SCHEDULE 2

PART II

DETERMINATION OF APLHA-AMYLASE IN WHOLE EGG OR YOLK

Introduction

1.—(1) A sample of whole egg or yolk which has been pasteurised by the process specified in sub-paragraph (a) of Part I of this Schedule shall be subjected to the alpha-amylase test to determine the efficacy of the pasteurisation process.

(2) The sample shall be subjected to the test as soon as possible after pasteurisation.

(3) At least one sample from each batch of egg product shall be taken.

Sample preparation

2. The sample shall consist of not less than 50 g of the whole egg or yolk. The sample to be examined shall be prepared for the test as follows:

(a) for whole egg, the original sample shall be used, save that any dried whole egg shall be reconstituted;

(b) for yolk—

(i) 5 ml yolk shall be diluted with 10 ml water,

(ii) any dried yolk shall be reconstituted before dilution.

Reagents

3. All reagents shall be of analytical reagent (AR) grade, water for the preparation of reagents shall be distilled or de-ionised, and the reagents shall comprise the following solutions—

(a) starch solution made up as follows—

(i) an amount of soluble starch of known moisture content and of the appropriate grade for the determination of alpha-amylase, equivalent to 0.70 g of dry starch mixed to a thin cream with cold water; transferred to about 50 ml of boiling water, boiled for one minute and then cooled by immersion in cold water; three drops of toluene shall be added and the whole diluted with water to 100 ml in a volumetric flask; the solution shall not be retained for longer than 14 days;

(b) solution of iodine made according to one of the following–

(i) an accurately weighed amount of 0.1269 g of iodine and 3.6 g of potassium iodide dissolved in water such that the final volume of the solution is 1 litre; the volumetric flask containing the solution shall be protected from light and a fresh solution shall be prepared daily;

(ii) the solution described in sub-paragraph (i) diluted from a stronger solution with appropriate adjustment of potassium iodide concentration; or

(c) solution of trichloroacetic acid made up as follows—

(i) 15% (w/v) of trichloroacetic acid dissolved in water.

Apparatus

4.—(1) Glassware shall be clean and dry before use and no mouth pipetting shall be carried out, and glassware that has come into contact with whole egg or yolk shall be sterilised after use.

(2) The components of the apparatus shall be the following:

(a) *analytical balance*

(b) *beakers:* 250 ml glass

(c) *volumetric flasks:* 100 ml, 1 litre

(d) *flasks:* 100 ml glass

(e) *water bath:* capable of maintaining a temperature of 44°C ± 0.5°C

(f) *pipettes:* 2 ml, 5 ml, 10 ml, 15 ml glass Grade A

(g) *test tubes:* glass

(h) *filter paper:* Whatman No 1 or equivalent

(i) *Lovibond Comparator:* plus disc 4/26 and 25 mm cell, or any other apparatus which offers the equivalent level of accuracy and performance as described in paragraph 6 of this Part.

Procedure

5. The test shall be carried out according to the following procedure:

(a) the sample shall be at room temperature immediately before the test;

(b) 15.0 g of the sample shall be put into a small flask, 2.0 ml of the starch solution described in paragraph 3(a) above shall be added and mixed thoroughly;

(c) the mixture shall be placed for 30 minutes in a water bath maintained at 44°C ± 0.5°C and anchored securely; then removed and cooled to room temperature;

(d) 5 ml of the mixture shall be added to 5 ml of trichloroacetic acid solution in a test tube fitted with a ground glass joint and suitable stopper (or an equivalent item of equipment) and shaken thoroughly;

(e) 15 ml of water shall be added and the mixture shaken again; then it shall be filtered and if the solution is cloudy or turbid, the first runnings shall be rejected;

(f) 10 ml of the clear filtrate shall be added to 2 ml of iodine solution contained in a test tube.

Interpretation

6.—(1) The sample passes the alpha-amylase test if the filtrate in the solution of iodine immediately turns a blue-violet colour.

(2) For this purpose colours more blue-violet than 3 of a standard Lovibond Comparator Disc 4/26, or of a comparable spectrophotometric standard, are taken as satisfactory.

Quality Control Procedures

7.—(1) The colour shall be compared in an all-purpose Lovibond Comparator using a 25 mm cell.

(2) The reagents and procedure shall be checked by preparing two control tubes at the same time, and in the first of these the egg product shall be replaced with an equivalent amount of water and in the second the starch shall be replaced with an equivalent amount of water.

(3) In order to be satisfactory the first tube should be a deeper blue, and the second tube a lighter blue, than any shade on the disc.