960). Pitting of Valencia oranges was reduced by preharvest sprays of Pinolene, a polyterpene film former (Albrigo *et al.*, 1970), and by postharvest application of commercial solvent-type wax (Chace *et al.*, 1969).

Coatings also affect the physiology of fruits. Ben-Yehoshua (1967) found that coatings lowered internal O_2 and raised internal CO_2 and suggested that weight loss and in-

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Table I. Effects of Coatings on Temple Oranges Held at 21.1° for 3 Weeks or at 4.4° for 3 Weeks plus 2 Weeks at 21.1°, Ten Fruits per Test

21.1°, 3 weeks	Acetaldehyde, mg/100 ml	Ethanol, mg/100 ml	O_2, %	CO_2, %	Wt loss, %		
Control[a]	0.3	21	17.9	2.0	12.2		
P1	0.2	13	16.3	2.8	8.9		
P2	0.4	29	16.8	2.7	8.1		
ST	0.2	18	17.9	1.9	8.6		
WW	0.6	63	10.0	7.5	8.8		
4.4°, 3 weeks, 21.1°, 2 weeks					On removal	1 week, 21.1°	2 weeks, 21.1°
Control	0.4	26	17.6	1.8	3.7	8.0	9.4
P1	0.5	29	18.0	1.1	2.7	6.6	9.4
P2	0.6	42	16.4	3.1	2.4	5.3	7.4
ST	0.5	29	16.8	2.8	2.6	5.7	8.4
WW	0.8	101	10.8	5.7	2.6	5.3	7.8

[a] P1 and P2 = polyethylene coatings; ST = solvent-type wax; WW = water wax.

ternal O_2 be used as guides in controlling the coating operation (Ben-Yehoshua *et al.*, 1970). Off-flavors were noted in fruits receiving repeated dippings. Under controlled atmosphere storage, waxed citrus fruits consistently had lower internal O_2 and higher CO_2 (Davis *et al.*, 1967) and higher ethanol content of juice (Davis, 1970) than unwaxed fruit.

The optimum amount of coating should provide sufficient gloss and minimize weight loss without producing off-flavors. Several new polyethylene emulsions are now available, and two of these were compared with two types of commercially used waxes.

EXPERIMENTAL SECTION

Two of several available polyethylene emulsion formulations imparted adequate gloss and were investigated further. One, herein designated "P1," contained high density polyethylene, oleic acid, and morpholine (25% solids). The second, "P2," contained polyethylene, oleic acid, morpholine, ammonium hydroxide, and food grade shellac (16% solids). Both were diluted and applied as emulsions containing 10% solids. The commercial waxes were a solvent-type, "ST," and a water wax, "WW." Temple and Valencia oranges from commercial groves were treated within 1 day after harvest. Fruits were weighed individually with a Sartorius Model 2402-T balance, washed by commercial-type brusher equipment, and coated by spraying. Internal O_2 and CO_2 were determined by gas chromatography using a thermal conductivity detector (Davis *et al.*, 1967). Ethanol content of juice was determined by gas chromatographic analysis of headspace (Davis and Chace, 1969) using a flame ionization detector. Both instruments were Micro-Tek GC-2000R chromatographs. All tests were made with lots of ten fruit per treatment. Coatings were supplied by Allied Chemical Corp., Food Machinery Corp., and American Machinery Corp. Taste evaluations were made by a panel of four laboratory personnel on composite juice of ten-fruit samples.

The oranges were held in a room maintained at 21.1° with a controlled relative humidity of 88–92% to simulate supermarket conditions. In one test, oranges were stored at 4.4° for 3 weeks prior to holding at 21.1°. The relative humidity of this room was also 88–92%. On removal from 4.4°, these fruits were allowed to warm to 21.1° before being weighed.

RESULTS AND DISCUSSION

Temple oranges were coated and held at 21.1° for 3 weeks. All coatings reduced weight loss to approximately the same degree (Table I). Internal O_2 was lowest and CO_2 was highest in WW-coated fruit, which also contained highest ethanol and acetaldehyde content of juice. In Temple oranges stored at 4.4° for 3 weeks and then held at 21.1° for 2 weeks, ethanol increased almost fourfold in WW-coated fruit. Weight loss was reduced by all coatings during 4.4° storage and for 1 week at 21.1°. After

Table II. Effect of Multiple Coatings of Solvent-Type (ST) and Water Wax (WW). Valencia Oranges, 1 Week at 21.1°, Ten Fruits per Treatment

	Wt loss, %	Ethanol, mg/100 ml
Control		
Not washed	4.2	85
Washed	4.9	110
ST, coats		
1	2.7	190
2	2.1	260
3	1.8	380
4	1.5	380
5	1.5	390
WW, coats		
1	3.7	250
2	3.8	490
3	3.9	560
4	3.9	560
5	4.0	580

Table III. Effect of Multiple Coatings on Ethanol Content of Juice and on Weight Loss. Valencia Oranges, 1 Week at 21.1°, Ten Fruits per Treatment

	Ethanol, mg/100 ml				
Coats	Control	ST	WW	P1	P2
1	130	140	310[a]	160	140
2		310[a]	370[b]	160	210
3		370[a]	490[b]	160	210
	Weight loss, %				
1	4.2	2.5	3.0	3.1	3.3
2		2.0	3.0	2.6	2.9
3		1.7	3.1	2.7	2.6

[a] Slight off-flavor. [b] Off-flavor. ST = solvent-type wax; WW = water wax; P1 and P2 = polyethylene coatings.

Table IV. Rate of Weight Loss of Fruits Compared to Weight or Surface Area. Valencia Oranges at 21.1°, Washed but Not Waxed

Weight, g	Surface area, cm^2	Weight loss		
		mg/fruit/hr	mg/100 g/hr	$mg/cm^2/hr$
134	107.1	52.3	39.0	0.49
154	121.3	55.5	36.0	0.48
162	124.5	60.2	37.2	0.48
164	126.5	51.5	31.4	0.41
173	134.8	72.9	42.1	0.54
181	140.0	48.8	27.0	0.35
182	136.8	66.0	36.3	0.48
184	136.8	82.4	44.8	0.60
186	140.0	57.5	30.9	0.41
194	145.8	67.6	34.8	0.46

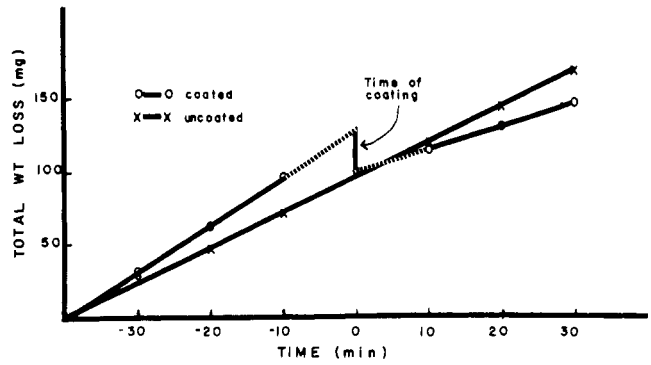

Figure 1. Illustration of weight loss patterns of individual Valencia oranges, one uncoated, one coated with solvent-type wax.

Table V. Relation of Weight, Surface Area, and Rate of Weight Loss. Valencia Oranges, Uncoated, Ten Fruits per Test

Test	Weight, g	Surface area, cm^2	Rate of loss, mg/hr	Correlation coefficients		
				wt, cm^2	wt, rate	cm^2, rate
1	134–194	107–146	48.8–82.4	.990	.473	.383
2	113–158	107–135	54.7–73.4	.935	.752	.651
3	144–193	125–148	42.8–55.9	.900	.540	.545
4	138–192	126–162	54.9–73.5	.963	.232	.276
5	128–199	125–165	54.4–70.3	.962	.589	.670

2 weeks at 21.1°, there was little difference between coated and control fruits.

Valencia oranges were given successive coatings of WW or ST wax and held for 1 week at 21.1° (Table II). Weight loss decreased with each additional coating of ST wax, but this advantage was offset by the accumulation of ethanol in juice, 380 mg/100 ml with three coatings. Weight loss was reduced from 4.9% in washed-only fruit to 3.7% by one coating of WW. Multiple coatings of WW did not further reduce weight loss but increased the ethanol content of juice to as high as 580 mg/100 ml.

Since little effect was noted from more than three coatings, subsequent tests were limited to three applications (Table III). Weight loss was least in ST-coated fruits and was reduced by multiple coatings. Polyethylene coatings also reduced weight loss, but multiple applications had slightly less effect than ST coatings. One coating of WW reduced weight loss about 25%, but additional coatings were without effect. Ethanol buildup in juice was greatest in fruits coated with WW and ST and least in polyethylene-coated fruits. Accompanying the ethanol buildup, off-flavors were noted in WW- and ST-treated fruits, but not in fruits coated with polyethylene.

The rate of loss of weight of individual fruits is apparently not necessarily due to original weight or to total surface area. Typical results, illustrated in detail in Table IV and summarized in Table V, indicate that while weight and surface area are closely related, little correlation was evident between weight and rate of loss or between surface area and rate of loss. These rates are comparable to those found by Jahn *et al.* (1967), losses of up to 2.9% during 84 hr of degreening at high humidities. They also reported that size and color had no apparent effect on weight loss.

The rate of weight loss, approximately 1–2 mg per minute for individual fruits, must be considered in determining the amount of coating per fruit by weighing procedures. Although the rate of loss cannot be predicted accurately from either weight or surface area of a particular fruit, the rate of loss of individual fruits over short times was practically constant (Figure 1).

This constant rate of weight loss can be used to advantage in determining the weight of coating applied to a fruit. As illustrated in Figure 1, a single fruit was weighed at 10-min intervals before and after coating with ST wax. The difference in weight just prior to coating and immediately after coating is the weight of coating applied. The weights can be calculated by plotting a graph or by use of the equation

$$\text{wt of coating} = (y + 10b) - (x - 10a)$$

where x = wt of fruit 10 min before coating, y = wt of fruit 10 min after coating, a = g/min wt loss before coating, and b = g/min wt loss after coating.

In the illustration, x = 150.7254, y = 150.7048, a = 0.00321, and b = 0.00164; thus, wt of coating = (150.7048 + 0.0164) − (150.7254 − 0.0321) = 27.9 mg.

The surface area of this fruit was 134 cm^2 (20.8 $in.^2$), and the coating was thus 0.21 mg/cm^2 (1.3 $mg/in.^2$), and was typical of fruits coated with ST wax in our tests. In a similar manner, single applications of 10% polyethylene emulsion coatings averaged 0.16–0.23 mg/cm^2 (1–1.5 $mg/in.^2$) and WW coatings averaged 0.31 mg/cm^2 (2 $mg/in.^2$). In practice, the amount of coating can be controlled easily by varying the concentration of material, size of spray nozzle, and length of time of spraying.

Minimum amounts of solvent-type or water wax which will impart sufficient gloss should be used in order to avoid off-flavors and should not exceed 0.2–0.3 mg/cm^2 (1–2 $mg/in.^2$). Ethanol buildup in juice accompanied off-flavor production and might be used as one parameter in establishing optimum amounts of coatings. Polyethylene coatings, while having somewhat lower gloss, did not produce ethanol buildup or off-flavors. The amount of coating per fruit in a given procedure can be determined easily by the method described.

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Canning and pH Stability of Epichlorohydrin-Treated Parboiled Rice

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Rice starch in the parboiled grain was etherified by epichlorohydrin in an alkaline environment. Samples were evaluated after canning and retorting for 60 min at 240°F in semiliquid media. The treatment vastly improved the kernel stability for canning in excess water, even under acidic conditions. Costly agitation-type retorting, which allows less severe processing due to improved heat transfer, was not required for this rice because of its high thermal stability. Cross-linked samples showed approximately 68% less leaching at pH 7 and approximately 82% less at pH 5, as opposed to untreated samples. Taste panel evaluation involving color, cohesiveness, flavor, and doneness of cross-linked samples indicates a high potential for its incorporation in various canned formulations.

At the present time, very few commercially canned products contain rice. This is due to two primary reasons. The first involves the stability of the rice grain and the second involves the high cost of agitated retorting equipment. Most canned products which could use rice in the formulation require processing for approximately 60 min at 240°F or a somewhat shorter process at 250°F in conventional retorting equipment. In the course of processing, a point is reached where the hydrogen bonds responsible for the starch granular integrity are weakened and an irreversible swelling occurs. If processing is continued, the starch granules will eventually rupture, resulting in leaching of solids and grain distortion. With regard to the above, white rice is less resistant to thermal degradation than that which is parboiled. However, due to lengthy processing in conventional retorts, parboiled rice is still considered unsatisfactory. With agitated retorting, parboiled rice can be canned in various soup formulations in a fairly acceptable manner. The cost of such equipment, however, is the main limiting factor. Many food operations are seasonal or operate with limited capital and cannot afford the agitated type of process.

The purpose of this study was to produce a rice which could withstand processing conditions encountered in still retorting while maintaining the desirable organoleptic properties associated with rice.

MATERIALS AND METHODS

The cross-linking treatment imposed on parboiled rice consists of three main steps: activation, cross-linking, and neutralization.

Activation. One-hundred grams of a canner's quality parboiled Bluebelle rice (obtained from Uncle Ben's, Inc., Houston, Tex.) was weighed in a 500-ml Erlenmeyer flask. Two-hundred milliliters of a 0.1 *N* NaOH solution and 10 g of NaCl were added to the rice. The mixture was allowed to stand for 2 hr.

Cross-Linking. After soaking, 26 ml of freshly prepared 1% epichlorohydrin solution (1 ml of epichlorohydrin made up to 100 ml with 0.1 *N* NaOH) was added to the flask and allowed to react on a shaker for 4 hr. The flask was closed with a rubber stopper to prevent loss of volatile epichlorohydrin.

Neutralization. After cross-linking, the alkali-salt-epichlorohydrin mixture was decanted into a 1000-ml volumetric flask. The rice was washed with distilled water several times and the washing was added to the volumetric flask for subsequent determination of unreacted epichlorohydrin. The washed rice was resuspended in 100 ml of distilled water; about 4.5 ml of 4 *N* HCl was added slowly drop by drop throughout 5 hr. The pH was maintained above 4, otherwise the rice grain may undergo acid modification, which is characterized by heavy starch leaching during canning. The grains were neutralized to a pH of 6.5 and thoroughly washed with tap water and air dried at room temperature.

Estimation of Unreacted Epichlorohydrin. The reaction mixture and the washing, which were collected in the 1000-ml volumetric flask, were brought to volume with distilled water and filtered after standing in a cold room at 5° for 18 hr for retrogradation of the starch, which aided filtration; 50 ml of the filtrate was diluted to 100 ml and a 10-ml aliquot was pipetted into a 50-ml volumetric flask, and 1 ml of 2 *N* sodium hydroxide was added. An epichlorohydrin standard and a reagent blank were prepared in a similar manner. The stoppered flasks were heated on a water bath at 80° for 1.5 hr and then cooled, and 1 ml of 10 *N* sulfuric acid was added, followed by 5 ml of 0.1 *M* sodium periodate. The flasks were placed in the dark for 10 min, after which 5 ml of 1.0 *M* sodium arsenite was added and the solutions were diluted to 50 ml with distilled water. One-milliliter aliquots were then pipetted into test tubes; 10 ml of chromotropic acid reagent was added rapidly, accompanied by mixing of each of the tubes, which were then heated in a boiling water bath for 30 min. The tubes were removed, cooled to room temperature, and poured into cuvettes, and the absorbance was read at a wavelength of 570 mμ in a Bausch & Lomb Spectronic 20 using a water blank set at zero absorbance. The procedure was similar to that described by Hamerstrand *et al.* (1960).

Canning Evaluation. Forty cans of rice were used in the evaluation, 20 containing the epichlorohydrin-treated rice and the remaining 20 using the untreated parboiled sample. Both treated and control rice were canned at pH 7 and 5, which resulted in a 2 × 2 factorial arrangement of treatments. Fifteen grams of rice was used in each can. The cans were filled to a ½ in. headspace with boiling water of the appropriate pH and processed at 240°F for 60 min in a conventional still retort, after which the cans were water cooled.

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