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# Indian Standard "gardez 9884" SPECIFICATION FOR "RE\_AFFIRMED 1995" MILK BREAD

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INDIAN STANDARDS INSTITUTION
MANAK BHAVAN, 9 BAHADÜR SHAH ZAFAR MARG
NEW DELHI 110002

#### Indian Standard

#### SPECIFICATION FOR MILK BREAD

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## Indian Standard SPECIFICATION FOR MILK BREAD

#### O. FOREWORD

- **0.1** This Indian Standard was adopted by the Indian Standards Institution on 29 March 1985, after the draft finalized by the Bakery and Confectionery Industry Sectional Committee had been approved by the Agricultural and Food Products Division Council.
- 0.2 Milk bread is prepared either by the straight dough method or sponge or 'no-time' mechanically developed dough method. Wheat flour (MAIDA) is the principal ingredient. Compressed Baker's Yeast (Saccharomyces cerevisiae) or its active dry form is used as a leavening agent for the dough. Brew containing yeast, or prepared from malthops or potatoes is also used in the fermentation of the dough. Milk solids from milk or dried milk products (skimmed or whole milk powder or butter milk powder) or condensed milk will be an essential ingredient. The other essential and optional ingredients to be used in the preparation of milk bread are indicated separately.
- 0.3 In the preparation of this standard due consideration has been given to the relevant provisions of the Prevention of Food Adulteration Act 1954 and Rules framed thereunder. Due consideration has also been given to the Standards of Weights and Measures (Packaged Commodities) Rules, 1977. However, this standard is subject to the restrictions imposed under these wherever applicable.
- **0.4** For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of a test or analysis, shall be rounded off in accordance with IS: 2-1960\*.

#### 1. SCOPE

1.1 This standard prescribes the requirements and the methods of sampling and test for milk bread.

<sup>\*</sup>Rules for rounding off numerical values ( revised ).

#### 2. ESSENTIAL INGREDIENTS

- 2.0 The following materials shall be used in the preparation of dough for milk bread.
- 2.1 Wheat Flour ( Maida ) ( see IS: 7464-1974\* ).
- 2.2 Leavening Agent Any of the following agents singly or in combination may be used:
  - a) Baker's yeast conforming to IS: 1320-1981; and
  - b) Barm and hops, fermented juice obtained from palms and lactic acid ferment.
- 2.3 Milk Solids Milk in any form or milk products such as skimmed milk or milk powder conforming to IS: 1165-1975‡ or condensed milk conforming to IS: 1166-1973§ or any other milk solids.
- 2.4 Edible Common Salt Conforming to IS: 253-1970||.
- 2.5 Water ( see IS : 4251-1967¶ ).

#### 3. OPTIONAL INGREDIENTS

- 3.1 In addition to essential ingredients specified above, the following ingredients may be added to the dough:
  - 1) Milk products whey, curd or milk fat;
  - 2) Gluten;
  - 3) Sugar and sugar products,
  - 4) Honey;
  - 5) Liquid glucose;
  - 6) Dextrose monohydrate;
  - 7) Malt products;
  - 8) Other edible flours and starches such as potato starch, corn flour (see IS: 1005-1976\*\*) and tapioca flour (see IS: 1318-1969††);
  - 9) Fat hydrogenated edible oils or refined edible oils of suitable type, butter or ghee or margarine or their mixture;
  - 10) Vitamins;

<sup>\*</sup>Specification for wheat flour for use in bread industry.

<sup>†</sup>Specification for baker's yeast ( second revision ).

<sup>‡</sup>Specification for milk powder (second revision).

<sup>§</sup>Specification for condensed milk (first revision).

Specification for edible common salt (second revision).

Quality tolerances for water for processed food industry.

<sup>\*\*</sup>Specification for edible maize starch (corn flour) (second revision).

<sup>††</sup>Specification for edible tapioca flour (first revision).

- 11) Lecithin albumin;
- 12) Lime water;
- 13) Fungal enzymes protease and diastase;
- 14) Lysine hydrochloride;
- 15) Sorbitol (food grade);
- 16) Glycerine and glyceryl monostearate;
- 17) Fruits and fruit products;
- 18) Nuts and nut product;
- 19) Defatted edible soya flour;
- 20) Defatted edible groundnut flour; and
- 21) Full-fat soyaflour ( see IS: 7837-1975\* ).

#### 3.2 Improvers

The following improvers in the quantities given against each may be permitted:

a)	Ammonium persulphate	Not exceeding 2.5 g/kg of mass o cereal mix
b)	Ammonium chloride	Not exceeding 0.5 g/kg of mass of cereal mix
<b>c</b> )	Potassium bromate	Not exceeding 0.05 g/kg of mass o cereal mix
d)	Calcium phosphate	Not exceeding 2.5 g/kg of mass o cereal mix
<b>e</b> )	Calcium carbonate	Not exceeding 5.0 g/kg of mass o cereal mix
f)	Sodium stearoyl — 2 Lactylate	Not exceeding 5 0 g/kg of mass o cereal mix

3.3 Mould Inhibitors - The following mould inhibitors in the quantities given against each may be used:

a)	Calcium	$\mathbf{or}$	sodium	Not	excecding	5.0 g/kg	of	mass	$\mathbf{of}$
	propion	ate		cei	real mix				
b)	Acetic ac	id (	glacial)	Not	exceeding	2.5  g/kg	of	mass	of

cereal mix

b) Acetic acid (glacial) or lactic acid

c) Vinegar

Equivalent to the concentration of acetic acid permitted [ see 3.3 (b)]

<sup>\*</sup>Specification for edible full-fat soya flour.

d) Acid calcium phosphate	Not exceeding cereal mix	10.0 g/kg of mass of
e) Sodium diacetate	Not exceeding cereal mix	4.0 g/kg of mass of
f) Acid sodium pyrophos- phate	Not exceeding cereal mix	5.0 g/kg of mass of
g) Sorbic acid or calcium sorbate or potassium sorbate	Not exceeding cereal mix	1.0 g/kg of mass of

#### 4. GENERAL REQUIREMENTS

- **4.1** The milk bread shall be baked in conventional form or in any other form as agreed to between the purchaser and the vendor. The crust shall be uniform golden brown to light brown in colour. The crust shall not be scorched and shall be free from soot and any other foreign matter.
- 4.1.1 The loaf shall have a good volume. The crumb shall be springy with small pores uniformly distributed throughout and with thin cell walls. It shall be free from non-porous mass, splits and large holes, lumps of flour or salt or any other evidence of incomplete mixing.

NOTE — The bread shall be considered as having good volume if its volume/weight ratio is not less than 2.5 when tested by the method prescribed in Appendix A of IS: 1483-1979\*.

- 4.1.2 There shall be no hollow between the crust and the crumb.
- **4.1.3** The flavour shall be characteristic of fresh, well-baked milk bread, free from staleness, bitterness or any other objectionable flavour or taste.
- **4.1.4** The bread shall also be free from indications of 'rope' or 'mould'. It shall also be free from added colouring matter.
- **4.2** The bread shall be manufactured in premises maintained under hygienic conditions ( see IS: 5059-1969†).
- 4.3 The bread shall also conform to the requirements of Table 1.

<sup>\*</sup>Specification for white bread ( second revision ).

<sup>†</sup>Code for hygienic conditions for large scale biscuit manufacturing units and bakery units.

TABLE 1 REQUIREMENTS OF MILK BREAD

(Clause 4.3)

SL	CHARACTERISTIC	REQUIREMENT	METHOD OF	METHOD OF TEST, REF TO		
No.			Appendix of this Standard	Appendix of IS:1483-1979*		
(1)	(2)	(3)	(4)	(5)		
i)	Total soild content, percent by mass, Min	60		В		
ii)	pH of the bread	5·3 to 6·0		$\mathbf{C}$		
iii)	Acid insoluble ash (on dry basis), percent by mass, Max	0.1		D		
iv)	Non-fat milk soilds, percent by mass (on dry basis), Min	6.0	A	_		
v)	Crude fibre (on dry basis), percent by mass, Max	0.2	· · · · ·	E		
vi)	Alcoholic acidity (as H <sub>2</sub> SO <sub>4</sub> ) in 90 percent alcohol percent by mass, Max	0.37	В			
*	Specification for white bread	( second revision ).				

#### 5. MASS

**5.1** The mass of the bread loaf shall conform to the provisions of the Standards of Weights and Measures (Packaged Commodities) Rules, 1977.

#### 6. PACKING

**6.1** Milk bread shall be wrapped or packed as such or in sliced form in clean waxed paper, grease proof paper or any other suitable non-toxic wrapper.

#### 7. MARKING

- 7.1 The following particulars shall be clearly and indelibly marked on each pack of bread on the outer side of the wrapper. If printed, the ink shall be non-toxic and non-transferable:
  - a) Name, trade name or description of material;
  - b) Name and address of the manufacturer;
  - c) Mass of loaf when packed;

- d) Statement 'Permitted Class-II Preservatives Added' whenever they are used;
- e) Other labelling requirements according to the provisions of the Standards of Weights and Measures (Packaged Commodities) Rules, 1977.
- 7.1.1 Each pack may also be marked with the ISI Certification Mark.

Note — The use of the ISI Certification Mark is governed by the provisions of the Indian Standards Institution (Certification Marks) Act and the Rules and Regulations made thereunder. The ISI Mark on products covered by an Indian Standard conveys the assurance that they have been produced to comply with the requirements of that standard under a well-defined system of inspection, testing and quality control which is devised and supervised by ISI and operated by the producer. ISI marked products are also continuously checked by ISI for conformity to that standard as a further safeguard. Details of conditions under which a licence for the use of the ISI Certification Mark may be granted to manufacturers or processors, may be obtained from the Indian Standards Institution.

#### 8. SAMPLING

8.1 Representative samples of the material for the test shall be drawn according to the method prescribed in Appendix F of IS: 1483-1979\*.

#### 9. TESTS

- 9.1 Tests shall be carried out as prescribed in 4.1.1 and appropriate appendices given in col 4 and 5 of Table 1.
- **9.2 Quality of Reagents** Unless specified otherwise, analytical grade reagents shall be employed in test and distilled water ( see IS: 1070-1977†) shall be used where the use of water as reagent is intended.

#### APPENDIX A

[ Table 1, Item (iv) ]

#### **DETERMINATION OF NON-FAT MILK SOLIDS**

A-0. METHODS — Two methods, namely lactose estimation (see A-1) and colorimetric (see A-2), are prescribed for the determination of non-fat milk solids in bread. Any of the methods may be used.

#### A-1. LACTOSE ESTIMATION METHOD

A-1.1 Principle — The fact that milk contains lactose, but the other constituents do not, is made use of in the detection and determination of milk in bread. Lactose is to be detected by the method which involves

<sup>\*</sup>Specification for white bread ( second revision ).

<sup>†</sup>Specification for water for general laboratory use ( second revision ).

extracting the sugars and destroying the other reducing sugars excepting lactose, by fermenting with yeast and determining the lactose by a suitable method such as the Munson and Walker Gravimetric Method. The non-fat milk solids are calculated from the percentage of lactose found, since skim milk powder has an average of 50 percent lactose with only slight variation.

#### A-1.2 Apparatus

- A-1.2.1 Centrifuge
- **A-1.2.2** 850 μm IS Sieve [ see IS : 460 ( Part 1 )-1978\* ].
- A-1.2.3 Volumetric Flask capacity 1-litre and 2-litre.
- **A-1.2.4** Beaker 800-ml and 400-ml.
- A-1.2.5 Filter Gel
- A-1.2.6 Gooch Crucible with Treated Asbestos for a Mat

#### A-1.3 Reagents

- A-1.3.1 Ammonium Sulphate
- A-1.3.2 Sodium Bisulphite
- **A-1.3.3** Baker's Compressed Yeast Conforming to IS: 1320-1981†.
- **A-1.3.4** Copper Sulphate Solution (Regular Fehling Solution A) Dissolve 34.639 g of pure copper sulphate (Cu  $SO_45H_2O$ ) in water, dilute to 500 ml and filter through asbestos, washed successfully with hydrochloric acid (1:3), 10 percent sodium hydroxide solution and alkaline tartrate solution and then wash thoroughly with water, after each treatment. This type of asbestos is termed treated asbestos.
- A-1.3.5 Alkaline Tartrate Solutions Dissolve 173 g of potassium sodium tartrate (Rochelle salt) and 50 g of sodium hydroxide in water, dilute to 500 ml, allow to stand for 2 days and filter.
  - **A-1.3.6** Sodium Hydroxide Solution (1:1)
  - A-1.3.7 Ether

#### A-1.4 Procedure

- A-1.4.1 Lactose Estimation
- A-1.4.2 Preparation of Sample Remove the crust of the bread, airdry the crumb, then grind sufficiently to pass through a 850  $\mu$ m IS Sieve.

<sup>\*</sup>Specification for test sieves: Part 1 Wire cloth test sieves ( second revision ). †Specification for baker's yeast ( second revision ).

A-1.4.2.1 Estimation — Digest 50 g of the prepared material in 400 ml of water at about 40°C for 3 hours and transfer the mixture to a large centrifuge tube. Centrifuge and decant the liquid portion into a 1-litre volumetric flask. Wash the residue four times using 75 ml of water each time, and separate solids by centrifuging. Decant after each washing and add the liquid portion to the first extract. Add 35 g of baker's compressed yeast, suspended in a small amount of water, 0.5 g of ammonium sulphate and 0.2 g of sodium bisulphite and let it stand over night at room temperature stoppered, but with a vent for the escape of carbon dioxide. The ammonium sulphate is used as a yeast stimulant, but the sodium bisulphite retards bacterial action.

After keeping it over night add 20 m! of copper sulphate solution (Regular Fehling A) and add sufficient sodium hydroxide solution to give a definite blue colour and clarify the solution. Make up to volume in a one-litre flask, shake and filter through a good filter paper.

Take 50 ml of the filtrate in 400-ml beaker and add 25 ml of each of the copper sulphate and alkaline tartrate solutions. Keep the beaker covered with a watch glass and heat the beaker on an asbestos gauge in such a manner that boiling begins in exactly 4 minutes. Continue the heating for exactly 2 minutes more. Some practice with 50 ml of the mixed reagents and 50 ml of water will enable the analyst to regulate the flame properly. Filter the hot solution at once through a prepared Gooch crucible having some of the treated asbestos for a mat. Wash the precipitate throughly with hot water (60°C), then with 10 ml of alcohol and finally with 10 ml of ether. Place the crucible in a constant temperature oven at 100-105°C for 30 minutes to dry. Cool in a desiccator and weigh as cuprous oxide. Determine the amount of lactose from Table 2.

A-1.4.22 Carry out a parallel determination with lactose alone or with a known sugar mixture.

#### A-1.5 Calculation

**A-1.5.1** The fat-free milk solids are calculated from the percentage of lactose found, since skim milk powders contains on an average 50 percent lactose with only slight variations. Therefore, twice the percentage of lactose found (after calculation on the dry basis) is equal to the percentage of fat-free solids on dry basis of the bread. Fifty ml of the filtrate (**A-1.4.2.1**) are equivalent to 2.500 g of bread after correction for the yeast is made.

TABLE 2 MUNSON AND WALKER SUGAR TABLE IN MILLIGRAMS (Clause A-1.4.2.1)

Cuprous Oxide (Cu <sub>2</sub> O)	$\begin{array}{c} \text{Lactose} \\ \text{C}_{12}\text{H}_{22}\text{O}_{11} + \text{H}_2\text{O} \end{array}$	Cuprous Oxide (Cu <sub>2</sub> O)	LACTOSE C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> +H <sub>2</sub> O
(1)	(2)	(1)	(2)
10	6.3	82	5 <b>4</b> ·2
12	7.5	84	5 <b>5</b> •6-
14	8.8	86	57•0
16	10.0	88	5 <b>8·4</b>
18	11.3	90	59.7
20	12.5	92	61·1
22	13.8	9 <b>4</b>	62.5
24	15.0	96	6 <b>3·</b> 8
26	16.3	98	65.2
28	17.6	100	66.6
30	18.8	102	6 <b>8·</b> 0
32	20.1	104	69·3
34	21•4	106	70.7
<b>3</b> 6	22.8	108	72.1
38	24.2	110	73.5
<b>4</b> 0	25.5	112	74.8
42	26.9	114	76.2
44	28.3	116	77•6
<b>4</b> 6	29.6	118	79 <b>·</b> 0
48	31.0	120	80.3
50	32.3	122	81.7
52	33.7	12 <b>4</b>	83.1
54	35.1	126	84.5
56 50	36 <b>·4</b> 37·8	128	85 <b>·8</b>
5 <b>8</b> 60	37·8 39·2	130 132	8 <b>7·2</b> 8 <b>8·</b> 6
62	40.5	134	90.0
64	41.9	136	91.3
66	43.3	138	92.7
68	44.7	140	94·1
70	46.0	142	95•5
72	47.4	1 <b>4</b> 4	96.8
7 <b>4</b>	48.8	1 <b>4</b> 6	9 <b>8·2</b>
76	50.1	148	99.6
78	51.5	150	101.0
80	52.9	152	102.3
			(Continued)

TABLE 2 MUNSON AND WALKER SUGAR TABLE IN MILLIGRAMS - Contd LACTOSE CUPROUS OXIDE CUPROUS OXIDE LACTOSE  $(C \sigma_2 O)$ C12H22O11+H2O ( Cu<sub>2</sub>O ) C12H22O11+H2O (1) (2)(1) (2)103.7 228 154.8 154 105.1 230 156.2 156 106.5 232 157.6 158 160 107.9 234 159.0 162 109.2 236 160.3 164 110.6 238 161.7 166 112.0 240 163.1 168 113.4 242 164.5 2:4 170 114.8 165.9 116.1 **24**6 167:3 172 117.5 174 248 168.7 118.9 250 170.1 176 120.3 252 178 171.5 121.6 254 172.8 180 123.1 256 182 174.2 124.3 258 175.6 184 125.8 260 186 177.0 127:2 262 178.4 188 190 128.5 264 179.8 129.9 266 192 181.2 194 131.3 268 182.6 270 184.0 196 132.7 185.3 134.1 272 198 274 186.7 200 135.4 136.8 276 188:1 202 278 189.5 204 138.2 139.6 280 190.9 206 208 141.0 282 192.3 210 142.3 284 193.7 286 195.1 143.7 212 288 196.5 214 145.1 290 197.8 216 146.5 218 147.9 292 199.2 200.€ 149.3 294 220 222 150.7 296 202.0 152.0 298 203.4 224 300 204.8 226 153.4 ( Continued )

TABLE 2 MUNSON AND WALKER SUGAR TABLE IN MILLIGRAMS-Contd

1.12111 1 11101101	on mile whomen	COAR TABLE IN MI	LLICKAMSConta
Cuprous Oxide (Cu <sub>2</sub> O)	$\begin{array}{c} \text{Lactose} \\ \text{C}_{12}\text{H}_{22}\text{O}_{11}\text{+}\text{H}_{2}\text{O} \end{array}$	Cuprous Oxide (Cu <sub>2</sub> O)	$\begin{array}{c} { m Lactosb} \\ { m C_{12}H_{22}O_{11}\!+\!H_{2}O} \end{array}$
(1)	(2)	(1)	(2)
302	206.2	376	257.9
30 <b>4</b>	207.6	378	259· <b>3</b>
306	209.8	380	260.7
308	210.4	382	262·1
310	211.8	384	263.5
312	213.2	386	264.9
314	21 <b>4</b> ·6	388	<b>2</b> 66 <b>·5</b>
316	216.0	390	<b>2</b> 67·7
318	217.3	392	269·1
320	218.7	394	270.5
322	220.1	396	271.9
324	221.5	398	273.3
326	222-9	400	274.7
328	2 <b>24·3</b>	402	276.1
330	225.7	<b>4</b> 0 <b>4</b>	277.5
3 <b>3</b> 2	227.1	<b>40</b> 6	<b>278</b> ·9
334	228.5	408	280.3
336	229.9	410	281.7
338	231.3	412	283.2
340	232.7	414	<b>284</b> ·6
342	234.1	416	286.0
344	235.5	418	287.4
346	236.9	420	288.8
<b>34</b> 8	238.3	<b>4</b> 22	290.2
350	239•7	424	291.6
352	241.1	426	293.0
354	242.5	428	294·4
356	2 <b>43·</b> 9	<b>4</b> 30	295.8
358	245.5	432	297.2
360	<b>24</b> 6·7	<b>4</b> 34	298.6
362	<b>24</b> 8·1	<b>43</b> 6	300.0
364	2 <b>4</b> 9·5	438	301.4
366	250.9	<b>44</b> 0	302.8
368	252.3	442	304-2
370	253.7	<b>444</b>	305·6 307 0
372	255.8	<b>44</b> 6	307 U 308·4
374	<b>2</b> 56·5	448	
			(Continued)

TABLE 2 MUNSON AND WALKER SUGAR TABLE IN MILLIGRAMS—Contd

Cuprous Oxide (Cu <sub>2</sub> O)	${ m L_{ACTOSE} \atop C_{12}H_{22}O_{11}+H_2O}$	Cuprous Oxide ( $Cv_2O$ )	${ m Lactose} \\ { m C_{12}H_{22}O_{11}+H_2O}$
(1)	(2)	(1)	(2)
<b>4</b> 50	309.9	472	325.3
<b>4</b> 52	311.3	474	<b>326·</b> 8
454	312.7	476	328.2
<b>45</b> 6	314.1	478	329.6
458	315.5	<b>48</b> 0	331.0
460	316.9	482	332 4
<b>4</b> 62	318:3	<b>4</b> 84	333.8
464	319.7	<b>4</b> 86	335.2
<b>4</b> 66	321.1	<b>4</b> 88	<b>336</b> ·6
468	<b>322</b> ·5	<b>4</b> 90	<b>3</b> 38·0
470	323.9		

#### A-2. COLORIMETRIC METHOD BASED ON OROTIC ACID

A-2.1 Principle — The method is a colorimetric one for estimating the non-fat milk solids in milk bread based on the orotic acid (2, 6-dihydroxypy-rimidine-4-carboxylic acid) content. The mean orotic acid content of non-fat milk solids is 62.5 mg/100 g (range 48.0-74.5 mg/100 g).

#### A-2.2 Apparatus

- A-2.2.1 Air-Drying Oven
- A-2.2.2 Homogeniser
- **A-2.2.3** Pipettes 5-ml, 10-ml and 25-ml.
- A-2.2.4 Glass Stoppered Test Tubes
- **A-2.2.5** Volumetric Flask capacity 500-ml, 100-ml, 50-ml and 10-ml.
- A-2.2.6 Waterbath
- A-2.2.7 Colorimeter

#### A-2.3 Reagents

- **A-2.3.1** Zinc Sulphate 23 percent m/v solution.
- **A-2.3.2** Potassium Hexacyanoferrate 15 percent m/v solution.
- **A-2.3.3** p-Dimethylaminobenzaldehyde (DAB) 3 percent (m/v) in propanol.

- **A-2.3.4** Standard Orotic Acid Dissolve 50 mg orotic acid in a mixture of 1 ml of 0.88 ammonia and 10 ml water. Dilute to 500 ml with water. Dilute 10 ml to 100 ml with water. Dilute 2.5, 5, 10 and 15 ml of this solution to 50 ml to produce solution containing 2.5, 5, 10 and 15  $\mu$ g orotic acid per 5 ml.
  - A-2.3.5 Saturated Bromine Water
  - A-2.3.6 Ascorbic Acid Solution 10 percent.
  - A-2.3.7 n-Butyl Acetate
  - A-2.3.8 Anhydrous Sodium Sulphate

#### A-2.4 Procedure

- **A-2.4.1** Weigh the bread on receipt accurately, nearest to 0.1 g. Cut the bread into 2-3 mm slices, spread on paper and allow to dry in a warm room overnight so that the bread is crisp and brittle. The sample should be in equilibrium with the atmosphere so that the moisture content remains constant during grinding. Then return quantitatively the air-dried bread to the balance and re-weigh. Grind to pass through a 850  $\mu$ m IS Sieve, mix and transfer to an air-tight container. Determine the total solids by drying 2 g at 130°C for one hour.
- A-2.4.2 Weigh 5 g of dried sample into the beaker of homogeniser, add 100 ml water and mix at the maximum speed for 1 min. Filter the supernatant liquor through a 15 cm Whatman No. 541 paper rejecting the first 10 ml. Five ml is required for the determination.
- A-2.4.3 Into a series of glass stoppered test tubes add by pipette 5 ml of test solution (containing 2-15 µg orotic acid), 5 ml of each of the standard orotic acid solutions and 5 ml of water to act as the blank. Add to each tube 1.5 ml of saturated bromine water and allow the mixture to stand at room temperature for not more than 5 minutes. As the addition of bromine water is made to the series of tubes the times will vary slightly between each, the time of reaction is not critical provided it is between 1 and 5 minutes. Add 2 ml of 10 percent ascorbic acid solution to each tube and place the tubes in a water bath at 40°C for 5 minutes. Add to each 3 ml p-dimethylaminobenzaldehyde and return to the bath for 10 minutes. Cool to room temperature, add to each tube 4.0 ml n-butyl acetate and shake vigorously for 15 seconds. Transfer the copper separated layers to dry test tubes containing 1 g anhydrous sodium sulphate, mix gently, add another gram of anhydrous sodium sulphate, mix gently and allow to separate. Transfer the clear butyl acetate layer to 1-cm cell and measure the optical density at 461 to 462 nm against the blank.

#### A-2.5 Calculation

**A-2.5.1** Draw a calibration graph of the standard orotic acid solution plotting the optical density on the X-axis against concentration of orotic acid on the Y-axis. Determine the orotic acid content in 5 ml of sample extract by interpolation of the colorimeter reading on the calibration graph, and hence the amount in the dry sample. For converting to milk assume that skim milk powder contains 62.5 g orotic acid per 100 g.

#### APPENDIX B

Table 1, Item (vi) ]

#### **DETERMINATION OF ALCOHOLIC ACIDITY**

#### **R-1. REAGENTS**

- **B-1.1 Neutral Ethyl Alcohol** 90 percent (v/v).
- **B-1.2 Standard Sodium Hydroxide Solution** approximately 0.05 N.
- **B-1.3 Phenolphthalein Indicator Solution** Dilute 0.1 g of phenolphthalein in 100 ml of 60 percent (v/v) rectified spirit.

#### **B-2 PROCEDURE**

**B-2.1** Weigh 5 g of sample into a conical stoppered flask and add 50 ml of neutral ethyl alcohol. Stopper, shake and allow to stand for 24 hours, with occasional shaking. Filter the alcoholic extract through a dry filter paper. Titrate the combined alcoholic extract against 0.05 N standard sodium hydroxide solution using phenolphthalein as indicator. Calculate the percentage of alcoholic acidity as sulphuric acid.

#### **B-3. CALCULATION**

**B-3.1** Alcoholic acidity (as  $H_2SO_4$ ) in 90 percent alcohol on dry basis, percent by mass  $\frac{25.42 \text{ } AN}{M} \times \frac{100}{T}$ 

where

A = volume in ml of standard sodium hydroxide solution used in titration;

 $\mathcal{N}$  = normality of standard sodium hydroxide solution; and

M =mass in g of the material taken for the test.

T = total solids percent