EVALUATION OF METHODS FOR FORTIFYING SKIM MILK POWDER WITH VITAMIN A

By

GAETAN MARC ANDRE PAQUETTE

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Department of Food Science

We accept this thesis as conforming
to the required standard

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Department of ___ FOOD SCIENCE ___

The University of British Columbia
1956 Main Mall
Vancouver, Canada
V6T 1Y3

Date ___ SEPTEMBER, 1985 ___
EVALUATION OF METHODS FOR FORTIFYING SKIM MILK POWDER WITH VITAMIN A

The fortification of skim milk powder with vitamin A has been found to be ineffective with available methods. The purpose of this study was to assess new methods and materials for their effectiveness in providing stability to vitamin A in fortified skim milk powder.

The first phase of the project involved trials in Pilot Plants which evaluated 14 different treatments for vitamin A stability during storage periods of twelve months at 22°C and six months at 37°C. The second and third phases of the experiment consisted of primary and instant powder trials in commercial plants using the most stable methods from the Pilot Plant trials. In the latter phases of the project, eight treatments were tested for primary powder and ten for instant type of powder.

Results show that levels of antioxidants were important to control the oxidative degradation of vitamin A in the milk powder. The vitamin A concentrate containing BHA (5 mg), BHT (55 mg) and α-tocopherol (12.5 mg) antioxidants produced the best results for primary powder. Ascorbyl palmitate-α-tocopherol combination of antioxidants was found to be more effective than the BHA-BHT-α-tocopherol blend for instant powder. The level of hydrogenated coconut oil (HCO) used as the vitamin carrier was also found to be important for stability, 0.2% being slightly better than 0.1% in primary powder. A 12% emulsion injected at such a rate as to add 0.027% oil in milk solids was the best treatment of the instant powder trials.

Hay-like flavour in reconstituted skim milk powder was correlated with vitamin A destruction.
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# TABLE OF CONTENTS

ABSTRACT \( \text{ii} \)

ACKNOWLEDGEMENTS \( \text{iii} \)

TABLE OF CONTENTS \( \text{iv} \)

LIST OF TABLES \( \text{vii} \)

LIST OF FIGURES \( \text{viii} \)

LIST OF APPENDICES \( \text{ix} \)

INTRODUCTION \( \text{1} \)

REVIEW OF LITERATURE \( \text{3} \)

- Vitamin A deficiency \( \text{3} \)
- Function and technology of vitamin A \( \text{4} \)
- Vitamin A fortification of NDM \( \text{8} \)
- Antioxidant technology \( \text{9} \)
- Off-flavour from vitamin A destruction \( \text{13} \)

NEED FOR THE STUDY \( \text{14} \)

PROJECT OUTLINE \( \text{16} \)

PHASE I - PILOT PLANT TRIALS \( \text{18} \)

- Introduction \( \text{18} \)
- Materials and Methods \( \text{18} \)
  - Method of drying and addition of the vitamins \( \text{18} \)
  - Storage and analysis \( \text{20} \)
  - Treatment description \( \text{20} \)
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Results and Discussion</td>
<td>24</td>
</tr>
<tr>
<td>Conclusions</td>
<td>28</td>
</tr>
<tr>
<td><strong>PHASE II - COMMERCIAL PLANT TRIALS - PRIMARY POWDER</strong></td>
<td></td>
</tr>
<tr>
<td>Introduction</td>
<td>29</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>30</td>
</tr>
<tr>
<td>Addition of the vitamins</td>
<td>30</td>
</tr>
<tr>
<td>Treatment description</td>
<td>30</td>
</tr>
<tr>
<td>Storage and sampling</td>
<td>32</td>
</tr>
<tr>
<td>Vitamin A analysis</td>
<td>32</td>
</tr>
<tr>
<td>Sensory evaluation</td>
<td>33</td>
</tr>
<tr>
<td>Moisture analysis</td>
<td>33</td>
</tr>
<tr>
<td>Method for graphing</td>
<td>34</td>
</tr>
<tr>
<td>Statistical analysis</td>
<td>37</td>
</tr>
<tr>
<td>Results and Discussion</td>
<td>39</td>
</tr>
<tr>
<td>Vitamin A stability</td>
<td>39</td>
</tr>
<tr>
<td>Haylike flavour</td>
<td>42</td>
</tr>
<tr>
<td>Comparison of production plants</td>
<td>42</td>
</tr>
<tr>
<td>Comparison of analytical methods of vitamin A</td>
<td>45</td>
</tr>
<tr>
<td>Conclusions</td>
<td>46</td>
</tr>
</tbody>
</table>
Phase III - COMMERCIAL PLANT TRIALS - INSTANT POWDER

Introduction 47
Materials and Methods 48
  Treatment description 48
  Preparation of vitamin mixes 48
  Addition of the vitamins 51
  Storage and sampling 51
  Vitamin A analysis 51
  Sensory evaluation 51
  Statistical analysis 51
Results and Discussion 53
  Vitamin A stability 53
  Haylike flavour 59
  Comparison of production plants 59
Conclusions 62

OVERALL CONCLUSIONS 64
LITERATURE CITED 65
APPENDIX -Additional table 70
LIST OF TABLES

1. Processing conditions in Pilot Plants A and D. 19
2. Vitamin A fortification treatments for Phase I - Pilot Plant Trial. 21
3. Initial levels of vitamin A and percent vitamin A losses during storage in primary skimmilk powder fortified by different treatments in Pilot Plants A and D after 3 and 12 months of storage at 22°C and after 3 and 6 months at 37°C. 25
4. Levels of antioxidants contained in Pilot Plant treatments 26
5. Description of vitamin A fortification treatments for Phase II - Primary Powder. 31
6. Estimated regression coefficients for Multiple Comparison Analysis for Primary Powder stored at 22 and 37°C. 40
7. Losses of vitamin A in Primary Skimmilk Powder fortified by different treatments in Plants A and B after 12 months of storage at 22°C and 6 months at 37°C. 43
8. Description of vitamin A fortification treatments for Phase III Instant Powder. 49
9. Estimated regression coefficients for Multiple Comparison Analysis for Instant Powder stored at 22 and 37°C. 56
10. Losses of vitamin A in Instant Skimmilk Powder fortified by different treatments in Plants A and B after 12 months of storage at 22°C and 6 months at 37°C. 60
1. Formulas of most common forms of vitamin A and provitamin A.  

2. Vitamin A depletion in Primary Skimmilk Powder during 12 months of storage at 22°C.  

3. Vitamin A depletion in Primary Skimmilk Powder during 6 months of storage at 37°C.  

4. Vitamin A depletion in Instant Skimmilk Powder during 12 months of storage at 22°C.  

5. Vitamin A depletion in Instant Skimmilk Powder during 6 months of storage at 37°C.
LIST OF APPENDICES

1. Regression equations for the Primary Powder treatments when stored at 22 and 37°C.  
   Page 70

2. Regression equations for the Instant Powder treatments during storage at 22 and 37°C.  
   Page 71
INTRODUCTION

Despite outstanding advances in vitamin fortification of food, vitamin A deficiency still occurs in endemic proportions in many developing countries and is occasionally seen in technologically developed societies (WHO, 1982; McLaren, 1980). Interest in the fortification of nonfat dry milk (NDM) with vitamin A has intensified in recent years as a result of growing concerns for nutritionally deficient populations resulting in the export of increasing quantities of this nutritious product to Third World Countries.

In 1984, the Food Aid Program of the Canadian International Development Agency (CIDA) shipped approximately 22,000 tonnes of fortified NDM to food-deficient areas of the world. Since 1979, following recommendations from the Protein Advisory Group of the U.N. Agency (Anonymous, 1976) and Marquardt (1979), CIDA has adopted the recommendation to fortify all NDM for Food Aid Programs with vitamin A to a level of 5000 I.U./100 g.

In Canada, the Food and Drugs Act requires fortification of instantized nonfat dry milk for consumers with vitamin A. Section B.08.014 of the Act (1979) stipulates that "the added vitamin A shall be in such amount that a reasonable daily intake of the milk contains not less than 1200 I.U. and not more than 2500 I.U. of vitamin A". The reasonable daily intake of milk is determined further in Schedule K of the Act as 852 ml.
Therefore the addition of vitamin A to instantized nonfat dry milk is usually between 1400 and 2900 I.U. 100g allowing for overage.

In spite of extensive research in developing methods to produce a stable vitamin A fortified NDM, this process has remained less than satisfactory. In an attempt to improve the stability of vitamin A in skim milk powder, a research project funded by the Canadian Dairy Commission was undertaken to evaluate the effectiveness of vitamin A fortification methods.

The main objective was to test proposed new methods of primary and instant NDM fortification by determining the vitamin A stability during storage. The development of haylike flavour was also examined.
REVIEW OF LITERATURE

Vitamin A deficiency

Vitamin A deficiency has been studied extensively in experimental animals and in humans. Early signs of vitaminosis A include loss of appetite, growth failure and impaired immune response with lowered resistance to infection. In humans the signs of clinical importance are the ocular manifestations (McLaren, 1984). Xerophthalmia, the term generally used to cover all the ocular manifestations of vitamin A deficiency, is the most common cause of blindness in young children throughout the world (WHO, 1976; Sommer et al., 1981). The Protein-Calorie Group of the United Nations has listed 73 countries and territories where it is considered that a vitamin A deficiency problem of public health significance occurs (Anonymous, 1976). That report recognized xerophthalmia to be endemic in many of the countries in southern and eastern Asia and parts of Latin America. In recent years, attention has also been drawn to its frequent occurrence in many countries in Africa and the Middle East.

In well-nourished societies, requirements are more than adequately met by an ample intake of both vitamin A and carotenoids from milk, vegetables and fruits, but in developing countries, requirements are frequently not met. The shipping of high protein foods alone to these nutritionally deficient populations is not sufficient. It is known that with diets having protein supplements without adequate vitamin A fortification, vitamin A depletion occurs in the liver and precipitates xerophthalmia (Srikantia, 1975; Pereira and Begum, 1976).
Skim milk powder being consumed in large quantities may be used as a vehicle to provide deficient populations with vitamin A and especially children who are most frequently in need of the antixerophthalmic and anti-infective vitamin.

**Function and technology of vitamin A**

The best-defined role of vitamin A is in vision. Other less defined nutritional roles are with growth and differentiation of cells, i.e., gene expression. Clinically, large doses of vitamin A have been shown to be effective in the treatment of many skin disorders and various kinds of cancer (Sporn and Newton, 1979; Bollag, 1979; McLaren, 1984). The term "vitamin A" includes all β-ionone derivatives, other than the provitamin A carotenoids. Figure 1 shows the formulas for the more common forms of vitamin A which include: all-trans retinol (Figure 1A); esters of all-trans retinol such as retinyl palmitate (Figure 1B) and retinyl acetate (Figure 1C); the aldehyde form of all-trans retinol commonly designated as retinaldehyde or retinal (Figure 1D); and retinoic acid (Figure 1E), the acidic form of vitamin A. Among the more than 400 characterized carotenoids, only 30 possess provitamin A activity. The most active carotenoid, all-trans β-carotene is shown in Figure 1F.

Vitamin A is fairly stable when heated to moderate temperatures in an inert atmosphere in the absence of light, but it is unstable in the presence of oxygen or air, or when exposed to ultraviolet light. Trace metals may also accelerate the oxidation of vitamin A. Moisture and pH are somewhat more critical for certain forms of the vitamin (acetate ester)(Bauernfeind, 1978). Therefore, some precautions in handling
Figure 1. Formulas of most common forms of vitamin A and Provitamin A.
A. all-trans retinol

B. retinyl palmitate

C. retinyl acetate

D. retinaldehyde or retinal

E. retinoic acid

F. all-trans β-carotene
vitamin A include: (a) exclusion of oxygen; (b) protection against light; (c) avoidance of pro-oxidant trace metals and strongly acidic environment.

The principal methods of stabilizing vitamin A involve (a) sealing under vacuum or inert gas, (b) storage at low temperatures, (c) the addition of antioxidants, (d) coating and sealing vitamin containing oil-droplets with a protective matrix such as gelatin or vegetable gums. The first two methods are common methods of storing any unstable compound, but they are not always convenient and practical.

The use of antioxidants to protect vitamin A is common practice. However, users are confined to those antioxidants that are acceptable for food addition. In the preparation of dry forms of vitamin A, powders, granulates, microspheres, beadlets and agglomerates have been prepared by a variety of processing methods involving absorption, granulation, spray congealing and encapsulating procedures as detailed by Klaui et al. (1970).
Vitamin A fortification of NDM

The addition of vitamin A to nonfat dry milk is not new; trials were reported in the literature over 30 years ago (Olson et al., 1949; Bauernfeind et al., 1953) soon after the successful synthesis of vitamin A in 1947. Since then, attempts to fortify dry milk products with vitamin A have been discouraging due to off-flavor and stability problems. The fact that the added vitamin A in NDM generally oxidizes easily can be attributed to factors, such as the rigors of the process to dry the product; the degree of air exposure and the frequently long length of storage at high temperature which all possibly contributes to the difficulty of producing a product with stable vitamin A.

Several studies contributed greatly to the practical vitamin fortification of NDM by examining the following factors: a) carrier formulation for the vitamin A (Conochie and Wilkinson, 1956), b) influence of homogenization (Shroff et al., 1954), c) effect of heat (Wilkinson and Conochie, 1958), d) effect of light (Dalle et al., 1969; Lerner et al., 1970; Sattar et al., 1976; Sattar et al., 1977; Thompson and Erdody, 1974; Smith and MacLeod, 1957; Stull, 1951), e) influence of reconstitution (Anantakrishan and Conochie, 1958) and f) importance of packaging (Wodsak, 1953).

Some of these earlier works were basis of studies Bauernfeind and Allen (1963) to improve methods of vitamin A fortification to NDM. In 1963, these researchers outlined two new methods for vitamin A fortification of NDM.
The first, the wet method, involved the addition of concentrated, stabilized vitamin A palmitate in a carrier of liquified hydrogenated coconut oil (HCO) to condensed skim milk prior to spray drying. The dry method involved the blending of dry, stabilized vitamin A palmitate beadlets to NDM. One year later, Bauernfeind and Parman (1964) demonstrated that both "wet" and "dry" methods offered a high degree of vitamin A stability and low off-flavor to the NDM. The key to their success now appears to be in the use of antioxidant-stabilized vitamin A.

It has been suggested that added synthetic vitamin A is less stable than naturally-occurring vitamin A (Thompson and Erdody, 1974). This is possibly due to the difficulty of blending small amounts of a lipid soluble substance into a non-lipid solution resulting in the synthetic vitamin A being in fewer dispersed particles than naturally-occurring vitamins. Another possibility is that skim milk might have lost its natural antioxidants that protect native vitamin A.

**Antioxidant technology**

It has long been observed that certain substances inhibit oxidation or antioxidant action. Various substances have been established as safe and effective as antioxidant; they can be divided into two categories comprised of primary antioxidants and of synergists. Primary antioxidants are those substances which function by inhibiting or interrupting the free radical stage or the initiation step of autoxidation (Sherwin, 1976). Thus by being preferentially oxidized, the antioxidants either prevent direct oxidation or provide indirect protection by breaking the oxidation chain reaction.
A list of commonly used primary antioxidants in vegetable oil include the tocopherols, gallates, nordihydroguaiaretic acid (NDGA), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary butylhydroquinone (TBHQ). The list of permitted primary antioxidants in vitamin A concentrates is limited to BHA, BHT and dl-α-tocopherol. In Canada, the Food and Drugs Act, Division B.16.100 under Class IV Preservatives (1979) stipulates that the addition of BHA and BHT to vitamin A preparations is limited to 5 mg of each BHA and BHT per million I.U. of vitamin A. There is no restriction on dl-α-tocopherol.

Synergists are substances which, in combination with other substances, result in a mixture whose activity is greater than the sum of the activity of the individual components. Synergists may possess no activity of their own (e.g. citric acid) or may themselves be antioxidants (e.g. ascorbyl palmitate). Their actual mode of action has not yet been fully explained but probably involves chelation of prooxidant metals, regeneration or sparing of primary antioxidants and/or inhibition of peroxide decomposition which interrupt the autoxidation process (Sherwin, 1976).

Synergists for primary antioxidants are acids such as citric, phosphoric, ascorbic and tartaric. Derivatives of the acids such as ascorbyl palmitate have better solubility in oil and are also effective. Lecithin is another substance that appear to exhibit an antioxidant effect. However, it is suggested that this effect may be a synergistic effect on the true antioxidant action of primary antioxidants (Sherwin, 1976).
Antioxidants and synergists thus protect not only fats from oxidizing, but also other substances of an "unsaturated" nature that are contained in foods such as vitamin A.

Evidence of the antioxidative effect of tocopherol in vegetable oils was observed over 40 years ago (Greenbank et al., 1944) and research for its possible use in dairy products has since been extensive (Abbot and Waite, 1965; Abbot and Waite, 1962; Battna et al., 1982; Timmen, 1978; Merzametov and Gadzhieva, 1982; Erickson et al., 1963; King, 1968). Dl-α-tocopherol is one of the most widely used antioxidants for its action as a primary antioxidant and vitamin E activity, but at least seven other types of tocopherol have been used as antioxidant with varying degrees of effectiveness.

Abbot and Waite (1965) tested dl-γ-tocopherol and mixtures of α,γ,δ-tocopherols along with dodecyl gallate in spray-dried whole milk powder. No tocopherol preparation was effective, but dodecyl gallate was. Previously, Lea and Ward (1959) and Lea (1960) found that out of seven tocopherols, γ-tocopherol gave a consistently good performance but α-tocopherol was not effective. Abbot and Waite (1965) reported that the tocopherols and dodecyl gallate did not impart any off-flavor to the powder. They were used at the 0.01% level in the powder.

Several other primary antioxidants and synergists have been used in the dairy industry. Abbot and Waite (1962) tested flavones, gallates, BHA and NDGA in spray-dried whole milk powder stored for up to 400 days at 20 and 37°C. Dodecyl gallate was shown to be a very effective antioxidant and, propyl gallate and NDGA, whilst gave good protection were less so;
BHA did little to improve keeping quality. All antioxidants were used at the 0.01% level in the powder. When powder is fortified at 5000 I.U./100g this level of antioxidant (0.01%) is equivalent to 20 mg/million units of vitamin A.

Pont (1964) showed that dodecyl gallate and NDGA are two times better than BHA, BHT and ascorbyl palmitate and three times better than α-tocopherol and lecithin in prolonging the induction period of oxidation in butter. Hammond (1970) found BHT, propyl gallate and NDGA effective antioxidants for butteroil. Stull (1951) showed the effectiveness of NDGA at retarding the oxidation of whole milk powder during 12 months of storage at 22°C. Those researchers also showed the enhanced antioxidant power of NDGA when used with citric acid as synergist.

Timmen (1978) tested in pure butterfat the antioxidative effect of gallates, BHA, BHT, α-tocopherol and ascorbyl palmitate singly or in combination. Simultaneous addition of α-tocopherol and ascorbyl palmitate drastically retarded oxidation; this effect being further intensified by lecithin and propyl gallate. As demonstrated in the above, the action of synergists in combinations such as ascorbyl palmitate, dl-α-tocopherol and lecithin have been widely studied. (Battna et al., 1982; Schuler, 1980; Stull, 1951).

The amount of antioxidant used to ensure a proper stabilization should be the smallest possible; for most food 100-500 ppm are sufficient. The addition of larger quantities may in certain cases produce the opposite result, i.e., accelerate oxidation. For example, it is important to pay attention to the original tocopherol content of food.
because in general, the total tocopherol should not exceed 1000 ppm or 1 mg per gram of oil (Schuler, 1980). Others (Klaui, 1979; Timmen, 1978) have also reported that a great excess of tocopherol no longer has an antioxidative effect on retinol or retinyl acetate; 50 ppm being considered optimum.

Ascorbyl palmitate, dl-\(\alpha\)-tocopherol (vitamin E) and ascorbic acid (vitamin C) are often added to foods for their dual purposes by acting as antioxidants and for their biological activity as vitamins when not oxidized. Ascorbyl palmitate has the full biological activity of vitamin C; it breaks down in the digestive tract releasing ascorbic acid. For that reason, Health and Welfare Canada has no restriction on the levels of \(\alpha\)-tocopherol, ascorbyl palmitate and ascorbic acid added to foods.

**Off-flavour from vitamin A destruction**

In addition to loss in vitamin A activity when destroyed by oxidation, the by-products of this reaction have been reported to cause an off-flavor called "haylike flavor". As described in the term, this off-flavor is comparable to the smell coming from a drying hay field on a sunny summer day. Haylike flavor may be described as alfalfa or carrot-like and is distinctly different than the oxidized flavor from fat oxidation or the activated flavor caused by ultraviolet light on milk protein (Nakai et al., 1983). Many researchers have published the association of hay-like flavor development with destruction of added vitamin A in fortified dairy products (Weckel and Chicoye, 1954; Bauernfeind and Allen, 1963; Thomas et al., 1965, Suyama et al., 1983; Nakai et al., 1983).
NEED FOR THE STUDY

Despite earlier trials demonstrating the effectiveness of Bauernfeind's suggested methods of fortification, still recently, the literature has cited several incidences in which researchers have observed rapid loss of vitamin A in NDM during storage (Woollard and Edmiston, 1983; Nakai et al., 1983; Suyama et al., 1983; deBoer et al., 1984) and simultaneous development of haylike flavor (Nakai et al., 1983; Thomas et al., 1965; Weckel and Chicoye, 1954; Suyama et al., 1983).

In a previous Canadian Dairy Commission (CDC) contract conducted at the University of British Colombia, Nakai et al. (1983) found from a market survey that 27% of instantized NDM samples obtained from various manufacturers in Canada and the U.S. had vitamin A levels less than the accepted minimum (14 I.U./g). Of the retail samples of both U.S. and Canadian origin, 73% had vitamin A levels less than 20 I.U./g. This indicated that there was still a problem with NDM fortification at the retail level. This same study found that dry-blending vitamin A beadlets with the powder at the agglomeration chamber during the instantizing process was the best method and the "wet" method adding the vitamin A in a hydrogenated coconut oil emulsion was the next best method.

The result of the above survey spelled out the need for further research. The need for an investigation into the effectiveness of proposed new methods of vitamin fortification was also suggested by Agriculture Canada researchers. Research was also requested by the milk powder industry as a result of the findings from the above research project. The Canadian International Development Agency (CIDA)
which buys large quantities of fortified NDM annually was another body requesting recommendations regarding this subject. The major portion of their milk powder purchase is fortified using the dry batch-blending method of incorporating a beadlet vitamin concentrate to the powder.

As mentioned earlier, this method has been shown to be effective in producing powder with reasonable vitamin A stability during storage, but is prohibitively expensive, i.e. $145/tonne. Since the major portion of this cost is due to the labour required for the debagging, batch-blending and re-bagging of the powder, the discovery of an equally effective method of fortification during or prior to production has significant saving potential. The cost of such a procedure (in-line addition) including the fortification materials is estimated at approximately $15-20/tonne of powder. For the fortification of 22,000 tonnes the saving for CIDA's Food Aid Program alone would be $2.75 millions for one year.
In an attempt to solve this seemingly unsolved problem of vitamin A instability, a research project was undertaken with the following objectives:

1. Prepare in Pilot Plants and test the storage stability of vitamin A added to skim milk by different methods before drying.
2. Prepare in Commercial Plants and test the storage stability of vitamin A added by the best pilot plant methods for instant and primary powder production.

A major U.S. powder manufacturer was visited in 1982 to obtain the latest U.S. technology on vitamin A fortification to NDM. For the fortification of instant powder, vitamin A in a beadlet form was metered with a dry vitamin feeder into the powder ahead of the agglomerator. They had developed this process following a problem of poor reconstitutability which arose from a previous method involving the spraying of a coconut-oil-based product into the agglomerator. The vitamin A stability of the beadlet method was claimed to be good, but produced some variability in levels.

The regular powder was fortified using a vitaminized coconut-oil concentrate following Bauernfeind's method except that approximately 0.06% was added to the powder instead of 0.2%. The USDA requires the fortified NDM to contain vitamin A in a range of 2200-4400 I.U./100g for their Food Aid Program. This method appeared to easily meet those requirements at the time of manufacture; they felt that it was the best and the simplest
method of adding vitamins to regular powder.

The methods of fortification tested in this project are based on the "wet" method of fortification. This basic procedure is of particular interest to both instant and primary powder manufacturers since it has the advantage of being added on a continuous on-line operation.

The experimental work of this project was divided into three phases; Phase I (Objective #1) involved the testing of several treatments at the Pilot Plant level in order to determine the best possible treatments for the subsequent phases of the project. Phases II and III were carried out in Commercial Plants, by applying the treatments chosen from the results of Phase I, as most likely to produce powder with stable vitamin A.
**PHASE I - PILOT PLANTS TRIALS**

Experiments for this phase of the project were conducted in two different Pilot Plants. Those Pilot Plants will be referred to as Pilot Plant A and Pilot Plant D in this report. Each treatment was replicated twice during different work weeks and at alternating Pilot Plants.

**Materials and Methods**

**Methods of drying milk and addition of vitamins**

Enough condensed skim milk of 36-44% total solids was obtained to spray dry at least 20 kg of powder for each treatment. To facilitate the incorporation of the vitamins, about 10% (6 kg) of the amount of condensed skim milk required for a batch, was heated to 50-52°C. As the generally accepted minimum Vitamin A level in fortified NDM is 22 I.U./g, to ensure this amount is found in the dried product, it is necessary to include a certain percentage of overage to the level of added vitamin A. Therefore 30 I.U. of vitamin A and 6 I.U. of vitamin D₂ per gram of dry powder were added. The vitamins concentrate containing vitamins A and D, and the antioxidants formulation in an oil carrier, was then added with constant agitation to the heated portion of the condensed milk which was immediately homogenized with a double stage homogenizer at pressures of 2000 and 500 psi. This vitaminized portion (6 kg) was then thoroughly blended with the rest of the batch by manual stirring and immediately spray-dried. The various processing conditions and types of dryer for both Pilot Plants are listed in Table 1. Following the spray-drying of each treatment,
Table 1. Processing conditions in Pilot Plant A and D.

<table>
<thead>
<tr>
<th>Processing Parameter</th>
<th>Pilot Plant A</th>
<th>Pilot Plant D</th>
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<tr>
<td><strong>Concentrate Preparation</strong></td>
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<tr>
<td>Total solids of Condensed Milk</td>
<td>40-44%</td>
<td>36-38%</td>
</tr>
<tr>
<td>Holding temperature of Condensed Milk (when held overnight)</td>
<td>2°C</td>
<td>2°C</td>
</tr>
<tr>
<td>Temperature of Oil-Vitamin mix</td>
<td>49°C</td>
<td>49°C</td>
</tr>
<tr>
<td>Temperature of Oil-Vitamin concentrate at homogenization</td>
<td>49°C</td>
<td>49°C</td>
</tr>
<tr>
<td>Temperature of vitaminized batch prior to drying</td>
<td>11°C</td>
<td>11°C</td>
</tr>
<tr>
<td><strong>Drying Conditions</strong></td>
<td></td>
<td></td>
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<tr>
<td>Name of drier</td>
<td>Anhydro Compact</td>
<td>Rogers</td>
</tr>
<tr>
<td>Type of drier</td>
<td>Tower co-current with cyclone collector</td>
<td>Inverted tear-drop co-current with cyclone collector.</td>
</tr>
<tr>
<td>Capacity (Kg powder/hr)</td>
<td>20</td>
<td>25</td>
</tr>
<tr>
<td>Inlet air temperature</td>
<td>200°C</td>
<td>155°C</td>
</tr>
<tr>
<td>Outlet air temperature</td>
<td>98°C</td>
<td>80°C</td>
</tr>
<tr>
<td>Type of nozzle</td>
<td>Centrifugal</td>
<td>#72-220</td>
</tr>
<tr>
<td>Method of removing powder from drier</td>
<td>Cyclone collector emptied every 15 min.</td>
<td>Scrape bottom box each 15 min plus cyclone collector.</td>
</tr>
</tbody>
</table>
the dryer box was swept clean to minimize carry-over from one treatment to another. Each lot of vitaminized powder was then blended for one hour in a butter-churn to ensure an homogeneous distribution of the vitamins.

Storage and analysis

Each lot of powder was subsequently divided into 250-g samples in foil-laminated bags. A number of those powder bags from each treatment were then stored at 22°C (room temperature) and 37°C (high temperature) to be analysed for vitamin A content at 0, 1, 2, 3, 4, 6, 9 and 12 months (22°C) and 0, 1, 2, 3, 4 and 6 months (37°C) of storage. The spectrofluorometric method as described by Thompson et al. (1978) was used by laboratory C to determine the vitamin A content. Moisture content was also determined on each lot at the beginning and at the end of the storage period.

Treatment description a)

The list of the treatments and processing conditions tested in this trial are shown in Tables 1 and 2. The following are brief descriptions of the materials used in the treatment preparations. Treatment A uses a commercial preparation with a vitamin concentration of 45,400 I.U. of vitamin A palmitate and 9090 I.U. of vitamin D₃ (cholecalciferol) per gram intended for non-instantized NDM. In addition, this product contains polysorbate 80 with BHA and BHT as antioxidant in a base of hydrogenated coconut oil (HCO). Due to its HCO content, this concentrate is not liquid at room temperature.

a) Trade names of commercial products were not used throughout this report to protect the identity of the implicated companies.
Table 2. Vitamin A fortification treatments for Phase I - Pilot Plant Trial.

A. Commercial HCO-based product  
B. Commercial milkfat-based product  
C. Milkfat carrier -0.1% (no antioxidant)  
D. Milkfat carrier -0.1% (500 ppm commercial antioxidant mixture)  
E. Commercial Vitamin A concentrate (vegetable oil-based)  
F. HCO carrier -0.05% (no antioxidant)  
G. HCO carrier -0.05% (500 ppm commercial antioxidant mixture)  
H. HCO carrier -0.10% (no antioxidant)  
I. HCO carrier -0.10% (500 ppm commercial antioxidant mixture)  
J. HCO carrier -0.10% (2000 ppm commercial antioxidant mixture)  
K. HCO carrier -0.10% (Commercial stabilized vitamin concentrate)  
L. HCO carrier -0.20% (Commercial stabilized vitamin concentrate)  
N. HCO carrier -0.20% (no antioxidant)  
O. HCO carrier -0.20% (500 ppm commercial antioxidant mixture)
Treatment B uses a commercial preparation containing the same vitamin A and D concentrations as treatment A, but is in a base of reconstituted non-fat dry milk and butter oil. This product is meant for instant skim-milk powder fortification and has no declared antioxidants in its content. Treatments C and D are lab-made preparations using butter oil from melted butter as the vitamin carrier added to condensed milk at a level of 0.1% of the dry weight. Vitamins A (all trans-retinyl palmitate) and D$_3$ were added to the butter oil from a highly concentrated source (1 million I.U. per gram in vegetable oil).

Treatment E uses a U.S. commercial preparation with vitamins A and D concentrations of 50,000 I.U. and 10,000 I.U. per gram, respectively. The vitamin carrier in this product is a blend of hydrogenated coconut oil and vegetable oil containing a food-grade emulsifier; it is orange in colour and remains almost liquid (very soft paste) at room temperature.

The antioxidants used for Treatments D, G, I, J and O are from a commercial mixture containing a blend of ascorbyl palmitate (23%) and dl-α-tocopherol (7.5%) with citric acid as synergist in a mono- and di-glycerides matrix. This product can be described as off-white to tan in colour and odourless with a waxy and lard-like consistency. Treatments F to O are all lab-made preparations using fully hydrogenated pure coconut oil (Peroxide Value = 0.20 and antioxidant-free) as the vitamins carrier to the respective percent (0.05, 0.1 or 0.2%) of the dry weight of condensed milk. The antioxidant and vitamin source used for these treatments is the same materials as described under Treatments C and D section, except
for Treatments K and L for which the vitamin A source contained its own antioxidants. The antioxidant content for these two treatments (K and L) is 5 mg of BHA, 55 mg BHT and 12.5 mg of dl-α-tocopherol per million I.U. of vitamin A. All expressions of the level of antioxidants in this thesis, thereafter, are based on milligrams per million I.U. of vitamin A.
Results and Discussion

Table 3 presents initial levels of vitamin A in powders from the 14 treatments and the percentage loss of the initial level during storage at 22 and 37°C.

Considering that the target fortification level was 30 IU/g, 12 treatments resulted in levels of more than 20 I.U. at the beginning of storage. One treatment (D) using vitamin A in milk fat with no antioxidant resulted in an initial level of 11.6 I.U./g. A similar laboratory-prepared mix adding 0.05% HCO to the powder (treatment F) and containing no antioxidant resulted in a level of 14.5 I.U./g. These treatments resulted in 90% or more destruction of the remaining vitamin A after three months of storage at both temperatures.

After 3 months at 22°C, treatments E, K, L, N and 0 had less than 25% destruction. These were respectively, a commercial preparation, antioxidant stabilized vitamins at 0.1 and 0.2% HCO in powder and vitamin A with no antioxidant and 500 ppm of a commercial antioxidant mixture at 0.2% HCO. It is apparent that higher levels of hydrogenated coconut oil and antioxidants are beneficial during initial storage at 22°C.

Further evidence of the effect of antioxidants lies in comparing treatments H, I, J and K, all at 0.1% HCO. Vitamin A losses after 3 months at 22°C were 80, 77, 52 and 15% respectively. Types and levels of antioxidants for non-commercial treatments are given on Table 4. Antioxidant levels were: zero (H); 1.3 and 5.2 mg α-tocopherol and 4 and 16 mg of ascorbyl palmitate (treatments I and J respectively);
Table 3. Initial levels of vitamin A and percent of vitamin A losses during storage in primary skim milk powder fortified by different treatments in Pilot Plants A and D after 3 and 12 months of storage at 22°C and after 3 and 6 months at 37°C.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Initial Level of Vitamin A (I.U.)</th>
<th>Percent vitamin A loss&lt;sup&gt;a/&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Room Temp (22°C)</td>
<td>High Temp (37°C)</td>
</tr>
<tr>
<td></td>
<td>3 mo</td>
<td>12 mo</td>
</tr>
<tr>
<td>A</td>
<td>46.8</td>
<td>67.6</td>
</tr>
<tr>
<td>B</td>
<td>11.6</td>
<td>89.7</td>
</tr>
<tr>
<td>C</td>
<td>27.7</td>
<td>93.0</td>
</tr>
<tr>
<td>D</td>
<td>23.2</td>
<td>92.6</td>
</tr>
<tr>
<td>E</td>
<td>36.8</td>
<td>22.6</td>
</tr>
<tr>
<td>F</td>
<td>14.5</td>
<td>91.3</td>
</tr>
<tr>
<td>G</td>
<td>20.2</td>
<td>95.5</td>
</tr>
<tr>
<td>H</td>
<td>22.1</td>
<td>80.7</td>
</tr>
<tr>
<td>I</td>
<td>24.9</td>
<td>77.4</td>
</tr>
<tr>
<td>J</td>
<td>28.2</td>
<td>52.5</td>
</tr>
<tr>
<td>K</td>
<td>32.1</td>
<td>15.4</td>
</tr>
<tr>
<td>L</td>
<td>29.9</td>
<td>21.2</td>
</tr>
<tr>
<td>N</td>
<td>24.6</td>
<td>24.5</td>
</tr>
<tr>
<td>O</td>
<td>27.6</td>
<td>23.7</td>
</tr>
</tbody>
</table>

<sup>a/</sup>= Mean of two trials in each of two Pilot Plants.
Table 4. Levels of antioxidants contained in Pilot Plant treatments

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Antioxidant</th>
<th>Concentration (mg/million I.U.Vit.A)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>(Commercial)</td>
<td>Undeclared</td>
</tr>
<tr>
<td>B</td>
<td>(Commercial)</td>
<td>Undeclared</td>
</tr>
<tr>
<td>C</td>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>D</td>
<td>Ascorbyl Palmitate</td>
<td>4.0mg 1.3mg</td>
</tr>
<tr>
<td>E</td>
<td>(Commercial)</td>
<td>Undeclared</td>
</tr>
<tr>
<td>F</td>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>G</td>
<td>Ascorbyl Palmitate</td>
<td>2.0mg 0.65mg</td>
</tr>
<tr>
<td>H</td>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>I</td>
<td>Ascorbyl Palmitate</td>
<td>4.0mg 1.3mg</td>
</tr>
<tr>
<td>J</td>
<td>Ascorbyl Palmitate</td>
<td>16.0mg 1.3mg</td>
</tr>
<tr>
<td>K</td>
<td>BHA</td>
<td>5.0mg 5.0mg 12.5mg</td>
</tr>
<tr>
<td>L</td>
<td>BHA</td>
<td>5.0mg 5.0mg 12.5mg</td>
</tr>
<tr>
<td>N</td>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>O</td>
<td>Ascorbyl Palmitate</td>
<td>8.0mg 2.6mg</td>
</tr>
</tbody>
</table>
and 5 mg BHA, 55 mg BHT and 12.5 mg α-tocopherol (K).

HCO levels of 0.05, 0.1 and 0.2% in the powder with no antioxidant were in treatments F, H and N; destruction after 3 months at 22°C for these treatments were 91, 81 and 24% respectively. Levels of HCO with 500 ppm of commercial antioxidant were 0.05, 0.1 and 0.2% in the powder for treatments G, I and O; destruction after 3 months at 22°C were 96, 77 and 24% respectively. Clearly higher levels of HCO resulted in greater stability of vitamin A. This is in agreement with the findings of Bauernfeind and Parman (1964).

There was little difference between the milkfat (C, D) and HCO (H, I) at 0.1% level as the vitamin carrier except at three months of storage at which time the latter showed less destruction.

The two commercial products with the vitamin in vegetable oil (A, E) showed poor and medium stability respectively. The commercial emulsion (B) was one of the least stable treatments. It is likely that the large difference in stability exhibited by the commercial preparations is due to their contents of antioxidants.

The crucial test for suitability of the treatments lies in the storage survival of the vitamin after 12 months at 22°C or 6 months at 37°C. Only treatments K and L resulted in about 30% destruction. All others resulted in 75% or more destruction for the same storage condition except treatment E which resulted in 58% destruction at 22°C, but 88% at 37°C.
Conclusions

1. The presence of antioxidant materials distinctly aided stability during storage. The most stable product used 5 mg of BHA, 55 mg of BHT and 12.5 mg of $\alpha$-tocopherol/10$^6$ I.U. of vitamin A. The antioxidant content of the commercial products were unknown. Different levels and combinations of antioxidants are used in the subsequent trials in Phases II and III.

2. Higher levels of the oil carrier (HCO) increased stability. The levels of 0.05% and 0.1% were unsuitable; the HCO level of 0.2% resulted in much better stability than the lower levels.

3. HCO resulted in slightly better stability than milkfat after three months of storage, but showed no difference in stability after six (37°C) and 12 (22°C) months of storage when little or no antioxidants are used. HCO is used in the subsequent trials.
Introduction

This phase of the project involves the testing of methods of vitamin A fortification to regular or primary type of skimmilk powder (SMP) when produced at the commercial scale. A brief review of the process is given in the following paragraph.

For primary powder production, milk is treated in an evaporator to remove much of the water before the drying process. There are a number of different types of evaporators in use, but regardless of which, the discharged fluid usually contains approximately 40-42% solids. It has probably been in a partial vacuum at a temperature of around 65°C. The condensed milk is then pumped with a high-pressure pump through a small orifice into the drier box. The air temperature in the spray drier is lowered from 200 to 85°C between the inlet and the outlet of the powder. From the drier, the powder is usually sacked or binned until needed and the temperature could be 40 to 50°C for several hours.
Materials and Methods

Addition of the vitamins

The vitamin concentrate was injected into the condensed skimmilk at a point just prior to the high-pressure pump leading to the spray-nozzle of the spray drier. A positive-pressure pump (Bran & Luebbe Inc., Model No. N-P31, Des Plaines, Illinois) was used to inject the vitamin mixture. It was necessary to heat the vitamin concentrate above 50°C (52-53) prior to injection in order to ensure good fluidity since most treatments contained hydrogenated coconut oil (HCO) which is not fluid at room temperature (22-25°C). The vitamin-concentrate injection was performed continuously for at least one hour, during which time, the first one-half hour production was not collected to ensure sufficient time for the powder to travel to the exit and proper equilibration of the system. Spread over the next one-half hour, at least 20 kg of powder was collected by drawing at five minutes intervals from the production line before bagging. Each treatment was done twice at both plants on separate days. In both cases, the dryers were not shut-down between treatments, because they operate continuously for three or four days.

Treatment description

The treatments tested for these trials are listed with a brief description in Table 5. Those formulations were either commercial preparations or lab-made preparations chosen from the most successful Pilot Plant (Phase I) treatments; the commercial preparations and the ingredients for the lab-made preparations were described in Phase I. Treatments E2 and H were additional treatments conducted as follow-up
Table 5. Description of vitamin A fortification treatments for Phase II - Primary Powder.

A - Commercial Vitamin A concentrate (45,400 I.U. Vit. A/ml in hydrogenated coconut oil)
B - Commercial Vitamin A concentrate (50,000 I.U. Vit. A/ml in vegetable oil)
C - 0.1% HCO in the powder with 2000 ppm of a commercial antioxidant mixture in the oil, i.e. 16 mg Ascorbyl palmitate and 5.2 mg α-tocopherol.
D - 0.2% HCO in the powder with 2000 ppm of a commercial antioxidant mixture in the oil, i.e. 32 mg Ascorbyl palmitate and 10.4 mg α-tocopherol.
E₁ - 0.1% HCO in the powder with a commercial stabilized vitamin concentrate containing 5 mg of BHA, 55 mg of BHT and 12.5 mg of α-tocopherol.
E₂ - Repeat of Treatment E₁.
F - 0.2% HCO in the powder with the same vitamin concentrate as in E₁, E₂.
H - Same as Treatment F, plus 2000 ppm of the commercial antioxidant mixture used in Treatments C and D, i.e. 5 mg BHA, 55 mg BHT, 23 mg α-tocopherol and 32 mg ascorbyl palmitate.

Note: Treatments E₂ and H were additional treatments conducted after eight months of storage of the other treatments.
trials from the most promising results obtained after 6 months of storage. The purpose of these additional treatments (E₂ and H) were to confirm treatment E₁ results and to determine the effect of higher levels of antioxidants on vitamin A stability.

Samples from freshly produced powder was obtained from a U.S. manufacturer. This powder was fortified using the same method and vitamin concentrate used for Treatment B in this trial. The results of the vitamin A stability from the U.S.-manufactured powder were not significantly different (P<0.05) than the results for Treatment B; for that reason only Treatment B results will be shown in this paper.

Storage and sampling

The powder samples produced for both Primary and Instant Trials were handled the same way as described for Phase I, i.e., they were transported to the Food Research Institute in Ottawa immediately after production for blending, bagging and storage at 22°C for 12 months and at 37°C for six months. Sub-samples were sent at regular intervals for vitamin A analysis, sensory evaluation and moisture analysis.

Vitamin A analysis

The determination of vitamin A content was conducted by three different laboratories at 0,1,2,3,6,9 and 12 months of storage at 22°C and by one of those laboratories at 0,1,2,3,4 and 6 months of storage at 37°C. Each of the three laboratories used a different method of analysis. In the past, the Carr-Price method (AOAC, 1975; Carr and Price, 1926) has traditionally been used to estimate the Vitamin A content in dried milk products. More recently a spectrofluorometric method
(Thompson et al., 1978, Indyk, 1982) and an HPLC method (Thompson et al., 1980; Woollard and Woolard, 1981; de Vries et al. 1979) have been developed and shown to produce more accuracy than the Carr-Price method. The powder samples stored at 22°C were analyzed by two laboratories (Lab A - HPLC method and Lab C - Spectrofluorometric method). Data from the third laboratory (Lab B - Carr-Price method) was obtained for powder samples from only one plant; this data was used for the comparison of results obtained by the different methods of analysis. The powder samples stored at 37°C were analyzed by only Lab C using the spectrofluorometric method of vitamin A analysis.

**Sensory evaluation**

Haylike flavour usually intensifies with increasing vitamin A destruction which is particularly noticeable and objectionable in a bland product such as skim milk. Sensory evaluation was performed on the reconstituted milk (10 g powder; 90 ml water) by five or six selected panelists who indicated the intensity of the haylike flavour using the following scale:

0 = no haylike flavour  
1 = doubtful  
2 = slight  
3 = moderate  
4 = strong  
5 = extreme

**Moisture analysis**

The moisture content of powder samples for the various treatments was determined by the forced-air-oven method (Agriculture Canada, 1977). This determination was performed immediately after production and at the end of each storage period (6 months at 37°C and 12 months at 22°C). This analysis was performed to ensure that the moisture content of the powder produced for the experiment was within commercially acceptable levels.
Method of graphing

The vitamin A depletion in powder can best be visualized graphically over the storage period. The method of graphing is as follows.

Figures 2 and 3 show the estimated quadratic curves for "the best fit" of vitamin A levels in powders plotted against storage time when stored at 22°C and 37°C respectively. The plotted line for each treatment accounts for the actual vitamin A content of the two replicate productions at two different plants as determined by one, two or three different methods. To obtain those curves, the data was fitted in a regression equation of the form:

\[ y = a + b_1x + b_2x^2 \]

where:
- \( y \) = I.U. of vitamin A
- \( x \) = storage time (month)
- \( a \) = regression intercept
- \( b_1 \) = slope
- \( b_2 \) = quadratic term (curvature)

The regression equations so produced for the curves shown on Figures 2 and 3 are given in Appendix 1. In order to facilitate the visual comparison of individual treatment, it is desirable for all treatments to have the same initial value. For that reason, the regression intercepts (a) in the regression equations were assigned the value of 30 as being the initial vitamin A content for all treatments in Figures 2 and 3, even though the actual regression intercepts were different for each treatment as shown in Appendix 1.
Figure 2. Vitamin A depletion in Primary Skim milk Powder during 12 months of storage at 22°C.
Figure 3. Vitamin A depletion in Primary Skim milk Powder during 6 months of storage at 37°C.
Statistical analysis

Due to the curvilinearity of the relationship between vitamin A content and time of storage (months) it was felt that linearization through data transformation would be useful to compare treatments and establish the reaction order of the vitamin A oxidation. A computer program was used for linearization of data. The super simplex optimization program of Routh et al. (1977) which was modified by Fujii and Nakai (1980) was used to search for the best fit values of A and B to find the highest $r^2$ values in linear regression analysis after data transformation using $t = (y + A)^B$ for linearization. This approach was abandoned when it was found that B value or the reaction order was very different among treatments. Unless a common transformation (B value) could have been found, it is not possible to use this elaborate technique to compare treatment results. Considering that the various treatments contained different types and levels of antioxidants and that antioxidants do affect the oxidation reaction, it is not surprising to obtain different reaction orders for individual treatments.

Due to the difficulty of comparing such a large number of treatments, a Multiple Comparison Analysis using the protected Least Significant Difference (LSD) test was employed (Snedecor and Cochran, 1967) on the estimated slopes from the linear regression equations. The quadratic terms of the second order equation were also treated in the same manner. This procedure is equivalent to using orthogonal polynomials and results in an independent test for linearity and the curvature. (Rowell and Walters, 1976). To study the parallelism of the lines, the results of
both tests were combined. A probable grouping was then subjected to the analysis of variance. The groupings which explained all but an insignificant part of the treatment sum of squares in the analysis of variance are discussed in the results. Note that the protected LSD test is considered to generally err on the side of too many significant differences. This was considered desirable in a screening experiment such as this.

Upon examination of the raw data, it was noted that results of vitamin A analysis from laboratory C were erroneous for powder samples of the additional trials (E2 and H) at time 0 (initial). For that reason, the statistical analysis was performed on the data with missing values at time 0 for treatments E2 and H. To confirm that the results of the Multiple Comparison Analysis (LSD test) were not affected by the omission of the missing values for E2 and H, the same test was performed on the data without time 0 values for all treatments. Ranking and grouping of non-significant treatments remained unchanged for either set of data. Therefore, results of the statistical analysis with missing values for treatments E2 and H are reported.
Results and Discussion

Vitamin A stability

Figures 2 and 3 show the estimated levels of vitamin A remaining in the powder during storage for 12 months at 22°C and for 6 months at 37°C. These plots are from the regression equations "of best fit" for the two trials in each of two plants.

From the statistical analysis, Table 6 lists Partial Regression Coefficients (linear and quadratic) in order of decreasing magnitude for each treatment for the two temperature storage conditions. It illustrates the grouping of non-significant differences among treatments based on the Multiple Comparison Analysis. The Partial Regression Coefficient (PRC) for the linear analysis is the slope and the PRC for the quadratic analysis indicates the degree of curvature.

An overview of the Figures 2 and 3 and Table 6 indicate that the treatments fall into four general levels of increasing stability: C; A; B,D; E₁, E₂,F,H. This grouping resulted in a highly significant difference between group sum of squares and insignificant differences within groups. A brief description of the treatments is as follows: C(0.1%HCO, α-t,AP); A(Commercial); B(Commercial) and D(0.2%HCO, α-t,AP); E₁ and E₂ (0.1%HCO, BHA-BHT-αt), F(0.2%HCO, BHA-BHT-αt) and H(0.2%HCO, BHA-BHT-αt + αt,AP)(see Table 5 for more details).

The results show that the levels of BHA-BHT-α-tocopherol used in treatments E's and F (5mg BHA, 55mg BHT, 12.5mg α-t) gave higher stability than the levels of α-tocopherol and ascorbyl palmitate (AP) contained in treatments C and D. However treatment C contained 15mg AP and 5 mg α-t and
Table 6. Estimated regression coefficients\(^1,\)\(^2\) for Multiple Comparison Analysis (LSD test) for Primary Powder stored at 22 and 37°C.

### 22°C Storage

<table>
<thead>
<tr>
<th>Linear</th>
<th>(\beta_1)</th>
<th>-1.95</th>
<th>-1.04</th>
<th>-.91</th>
<th>-.52</th>
<th>-.47</th>
<th>-.43</th>
<th>-.40</th>
<th>-.39</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>C</td>
<td>A</td>
<td>D</td>
<td>B</td>
<td>H</td>
<td>E(_1)</td>
<td>E(_2)</td>
<td>F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grouping</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>------</td>
<td>------</td>
<td>---</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Quadratic | \(\beta_2\) | 0.153 | 0.088 | 0.068 | 0.042 | 0.041 | 0.011 | 0.000 | 0.007 | .050      |
| Treatment | C   | A    | D    | E\(_1\) | D | F | E\(_2\) | H |
| Grouping | --- | ---  | ---  | --------- | --- | --- | ------ | --- |

**Grouping p<.001**

### 37°C Storage

<table>
<thead>
<tr>
<th>Linear</th>
<th>(\beta_1)</th>
<th>-4.51</th>
<th>-4.14</th>
<th>-1.64</th>
<th>-1.59</th>
<th>-1.33</th>
<th>-1.27</th>
<th>-1.21</th>
<th>-1.21</th>
<th>.84</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>C</td>
<td>A</td>
<td>D</td>
<td>B</td>
<td>H</td>
<td>E(_1)</td>
<td>E(_2)</td>
<td>F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grouping</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>------</td>
<td>------</td>
<td>---</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Quadratic | \(\beta_2\) | 0.476 | 0.437 | 0.236 | 0.215 | 0.087 | 0.001 | -0.042 | -0.073 | .23      |
| Treatment | C   | A    | D    | F    | D | E\(_1\) | E\(_2\) | H |
| Grouping | --- | ---  | ---  | ---  | --- | ------ | ------ | --- |

**Grouping p<.001**

---

\(^1\) Equations for the 22°C storage are of the form \(y=\beta_0+\beta_1x_1+\beta_2x_2\) where \(x_1=0,1,2,3,6,9,12\) and \(x_2=x_1\), \(-11.83x_1=0,10.83,7.83,2.83,24.17,69.17,132.17\). Thus \(\beta_1\) corresponds to the slope of the fitted straight line and \(\beta_2\) the coefficient of the quadratic term in the fitted second order equation.

\(^2\) Equations for the 37°C storage are of the form \(y=\beta_0+\beta_1x_1+\beta_2x_2\) where \(x_1=0,1,2,3,4,6\) and \(x_2=x_1^2-6x_1=0,-5,-8,-9,-8,0\).
treatment D contained double those amounts with 30mg AP and 10mg α-t. The lower level of AP-α-t antioxidants present in treatment D compared to the BHA-BHT-α-t antioxidants level present in treatment F (at the same HCO level) do not permit the comparison of the effectiveness of those antioxidant mixtures. Perhaps equivalent levels of AP-α-t and BHA-BHT-α-t would produce comparable vitamin stability.

The addition of α-tocopherol and ascorbyl palmitate to the BHA-BHT-α-t mixture gave no significant additional improvement in stability of vitamin A (H versus F).

The 0.2% of HCO (D) gave significantly (P<0.05) higher stability than the 0.1% level (C) with α-tocopherol and ascorbyl palmitate, whereas with the BHA-BHT-α-tocopherol antioxidant mixture (E₁, E₂ and F) there was no difference. The Food and Drugs Act of Canada stipulates a maximum level of 0.1% HCO in NDM. Due to this limitation, when excluding treatments containing HCO levels above 0.1% the BHA-BHT-α-tocopherol antioxidants at the levels in treatments E₁, E₂, F and H gave the higher stability at 0.1% HCO in the powder.

In summary, it appears that a vitamin A concentrate in HCO containing BHA, BHT and α-tocopherol at the levels in treatments E₁, E₂, F and H produced the highest stability, being significantly better (P<0.05) than one commercial product (A) and only slightly better than the other commercial product (B). As shown on Table 6, the quadratic analysis at both temperature storages of treatment B is significantly different (P<0.05) than E₂ and H, but the linear analysis did not find significant difference between these treatments. Treatments E₁, E₂, F and H were also
significantly better (P<0.05) than C which contained α-tocopherol and ascorbyl palmitate in 0.1% HCO. However, these same antioxidants used at the higher levels in 0.2% HCO (D) produced a non-significant difference (P<0.05) in stability over E₁,E₂,F and H.

Therefore, it would appear that when utilizing 0.2% HCO with the antioxidants tested in these trials or 0.1% HCO with the BHA-BHT-αt antioxidants combination and level used in these trials, with a level of 30 I.U. of vitamin A per gram present in the powder after production, one could expect to retain approximately 26 I.U./gm when stored under conditions equivalent to 22°C for 12 months. Approximately 23 I.U. can be expected to be retained from these same treatments when stored under conditions equivalent to 37°C for six months.

**Haylike flavour**

Sensory evaluation was used to assess the development of haylike flavour in powder samples during the storage period. On a scale from 0 to 5, 0 being no HLF detected and 5 being extreme HLF, the panelists generally detected increasing levels of HLF during storage. Statistically, the correlation between units of vitamin A losses and HLF was found to be significant at the 1% level for this type of powder (r=0.592 d.f.=82). Those results are consistent with the previous findings of Nakai et al.(1983).

**Comparison of Production Plants**

Table 7 shows the percent vitamin A loss for each treatment from the two different production plants during 12 months of storage at 22°C and 6 months at 37°C. Results indicate that the powder produced at Plant A had
Table 7. Losses of vitamin A in Primary Skimmilk Powder fortified by different treatments in Plants A and B after 12 months of storage at 22°C and 6 months at 37°C.

<table>
<thead>
<tr>
<th>Treatment</th>
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<tr>
<td></td>
<td>22°C</td>
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<td>A</td>
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<tr>
<td>B</td>
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<tr>
<td>C</td>
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<tr>
<td>H</td>
<td>27.7</td>
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</table>
roughly twice the percentage vitamin A loss of Plant B for the same treatments when stored at 22°C for twelve months. The losses were also generally higher for Plant A powders when stored at 37°C for 6 months. However, the difference between the two plants was less marked at the higher temperature storage.

The reasons for the lower losses in Plant B are not immediately apparent. One possibility is the slightly higher temperature in the dryer of Plant B (400°F; 200°F outlet) than in Plant A (290-310°F inlet; 190-195°F outlet; and the difference in the pre-heat treatments, both contributing to greater whey protein denaturation in Plant B powder. The hi-heat powder thereby produced in Plant B would contain more free sulphydryl groups which provides a natural antioxidant effect later in the stored product. This phenomena has previously been recognized to occur in products such as highly-heated milk (UHT) (LeMaguer and Jackson,1983), whole milk powder (Hollender and Tracy,1942; Holm et al.,1926; Mattick et al.,1945; Waite et al.,1947) and in skim milk powder (Pyenson and Tracy,1948). The relevance of this hypothesis to our situation was supported when several powder samples from Plant B were found to contain less than half the undenatured whey protein nitrogen (WPN of 3 versus 7 mg N/g) found in Plant A samples; the lower whey protein nitrogen level indicating the greater extent of denaturation. Further trials would be required to confirm this hypothesis.
Comparison of analytical methods of vitamin A

The statistical analysis of the data indicated that the difference among methods of vitamin A analysis was significant (P<0.05).

Values were tabulated with the data produced from each of the three methods of analysis done on the same powder samples at different laboratories. A ratio of results to be expected among methods of analysis was then calculated to be 0.82: 1.0: 1.16 for the Carr-Price, spectrofluorometric and HPLC methods respectively. A similar ratio was obtained throughout the storage periods and for various vitamin A levels.
Conclusions

1. It was demonstrated in these trials that the use of antioxidants is important to control the oxidation of vitamin A in primary skim milk powder. The antioxidants BHA-BHT-α-tocopherol at levels of 5, 5.5, and 12.5 mg/10^6 I.U. respectively in 0.1 or 0.2% HCO permitted the retention of approximately 80% of the vitamin A when stored at 22°C for 12 months and approximately 70% when stored at 37°C for 6 months.

2. Haylike flavour development was correlated with vitamin A destruction.

3. A large difference in vitamin A stability was observed between production plants. This difference was attributed to the type of dryer used and pre-heat treatment given to the milk prior to spray drying.

4. The three different methods of vitamin A analysis utilized in these trials consistently produced results proportionally different from each other. Their respective degree of accuracy was not determined. The HPLC method consistently produced higher values and the Carr-Price method produced lower values than the spectrofluorometric method.

5. The level of HCO added to the powder was important for vitamin stability when low levels of antioxidants were used. In treatments with properly stabilized vitamins with antioxidants, the higher HCO level (0.2%) did not affect stability.
Phase III-Commercial Plant Trials-Instant Powder

Introduction

Experiments for this phase of the project involved the testing of methods of vitamin A fortification to instant type of skim milk powder (SMP). Trials for this type of powder fortification were carried out simultaneously with the Commercial Primary Powder trials (Phase II) since both types of powder were produced at the same plants. A brief review of the instant powder manufacturing process is given in the following paragraph.

For instant powder production, the instantizers used are a tunnel-like construction where the dry powder travelling in a stream of air is sprayed at its entrance with either a fine jet of water or steam to cause the milk particles to agglomerate with a moisture content of 10-15%. These particles passing through the tunnel or tube structure are subjected to temperatures of 150 to 175°C to reduce the moisture content to 4-5%. They are then sized, with the fines being recycled back to the start of the instantizing process and the rest is bagged.

Trials for this phase of the project were conducted by two different commercial skim milk powder manufacturers. Each treatment was replicated twice at alternating work weeks and plants.
Materials and Methods

Treatment description

The treatments tested for these trials are listed with a brief description in Table 8. Treatments A, H and K are commercial vitamin concentrates, the rest are lab-made formulations containing different combinations of kinds and levels of antioxidants blended in an emulsion of HCO, liquid skimmilk and the vitamins A and D.

Treatment A is a commercial preparation containing vitamins A and D in a base of reconstituted non-fat dry milk and butter oil. Its content of antioxidants was not revealed. This product was part of Phase I trials.

Treatments H and K are a commercial dry beadlet vitamin concentrate containing 250,000 I.U. of vitamin A and 50,000 of vitamin D per gram on a dry basis. This product was not previously tested in Phase I trials. Commercially, this product is added to the primary NDM on a dry basis by metering it to the powder on the way to the instantizer. In this experiment, this product was dissolved in water to make a 20% solution for treatment H and a 10% solution for treatment K. These dilutions caused a five- or ten-fold reduction in vitamin concentration and made it possible to inject this material on a "wet" basis using the same procedure as the other treatments.

Preparation of vitamin mixes

The lab-made preparations (treatments B to J) were prepared by melting HCO at 50°C to which the vitamins and the antioxidants were added. This vitaminized HCO was then added to liquid skim milk to make 6, 12 or 25% mixtures which was subsequently homogenized with 52-55°C with 2500
Table 8. Description of Vitamin A fortification treatments for Phase III - Instant Powder.

A. Commercial vitamin A and D concentrate (45,400 I.U. vitamin A/ml in 6% milkfat)

B. 6% HCO - Skimmilk emulsion with a commercial stabilized vitamin A and D concentrate containing 5 mg BHA, 55 mg BHT and 12.5 mg α-tocopherol per gram.

C. 12% HCO - Skimmilk emulsion with the same commercial stabilized vitamin concentrate as in B.

D. 25% HCO - Skimmilk emulsion with the same commercial stabilized vitamin concentrate as in B and C.

E. 6% HCO - Skimmilk emulsion with vitamins A and D and 2000 ppm of a commercial antioxidant mixture added separately. i.e. 0.6mg AP and 0.2mg α-tocopherol.

F. 12% HCO - Skimmilk emulsion with vitamins A and D and 2000 ppm of a commercial antioxidant mixture added separately. i.e. 1.2mg AP and 0.4mg α-tocopherol.

G. 12% HCO - Skimmilk dispersion (same ingredients as in C, except homogenization was not used during preparation).

H. Commercial Beadlets dissolved in water to make a 20% solution.

J 1/ - 12% HCO - Skimmilk emulsion with the same ingredients as in C, but with lower vitamin concentration to inject four times more HCO into the powder.

K 1/ - Commercial Beadlets dissolved in water to make a 10% solution.

1/ Treatments J and K were additional treatments conducted after eight months of storage results from other treatments.
and 500 psi of pressure in a laboratory homogenizer. The vitamins added to these preparations were such to create a product of similar vitamin concentration (50,00 I.U. Vitamin A/ml) as the commercial products (treatments A, H and K).

The percentage of oil stated for each treatment indicates the fat content in the vitamin concentrate which unlike primary powder represents a very small addition to the dried powder. In fact, for example, the 12% oil-skim emulsion will add only 0.0067% or 67 ppm oil to the fortified powder.

Treatment G was prepared differently than other lab-made treatments, in that the mixture was not homogenized; the liquid was simply circulated through the homogenizer at very low pressure (circa 100-200 psi). Because this preparation was not homogenized, it was necessary to agitate constantly during injection to prevent the vitamin-containing oil from rising to the surface. The purpose of this treatment was to determine the effect of homogenization on the vitamin stability.

As in Phase II, additional treatments were conducted as follow-up trials based on results obtained after 6 months of storage. They were treatments J and K; J was to evaluate the effect of using more HCO on the vitamin stability and K was a repeat of treatment H (beadlets in water) in a more dilute solution.
Addition of the vitamins

For this type of powder, the vitamin concentrate was injected directly into the instantizing chamber through an opening at the middle top section of the cylinder. A small pulsating pump (Waltham Chemical Pump Model No. 10611-361) was used to transfer the material through a nozzle into the instantizer. This pump has the capacity to deliver small amounts of liquid (range 12 to 80 ml/min) with a maximum number of strokes per minute which produces a more constant flow for an even distribution of vitamins to the powder. This method of injection was used in both plants for their normal operation. The use of a different pump was the only modification to the existing system. The powder sample collection was conducted the same way as described for Primary Powder; vitamin concentrate was injected for one hour and 20 Kg of powder was collected directly from the bagging line, during the last half-hour.

Storage and sampling - see Phase II

Vitamin A analysis - see Phase II

Sensory evaluation - see Phase II

Statistical analysis

The same method of analysis was used as for the primary powder trials - Phase II ie. Multiple Comparison Analysis (LSD) (see Phase II for description).

As observed in Phase II trials, results of vitamin A analysis from laboratory C were also erroneous for the additional trials (treatments J and K) at time 0 (initial level). Again, the Multiple Comparison
Analysis was performed on the data with missing values at time 0 for treatments J and K and on the data omitting time 0 values for all treatments. However, in this case the ranking and grouping of non-significant treatments remained unchanged for the two sets of data of the 22°C storage but was different for the 37°C storage. The omission of time 0 values of laboratory C has a more important effect on the 37°C storage temperature. Therefore an adjustment based on slopes and quadratic terms differences between the two sets of data was executed on the statistical results of treatments J and K for the 37°C storage. The adjustment is reflected in the results discussed later.
Results and Discussion

Vitamin A stability

Figures 4 and 5 show the estimated levels of vitamin A remaining in the powder during storage for 12 months at 22°C and 6 months at 37°C. As for the primary powder trials (Phase II), these plots are from the regression equations obtained from the data of two duplicate trials in each of two plants. Appendix 2 lists the regression equations for each treatment at the two temperature storages.

From the statistical analysis, Table 9 lists both Partial Regression Coefficients (linear & quadratic) in order of decreasing magnitude for each treatment at the two temperature storage conditions. This table also illustrates the grouping of non-significant differences among treatments based on the Multiple Comparison Analysis. The Partial Regression Coefficient ($\beta_2$) analysis indicate the degree of curvature.

For this type of powder, Figures 4 and 5 show that only slight differences were found among treatments, especially at 22°C storage. The statistical analysis determining the grouping of non-significant difference among treatments shown on Table 9 supports this observation. Only treatment A (Commercial milkfat emulsion) was shown to be significantly ($P<0.05$) different than all other treatments and this only at the 22°C storage. The statistical analysis of the quadratic components revealed no significant differences ($P>0.05$) among treatments for the 22°C storage which indicate that one can rely solely on the results of the linear analysis for the evaluation of treatments at that temperature.
Figure 4. Vitamin A depletion in Instant Skim milk Powder during 12 months of storage at 22°C.
Figure 5. Vitamin A depletion in Instant Skim milk Powder during 6 months of storage at 37°C.
Table 9. Estimated regression coefficients\(^1\) for Multiple Comparison Analysis (LSD) for Instant Powder stored at 22\(^\circ\)C and 37\(^\circ\)C.

### 22\(^\circ\)C Storage

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### 37\(^\circ\)C Storage

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Equations for the 22\(^\circ\)C storage are of the form \(y = \beta_0 + \beta_1X_1 + \beta_2X_2\) where \(X_1 = 0,1,2,3,6,9,12\) and \(X_2 = X_1^2 -11.83X_1 = 0,-10.83,-7.83,-2.83,24.17,69.17,132.17\). Thus \(\beta_1\) corresponds to the slope of the fitted straight line and \(\beta_2\) the coefficient of the quadratic term in the fitted second order equation.

Equations for the 37\(^\circ\)C storage are of the form \(y = \beta_0 + \beta_1X_1 + \beta_2X_2\) where \(X_1 = 0,1,2,3,4,6\) and \(X_2 = X_1^2 -6X_1 = 0,-5,-8,-9,-8,0\).
In overview, the treatments containing higher HCO and/or the antioxidant mixture of α-tocopherol and ascorbyl palmitate produced the highest stability during both temperature storages. Those treatments were J(4×HCO,BHA-BHT-αt), D(25%HCO,BHA-BHT-αt), E(6%HCO, αt,AP) and F(12%HCO,αt,AP) (see Table 8 for description of treatments).

To assess the effect of HCO on vitamin stability one must examine the results of treatments B, C, D and J which contained increasing levels of HCO with the same types and level of antioxidants(5mg BHA,55mg BHT,12.5mg α-t). The statistical analysis shown in Table 9 ranked those treatments as higher in stability with increasing HCO levels for both storage temperatures. This distinction is also noticeable in Figures 4 and 5.

There was generally no significant difference among HCO levels at 22°C but the treatment with 25% HCO level (treatment J) showed significantly higher stability than the 6 and 12% HCO emulsions (B and C) when stored at 37°C.

To evaluate the effect of different types of antioxidants, treatment B(6%HCO,BHA-BHT-αt) is compared with E(6%HCO,αt,AP) and C(12%HCO,BHA-BHT-αt) against F (12%HCO,αt,AP). The results of the linear statistical analysis show that at 37°C, treatments E and F produced significantly (P<0.05) higher stability than treatments B and C. Although those treatments were not found to be significantly different (P<0.05) for the 22°C storage, however treatments E and F were ranked with slightly higher stability than treatments B and C. Based on these results, we may conclude that the commercial antioxidant mixture containing α-tocopherol and ascorbyl palmitate was more effective than the BHA-BHT-α-tocopherol
mixture for stabilizing the powder especially when used in the lower HCO emulsion (6%). Only a slight difference was found between the two mixes of antioxidants in the 12% HCO emulsion for the two temperature storages. Considering that the antioxidant levels present in treatments E and F (0.6mg AP, 0.2mg α-t and 1.2mg AP, 0.4mg α-t), were much lower than the level used in treatments B and C (5mg BHA, 55mg BHT and 1.2mg α-t) it may be concluded that ascorbyl palmitate is required in smaller quantities than BHA-BHT to stabilize instantized powder.

Treatments H and K were Commercial Beadlets dissolved in water. Results of those treatments were among the poorest for both temperature storages as illustrated on Figures 4 and 5. Dry beadlets have been found by Nakai et al. (1983) to produce a stable powder when it is incorporated by dry blending. The dissolving of this product is water which remove the protective gelatin coating over the oil droplets is likely the reason for its lower stability.

Another parameter which was studied in these trials relates to the effect of homogenization of the emulsion (HCO-Skimmilk) on vitamin stability. To evaluate the effect of this process, treatment C is compared against G; both contain the same ingredients but G was prepared prepared without homogenization. The statistical analysis, both linear and quadratic for the two temperature storages did not find significant (P<0.05) differences between these two treatments. However, a vitamin concentrate prepared without proper homogenization produces a very weak emulsion which requires constant stirring during injection to prevent separation.
Therefore, based on these trials, it would appear that when using treatment J (4xHCO,BHA-BHT-at) to fortify skimmilk powder with vitamin A, if the powder contains 30 I.U. of vitamin A per gram after production, one could expect to retain approximately 23 I.U./gm when stored under conditions equivalent to 22°C for 12 months. About 21 I.U./gm can be expected to be retained when stored under conditions equivalent to 37°C for six months.

### Haylike flavour

The results of the sensory evaluation for this type of powder indicated that the correlation between haylike flavour and units of vitamin A losses was significant at the 5% level. \( r=0.194, \text{d.f.}=111 \) On a scale of 0 to 5, haylike flavour being 0=no haylike flavour; 1=doubtful; 2=slight; 3=moderate; 4=strong; 5=extreme, panelists judged that approximately 9.5 I.U. of vitamin A had to be destroyed before a powder sample was labelled doubtful(1) in haylike flavour. Only an additional 2.5 I.U. had to be lost before the powder was judged slight (2) in haylike flavour. However, despite the relatively large number of samples loosing higher amounts of vitamin A, only a few were judged as high as "moderate" in haylike flavour and none were strong or extreme in that flavour.

### Comparison of production plants

Table 10 shows the percent vitamin A losses for each treatment from two different production plants during 12 months of storage at 22°C and 6 months at 37°C. Results of vitamin A losses for the same treatments were very similar with variations generally lower than 20% between plants.
Table 10. Losses of vitamin A in Instant Skimmilk Powder fortified by different treatments in Plants A and B after 12 months of storage at 22°C and 6 months at 37°C.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Percent vitamin A loss</th>
<th>22°C</th>
<th>37°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Plant A</td>
<td>Plant B</td>
</tr>
<tr>
<td>A</td>
<td></td>
<td>43.4</td>
<td>55.5</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td>44.3</td>
<td>42.9</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td>40.2</td>
<td>29.6</td>
</tr>
<tr>
<td>D</td>
<td></td>
<td>39.9</td>
<td>22.3</td>
</tr>
<tr>
<td>E</td>
<td></td>
<td>48.8</td>
<td>50.4</td>
</tr>
<tr>
<td>F</td>
<td></td>
<td>57.4</td>
<td>55.7</td>
</tr>
<tr>
<td>G</td>
<td></td>
<td>36.2</td>
<td>35.2</td>
</tr>
<tr>
<td>H</td>
<td></td>
<td>27.7</td>
<td>26.1</td>
</tr>
<tr>
<td>J</td>
<td></td>
<td>22.4</td>
<td>16.7</td>
</tr>
<tr>
<td>K</td>
<td></td>
<td>42.6</td>
<td>50.2</td>
</tr>
</tbody>
</table>

1/ Values are means of two replicate trials analyzed by two laboratories for 22°C and by one laboratory for 37°C storage.
under both storage conditions. In fact an analysis of variance of the data indicated that no significant difference existed between the two plants.

Those results are different than for the primary powder trials (Phase II) in which powder produced at Plant B resulted in significantly higher vitamin A stability during storage. The reason for this change in stability between plants for instant type of powder is not obvious. Perhaps an explanation for this discrepancy lies in the difference in micro-environments of the oil droplets containing the vitamins and antioxidants for the two types of powder. Presumably, during spray drying of fortified condensed milk, the vitamin containing oil would be incorporated within powder particles which would provide some protection to the vitamins against oxidation by restricting oxygen access and being in close contact with the protein component of the milk. As previously suggested, highly-heated milk would have higher protein denaturation, hence more free sulphydryl groups acting as antioxidants which would provide close-by vitamins greater protection against oxidation.

For instant powder fortification, the vitamin containing emulsion is sprayed on the agglomerated powder particles and the vitamin-containing oil droplets will probably adhere to the exterior of the agglomerates. In this case, the vitamins have greater exposure to oxygen and are not surrounded by proteins from the milk as for primary powder; therefore, free sulphydryl groups would have little effects on vitamin stability.
Conclusions

1. These trials demonstrated the positive effect of higher levels of hydrogenated coconut oil on vitamin A stability. The highest HCO level tested (treatment J) limited the vitamin A loss to approximately 20% during 12 months of storage at 22°C and approximately 30% during 6 months at 37°C.

2. The commercial vitamin concentrate containing milkfat as the vitamin carrier in an emulsion form did not produce a stable fortified powder. The poor results of this treatment are consistent with previous work of Nakai et al. (1983).

3. Antioxidants were again shown to be important additives to vitamin concentrates in order to delay vitamin A degradation during storage. Ascorbyl palmitate in conjunction with α-tocopherol was shown to be required in smaller quantities than BHA-BHT to stabilize instantized powder.

4. It was found that when the dry beadlet vitamin concentrate was dissolved in water for the wet method of fortification, this product did not produce a stable powder.

5. Homogenization of the vitamin concentrate did not affect the stability of the added vitamin to powder. However, when only dispersion is used, the need for constant stirring during injection of this material makes it impractical in commercial operations.
6. Hay-like flavour development during storage was again correlated with vitamin A destruction.

7. The large difference in vitamin A stability between production plants in the primary powder trials (Phase II) was not found for the instant type of powder.
OVERALL CONCLUSION

The previously reported poor stability of presently used commercial vitamin concentrate was reproduced in these trials. The "wet" method of fortifying NDM with vitamin A can be effective for both regular and instant types of powder providing sufficient antioxidants and hydrogenated coconut oil are added with the vitamins.

Powder purchased for Food Aid Programs is fortified at a higher vitamin A level than powder intended for Canadian market and is generally submitted to storage conditions conducive to faster vitamin A destruction. Therefore, unless higher levels of antioxidants are used for this powder, greater losses of vitamin A can be expected.
LITERATURE CITED


Holm, G.E., Greenbank, G.R. and Deysher, E.J. 1926. Results of preliminary experiments upon the effect of separating and clarifying and pasteurization of a milk upon the keeping quality of its powder. J. Dairy Sci. 9:512.


Marquardt, H.G. 1979. Distribution of dried skim milk as food relief measure and new findings on vitaminization of dried skim milk. Deutsche Milchwirtschaft 30(4) 118.


Appendix 1 - Quadratic equations for the Primary Powder treatments when stored at 22°C and 37°C.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>22°C</th>
<th>37°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>$y = 27.26 - 2.04x + 0.088x^2$</td>
<td>$y = 29.22 - 6.76x + 0.437x^2$</td>
</tr>
<tr>
<td>B</td>
<td>$y = 20.31 - 1.29x + 0.068x^2$</td>
<td>$y = 20.21 - 3.01x + 0.068x^2$</td>
</tr>
<tr>
<td>C</td>
<td>$y = 28.94 - 3.70x + 0.153x^2$</td>
<td>$y = 31.40 - 7.36x + 0.476x^2$</td>
</tr>
<tr>
<td>D</td>
<td>$y = 27.55 - 1.27x + 0.041x^2$</td>
<td>$y = 28.55 - 2.16x + 0.087x^2$</td>
</tr>
<tr>
<td>E₁</td>
<td>$y = 25.75 - 0.87x + 0.042x^2$</td>
<td>$y = 26.02 - 1.22x + 0.001x^2$</td>
</tr>
<tr>
<td>E₂</td>
<td>$y = 28.55 - 0.47x + 0.011x^2$</td>
<td>$y = 25.69 - 0.98x + 0.215x^2$</td>
</tr>
<tr>
<td>F</td>
<td>$y = 25.78 - 0.32x - 0.001x^2$</td>
<td>$y = 30.11 - 2.41x - 0.042x^2$</td>
</tr>
<tr>
<td>H</td>
<td>$y = 28.69 - 0.52x - 0.007x^2$</td>
<td>$y = 27.07 - 0.81x - 0.073x^2$</td>
</tr>
</tbody>
</table>
Appendix 2 - Regression equations for the Instant Powder treatments during storage at 22°C and 37°C.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Regression Equation 22°C</th>
<th>Regression Equation 37°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>$y = 30.93 - 1.26x - 0.020x^2$</td>
<td>$y = 28.68 - 6.94x + 0.634x^2$</td>
</tr>
<tr>
<td>B</td>
<td>$y = 25.46 - 1.34x + 0.036x^2$</td>
<td>$y = 24.80 - 5.81x + 0.509x^2$</td>
</tr>
<tr>
<td>C</td>
<td>$y = 26.46 - 1.03x + 0.026x^2$</td>
<td>$y = 26.78 - 4.30x + 0.263x^2$</td>
</tr>
<tr>
<td>D</td>
<td>$y = 27.68 - 0.48x - 0.021x^2$</td>
<td>$y = 25.30 - 1.59x - 0.050x^2$</td>
</tr>
<tr>
<td>E</td>
<td>$y = 16.96 - 1.29x + 0.055x^2$</td>
<td>$y = 16.38 - 2.82x + 0.207x^2$</td>
</tr>
<tr>
<td>F</td>
<td>$y = 16.59 - 1.41x + 0.064x^2$</td>
<td>$y = 16.43 - 3.74x + 0.340x^2$</td>
</tr>
<tr>
<td>G</td>
<td>$y = 24.97 - 1.07x + 0.028x^2$</td>
<td>$y = 24.21 - 5.36x + 0.487x^2$</td>
</tr>
<tr>
<td>H</td>
<td>$y = 33.29 - 0.66x - 0.010x^2$</td>
<td>$y = 33.93 - 6.89x + 0.648x^2$</td>
</tr>
<tr>
<td>J</td>
<td>$y = 24.63 - 0.53x + 0.004x^2$</td>
<td>$y = 25.06 - 4.36x + 0.554x^2$</td>
</tr>
<tr>
<td>K</td>
<td>$y = 23.75 - 1.34x + 0.033x^2$</td>
<td>$y = 25.09 - 5.64x + 0.512x^2$</td>
</tr>
</tbody>
</table>