Determination of Incubator-Reject Eggs in Egg Products by means of the 3-Hydroxybutyric Acid Content. 2. Levels of 3-HBA in Different Kinds of Eggs and Egg Products and the Development of 3-HBA during Incubation

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Incubated and unincubated table eggs (from battery cages and from deep litter), hatchery eggs (clears and forced or spontaneously dead), and egg products were analyzed with an enzymatic method for the presence/development of 3-hydroxybutyric acid (3-HBA). The average amount of 3-HBA for incubator rejects held in incubators for longer than 6 days, even after being candled very carefully, was much higher (approximately 30 mg/kg) than in table eggs (approximately 0.7 mg/kg). The weighted mean for 6-day incubator clears as well as for 6-day incubator clears plus "dubious" is 1.0 mg/kg. The effect of the moment of embryo growth termination on the 3-HBA content was also investigated.

INTRODUCTION

In The Netherlands 600 million eggs are hatched annually. From this number 10–15% fail to develop into chicks because the eggs are unfertilized or the embryo died prematurely. The clear incubator-reject eggs, worth more than $1 000 000, are processed into egg products. The use of these incubator-reject eggs in egg products intended for human consumption is banned in the U.S. and Canada. In the European Economic Community (EEC) the use of *clear unfertilized* incubator-reject eggs held in incubators for not longer than 6 days is allowed at the present time (EEC, 1975). Some of the EEC members insist on a complete prohibition while others prefer this regulation or even a less restrictive one.

The enforcement of any regulation is difficult without a satisfactory analytical control method. On the basis of existing enzymatic (Parry et al., 1980) and gas chromatographic methods (Heaney and Curtis, 1976; Littmann et al., 1982) for the determination of 3-hydroxybutyric acid (3-HBA), an acid related to embryo development (Salwin et al., 1972), modified methods have been developed, as described by Elenbaas et al. (1986).

This paper describes the enzymatically estimated 3-HBA content in eggs of different origin. Incubated and unincubated table eggs from battery cages and deep litter, as well as hatching eggs (clears and eggs with spontaneously and—in different stages of incubation—forced-died embryos) have been analyzed.

EXPERIMENTAL SECTION

Materials. To investigate the 3-HBA content in eggs and in egg products the following samples were prepared and analyzed:

(1) Unfertilized table eggs, during at most 18 days at room temperature stored unincubated fertilized eggs and samples of unpasteurized and pasteurized liquid whole egg containing different amounts of salt or sugar and samples whole egg powder.

(2) Samples of fertilized eggs from 19 commercial flocks (120 eggs/flock) incubated at the Spelderholt Centre for 6 days. After incubation the eggs were candled and the eggs without a living embryo were divided into three groups: a group of clear eggs, a group with a dead embryo, and a group of eggs that did not belong to either of these two groups (referred to as "dubious").

(3) Thirty samples of 60 clear incubator-reject eggs each supplied by different hatcheries and defined as the clear fraction of the total of rejects. The rejection time varied from 7 to 18 days. The 7–18-day-incubated clears were again candled carefully at Spelderholt and, if possible, divided into three groups: a group of clear eggs, a group of eggs that showed any embryo development, and a group of eggs that did not belong to the first two groups and was called dubious. A mixture containing half of each of these groups was prepared to obtain a representative sample of the original "clear" rejects. The other half of each group was analyzed separately.

(4) Fertilized eggs of three different strains (over 500 eggs per strain) incubated to investigate the influence of the moment of embryo growth termination on the 3-HBA content of incubated eggs. After 3 days, the eggs were candled and the unfertilized and/or already dead embryos were rejected. The fertilized eggs were reincubated except for 75 eggs per strain, which were cold treated (30 min in ice and 4 h at 0 °C in a cold storage room) to terminate the embryo growth. Fifteen of the treated eggs per strain were analyzed, and incubation of the remaining eggs was prolonged. After 7, 10, 13, and 18 days in the incubator again, 15 cold-treated eggs were analyzed. A similar procedure was performed with eggs in which embryo development was terminated after 4, 5, 6, 7, 10 and 13 days.

Sample Preparation and Analysis. All the eggs of each sample were broken open immediately after gathering and the contents homogenized, frozen and stored at −20 °C until analyzed.

The samples were thawed at 18 °C and analyzed by the enzymatic method—as this method was ready to use first—as described by Elenbaas et al. (1986). Most samples were analyzed singularly.

RESULTS AND DISCUSSION

Influence of Origin, Storage, and Treatment/Processing of Eggs on the 3-HBA Content. From the literature it is known that 3-HBA can be found not only in incubated eggs but in all eggs (Heaney and Curtis, 1976). In order to decide whether or not incubator-reject eggs, not obeying the EEC legislation, have been processed in

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Table I. Enzymically Determined 3-HBA Content (mg/kg) in Table Eggs, Not Incubated Fertilized Eggs and Egg Products

sample type	no. of samples	amt of 3-HBA, mg/kg av	min	max
table eggs[a]				
battery cages	15	0.7	0.4	1.0
deep litter	5	0.7	0.4	1.0
18-days incubated	5	0.7	0.4	0.8
not incubated fertilized eggs[b]	3	1.0		
stored at 20–25 °C				
6 days	3	1.0		
12 days	3	0.8		
18 days	3	1.1		
egg products				
liquid whole egg[c]	24	2.0	0.4	7.4
liquid whole egg[d]	15	29.6	6.0	120
whole egg powder[e]	5	0.7	0.5	0.8

[a] Each sample consisting of 30 randomly chosen eggs from different flocks. [b] Each sample consisting of 30 randomly chosen eggs from different hatcheries. [c] According to the manufacturers without incubator rejects. [d] Containing 5–100% clear incubator rejects. [e] Calculated as liquid whole egg.

egg products intended for human consumption, it is necessary to know the 3-HBA content in different kinds of eggs and the influence of storage and treatments. The results of all examined egg samples are summarized in Table I.

In this investigation *table eggs* have been chosen as "zero" level. The 3-HBA content in eggs from hens kept in battery cages as well as on deep litter is 0.7 mg/kg (Table I), which is in good agreement with the gas chromatographic results of Heaney and Curtis (1976) and Littmann et al. (1982). The standard deviation of the 3-HBA content in table eggs from different flocks analyzed in duplicate is equal to the standard deviation of a 10-fold analysis of one sample with 0.8 mg/kg, being 0.16 mg/kg (Elenbaas et al., 1986). The standard deviation of the content in table eggs is smaller than that within the analysis, which means that there is no influence of the flocks. An 18-day stay of table eggs in incubators did not influence the 3-HBA content. *Storage of fertilized eggs* at temperatures not exceeding 25 °C for up to 18 days does not influence the 3-HBA content, which agrees with the experiences of Thomas and Stock (1982).

The manufacturers of the liquid whole egg samples claimed that their products did not contain incubator rejects. However, 6 out of 24 samples showed a 3-HBA content up to 7.4 mg/kg, which means, relying on the experience so far, that incubator rejects were probably present in the samples. Addition of salt and sugar does not influence the 3-HBA content but does influence the enzymatic reaction time (Elenbaas et al., 1986).

Liquid whole egg containing 5–100% of clear incubator-reject eggs showed an obvious increase in the 3-HBA content (Table I). The five analyzed samples of whole egg powder did not show increased amounts of 3-HBA based on liquid whole egg. Technological treatment of eggs (pasteurization, drying, hot room) did not influence the 3-HBA content (Elenbaas et al., 1985).

3-HBA Content in Incubator-Reject Eggs. The results of the eggs incubated at Spelderholt Centre during 6 days are summarized in Table II.

During candling at the hatcheries part of the dubious eggs may be mixed up with the clears. Table II shows clearly that this mixing does not significantly influence the 3-HBA content. Even an outlier does not yield a significant difference; one sample of the category dubious, containing just one egg, showed a 3-HBA content of 19.1 mg/kg, probably due to a mistake during candling or analysis.

Table II. Enzymically Determined 3-HBA Content (mg/kg) in Eggs Rejected at Day 6 and in between Days 7 and 18 of Incubation Rejected Eggs

sample type	no. of samples	no. of eggs	amt of 3-HBA, mg/kg av	wt mean	min	max
		6 Days				
clear (C)	19	253	1.0	1.0	0.4	1.8
dubious (D)	7	10	1.4	1.4	0.7	2.4
C + D	26	263	1.1	1.0	0.4	2.4
with dead embryos	18	53	10.2	9.7	2.9	41.4
		7–18 Days				
orig clear rejects[a]	30	1800	76		1.5	338
recandled						
clear	30	1512	29		0.7	143
dubious	28	270	242		10	424
not clear	7	18	98		46	214

[a] By candling at the hatcheries defined as clear, each sample consisting of 60 eggs.

Due to the nonnormal distribution of the eggs in the samples, a correct calculation of the standard deviation is impossible. The standard deviation of the method for the range from 0 to 5 mg/kg is 0.33 mg/kg (Elenbaas et al., 1986). From this, an estimated standard deviation for the 6-day-incubated eggs can be calculated as well as the upper tolerance limit, resulting in weighted standard deviations of 0.36 and 0.37 for the categories "clear" and "clear + dubious", respectively. For $\alpha < 0.05$ the upper tolerance limit is 1.6 mg of 3-HBA/kg for the clear as well as for the clear + dubious fractions and for $\alpha < 0.01$, 1.8, and 1.9 mg of 3-HBA/kg, respectively.

In Table II the overall results are given of the eggs rejected between days 7 and 18 of incubation. This table shows clearly the effect of recandling. The original clear rejects show an average 3-HBA content of 76 mg/kg vs. 29 mg/kg for the recandled clears. So, even after recandling the 3-HBA content is very high with regard to table eggs. A frequency distribution of the original clears, indicating the broad range of 3-HBA contents, is shown in Figure 1. Beside the decrease of the 3-HBA content from original clears to recandled clears, Table II shows a striking high 3-HBA content in the group called "dubious" in comparison with the "not clears". This is possibly caused by the moment of embryo growth termination, which will be discussed further on in this paper.

From Tables I and II and the calculated upper tolerance limits, it can be concluded that egg samples with less than 1.9 mg of 3-HBA/kg may contain unfertilized eggs that have been incubated for longer than 6 days.

Egg samples with more than 1.9 mg of 3-HBA contain "not clear 6-day-incubated" eggs or longer than 6-day-incubated eggs.

Influence of the Moment of Embryo Growth Termination on the 3-HBA Content. The results of this part of the investigation are graphically summarized in Figure 2. Most striking is the fact that eggs with embryos that died at days 3 and 4 of incubation show a continuous increase of the 3-HBA content up to day 18 of incubation; the strongest increase for the eggs with embryos died at day 4. This phenomenon, also observed by Salwin et al. (1972), gives an explanation for the high 3-HBA content in more than 6-day-incubated eggs of the category dubious (see previous section). As the category clear included a

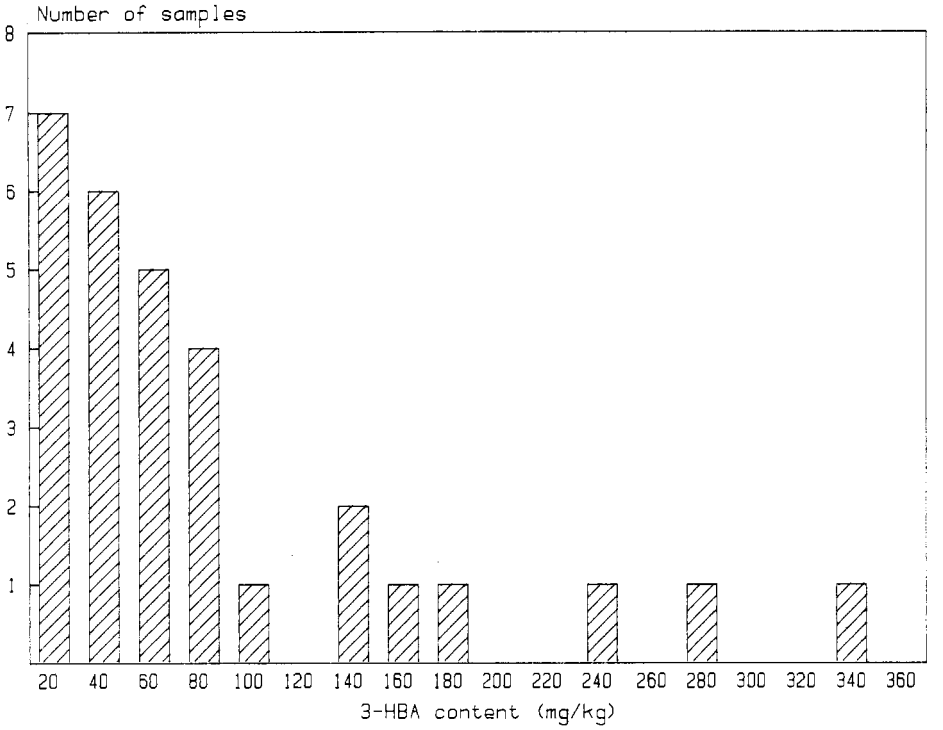

Figure 1. Frequency distribution of the 3-HBA content in commercial samples of incubator-reject eggs after 7–18 days of incubation.

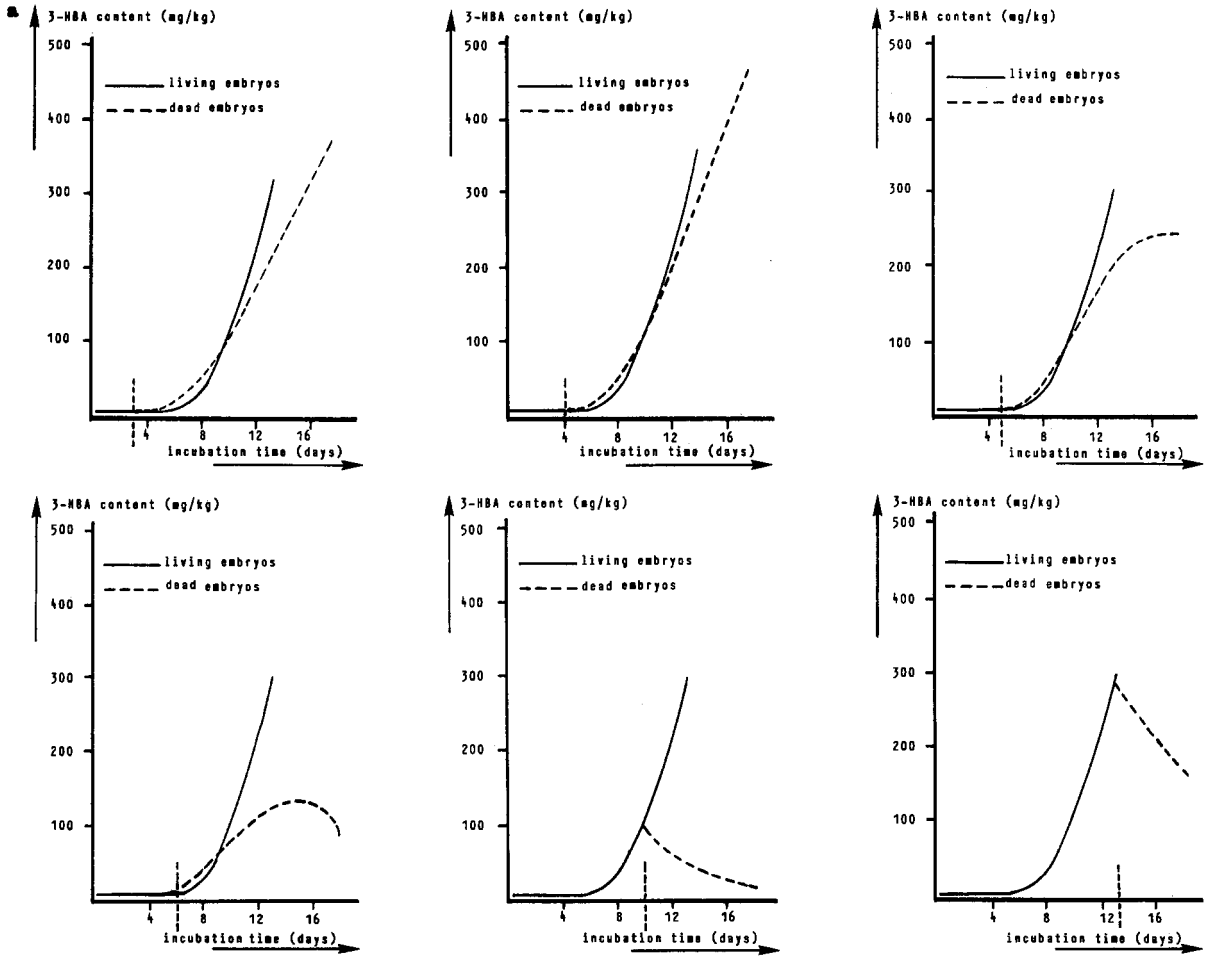

Figure 2. Influence of the moment of embryo growth termination on the 3-HBA content. The vertical dotted lines indicate the day of cold treatment.

significant number of fertilized eggs, in which embryo development stopped in an early stage of incubation (Salwin et al., 1972; Heaney and Curtis, 1976), the 3-HBA content in this category is much higher than the content in table eggs (29 vs. 0.7 mg/kg). The influence of the fertilized eggs with untimely dead embryos is even noticeable for clear eggs rejected at day 6: the 3-HBA content of these eggs differs significantly (Wilcoxon test) from that

of table eggs (1.0 vs. 0.7 mg/kg). This means that 3-HBA development starts *before* day 6 of incubation but not *after* 6 days as stated by Littmann et al. (1983).

Some Final Conclusions. The development of 3-HBA, which strongly depends on the moment of embryo growth termination, makes it impossible to calculate the amount of incubated eggs in egg product based on the 3-HBA content. Finally, in the present EEC legislation there is talk of incubated eggs. This work has shown that 3-HBA is not so much an indicator for "incubation" as (a) unfertilized incubated eggs do not show an increase in the 3-HBA content, not even after 18 days of incubation and (b) fertilized eggs stored at tempertures higher than 25 °C, which is a form of incubation, show an increase of the 3-HBA content. Especially for the latter it should be advisable not to refer to incubation alone, but also to embryo development or to storage at temperatures not exceeding 25 °C. However, the 3-HBA method as a control method for the misuse of longer than 6-days-incubated clears in egg products has been confirmed. In practice it is not possible to candle the incubator clears in such a way that only unfertilized eggs are selected.

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Functional Properties of Phosphorylated Yeast Protein: Solubility, Water-Holding Capacity, and Viscosity

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Phosphorylation of 30% of the lysine groups of yeast nucleoprotein facilitated the preparation of phosphoprotein (72%) with only 2.7% nucleic acid. Phosphorylation improved solubility slightly between pH 6.0 and 7.0 and greatly enhanced the water-holding capacity from 10 to 25 g of water/g of protein between pH 6 and 7.5. Viscosity increased with protein concentration and with pH, especially above pH 6.5 reflecting increasing electrostatic repulsion of the modified proteins. The phosphoproteins showed shear thinning at low shear rates, and viscosity decreased with increasing temperatures. The data indicated that phosphorylated yeast proteins might be useful as thickening agents in certain foods.

INTRODUCTION

To fully exploit their desirable functional properties, it is necessary to separate yeast proteins from the cell biomass (Kinsella and Shetty, 1979). However much of the isolated proteins exist as ribosomal, i.e. ribonucleic acid–protein, complexes and ribosomes (Shetty and Kinsella, 1979, 1980, 1982a,b; Damodaran and Kinsella, 1984). On the basis of the concept that electrostatic interactions are important for the integrity of these nucleoprotein complexes, we developed methods based on chemical derivatization for the separation of proteins from contaminant nucleic acids (Kinsella and Shetty, 1979; Shetty and Kinsella, 1982a, 1982b) and recently we showed that chemical phosphorylation of yeast proteins facilitated the recovery of protein concentrates (75–80% protein) from yeast with approximately 2% contaminant RNA (Damodaran and Kinsella, 1984).

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The successful adoption and use of proteins isolated from microbial biomass depend on their physicochemical properties, which determine their usefulness as functional food ingredients (Kinsella, 1976). There is limited information available concerning the functional properties of isolated yeast proteins (Vananuvat and Kinsella, 1975; Huang and Rha, 1971; Cooney et al., 1980), and more information is required to assess their potential in food systems (Litchfield, 1983; Batt and Sinskey, 1984).

Many of the important functional properties of food proteins are related to their interactions with water. Solubility provides an index of native structure and is a desirable prerequisite of proteins in beverages, in liquid foods, and for emulsion and foam formation (Kinsella, 1982). Water-holding capacity, a quantitative indication of the amount of water retained within a protein matrix under a defined condition, is important in doughs, batters, and comminuted meat systems. Knowledge of the viscosity of protein dispersions is of practical significance in relation to processing, process design, mouthfeel of viscous fluid products, and new product development (Hermansson,

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