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TECHNICAL REPORT

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PARAMETERS FOR MOISTURE CONTENT FOR STABILIZATION OF FOOD PRODUCTS

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Frank Hollis, Milton Kaplow,

Robert Klose, Joseph Halik

General Foods Corporation,

White Plains, New York

Contract No. DAAG 17-67-C-0098

August 1968

UNITED STATES ARMY
NATICK LABORATORIES
Natick, Massachusetts 01760



Food Laboratory

FL-77

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FOREWORD

The ever changing tactics of modern warfare, both conventional and unconventional, must be supported by effectual systems for assuring adequate supplies of acceptable, nutritious and safe food. These requirements pose formidable problems in supplying a soldier who must eat alone at the end of a long supply line through zones where refrigeration is non-existent. These problems are intensified if the soldier must carry or be air-dropped with his entire food supply for a prolonged period. Provision must also be made for military situations which prevent diversion of time or attention to the preparation of food for consumption. In the design of a food supply for such circumstances the requirements of acceptability, nutrition and safety must be extended to provide for a high level of nutrition per unit weight and bulk, consumption without preparation, and safety or wholesomeness notwithstanding damage to the package as may occur in air-drop.

Except for confectionary bars no item of subsistence in current supply fulfills the cited criteria. A variety of compressed freeze-dried food bars are under development. These are expected to meet the aforementioned criteria but with marginal acceptability due to dryness when consumed directly from the compressed state.

This investigation seeks to explore the feasibility of preserving a variety of foods for military use by adjusting their water activity to levels within the so-called intermediate moisture range. Historically reduced water activity has provided the basis for the preservation of a limited number of foods, such as jams and jellies, partially dried prunes, figs and other fruits, dry sausage and country ham, and a few bakery products. Recently reduced water activity combined with an effective antimycotic to suppress growth of yeast and mold have been exploited extensively for the production of commercial pet foods - the so-called soft-moist pet foods. These products generally contain 25-30 percent moisture and sufficient sugar to depress water activity to around 0.85. Notwithstanding inexpensive and unsophisticated packaging, soft-moist pet foods have achieved an excellent record for stability under market conditions.

The investigation was conducted at the Technical Center of the General Foods Corporation at Tarrytown, New York. The experimental program was under the general guidance of Mr. Frank Hollis with Mr. Milton Kaplow serving as Principal Investigator and Messrs. Robert Klose and Joseph Halik as Collaborators. The investigation was funded under a Project Titled: "Food Processing and Preservation

Techniques" (Number 1M624101D553) through Contract Number DAAG 17-67-C-0098, "Parameters for Moisture Content for Stabilization of Food Products." Project Officer for the U. S. Army Natick Laboratories was Dr. Maxwell C. Brockmann of the Food Laboratory. Alternate Project Officer was Mr. Justin M. Tuomy, also of the Food Laboratory.

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ABSTRACT

The following food items were prepared at water activities (A_w) between 0.71 and 0.81: diced chicken, ground beef, diced carrots, beef stew, barbecued pork and apple pie filling. All items were rated acceptable for consumption at reduced water activity, and acceptability was enhanced by restoration to near-normal moisture levels. No evidence of microbial deterioration developed during 4 months storage at 38°C; however, the apple pie filling exhibited excessive browning and the sauce for the beef stew underwent phase separation. Two general procedures were used to adjust foods to intermediate moisture levels: (1) infiltration of dehydrated, usually freeze dried, food with aqueous glycerol solution containing small amounts of propylene glycol, potassium sorbate (antimycotic), salt, sugar and other seasonings as appropriate and adjusted to desired A_w , and (2) a soak-cook procedure in which the raw food of normal moisture content was equilibrated against a similar but more concentrated solution to yield the desired A_w . Except for the apple pie filling, the latter procedure is preferred.

Statement of the Problem

The objective of this project was to develop and demonstrate procedures for preparing a variety of potential ration components of the "intermediate moisture" type. The moisture range was specified as corresponding to water activities from 0.75 to 0.90 as measured at 25°C. It was further specified that such moisture range should assure wholesomeness and preserve the essential physical, chemical, nutritional and sensory characteristics of the food during storage. The following products were to be investigated:

- a. Diced chicken, white meat only
- b. Coarse ground beef
- c. Diced carrots
- d. Beef stew
- e. Barbecued pork
- f. Apple pie filling

The specifications included the requirements that approved additives could be used to modify water activity and to control adverse changes incident to storage, and also that each food would be treated with an FDA approved antimycotic to suppress mold contamination.

Intermediate moisture products were required to be acceptable after storage for 4 months at 38°C and, following such storage, to be evaluated for (a) moisture content; (b) lipid content; (c) water activity; (d) total plate count; (e) rehydration characteristics; and (f) evidence of deterioration.

An additional requirement was that approximately 500 grams of each product listed above would be supplied to the Project Officer upon completion of Phase I of the project.

Background

We define "intermediate moisture" foods as foods that are partially dehydrated and have a suitable concentration of dissolved solids to bind the remaining water sufficiently to inhibit growth of bacteria, mold and yeast. Such foods are not new. The pemmican of the American Indians was a form of "intermediate moisture" food, and dried fruits, sweetened condensed milk and fruit preserves are more common examples.

However, these are specialized foods, and until recently the "intermediate moisture" category was limited to a few such specialized foods. Lately there has been the development of what is called "soft, moist pet food" (1,2). This product is made by mixing wet and dry ingredients with suitable soluble solids and antimycotics to obtain a product that is moist enough to be eaten without rehydration and yet is resistant to microbiological spoilage. The parameters of moisture content, antimycotics and soluble solids have been studied quite extensively, (3) and there is considerable technology available on this type of "intermediate moisture" food. While this technology can be extended to human foods, it is limited to fabricated products formed from comminuted ingredients. In the six items required for this project, the natural structure and texture of the fresh material was required, and therefore the "soft, moist" technology could not be applied directly. However, the principles related to preservation and food quality could be applied.

Approach to the Problem

An initial approach to the problem was to start with dehydrated pieces of the food and infuse them with the water and additives required for preservation and palatability in the intermediate moisture range (15-25%). Several methods of drying were investigated, and it was found that drying methods other than freeze drying (air, microwave, dielectric, infrared, vacuum with desiccant) all caused shrinkage and toughness in the food.

Freeze dried precooked chicken white meat ($\frac{1}{4}$ - $\frac{1}{2}$ " thick) and carrot pieces ($\frac{3}{8}$ " dices) were partially rehydrated to the intermediate moisture range and evaluated. Freeze-dried and intermediate moisture freeze-dried samples had similar rehydration and eating properties when rehydrated five minutes in water. The "as is" intermediate moisture eating properties were dry and unpalatable. Intermediate moisture samples processed by freeze drying were superior to intermediate moisture samples dried by other methods mentioned above. It was realized that the addition of additives would improve the palatability of all samples, but in most cases freeze-dried starting material would be required.

Since freeze drying is an expensive processing step, it was desired to find some other method of infusion that could be used with raw or full-moisture material. Preliminary tests using raw food and an infusing solution more concentrated than that desired in the final product showed promise, and a method based on this approach was worked out and is described in the next section.

Experimental Procedures and Results

Two workable methods were devised whereby food pieces could be infused while retaining the appearance, texture and flavor of the natural food. The two methods differ primarily in the initial moisture of the food piece being used in the process. It was found that both dehydrated and wet foods could be effectively infused.

One method treats foods that were vacuum-dried or freeze-dried by soaking in an infusing solution to a point where after draining, the food pieces have the proper moisture content and water activity to make them edible without further hydration and to maintain them microbiologically stable at room temperature. It was found that if a dehydrated porous, absorptive food is soaked in a solution whose viscosity is not too high to penetrate, the food will absorb an amount of solution approximately equal to the amount of water it would normally absorb.

The other method consists of soaking a normal moisture food in an equilibration solution so that after draining, the moisture content and the water activity have been reduced to the desired levels to make the food edible without rehydration and to maintain it microbiologically stable at room temperature. The food can be precooked or raw. It was found that raw food can be cooked in the equilibration solution. The solution's composition is based on the "target" formula of the finished product and the amount of water in the food to be treated. The processing formula is based on the assumption that the additives will diffuse into the food to an extent that the final concentration in the food and in the solution will be similar. The amount of solution should be enough to immerse the food. The processing formula can be expressed by the mathematical relationship:

$$C = \frac{W_s C_s}{W_s + W_f}$$

where C = desired concentration of non-aqueous solutes in equilibrated solution

C_s = initial concentration of non-aqueous solutes in external (infusing) solution

W_s = initial weight of above

W_f = initial weight of water in food

An analogy that can be made to help visualize what is occurring during the two methods is to think of the food structure as a sponge type having a liquid holding capacity. In the dry state it absorbs the infusing solution and in the wet state part of the water is "washed out" with another liquid by diffusion.

Early work using carrot pieces in sodium chloride solution followed by chemical analysis showed that a soak-equilibration technique was feasible. Subsequent work involved other ingredients, primarily glycerol. Glycerol alone was too viscous to penetrate into the interior of the food piece (dehydrated apple) but was effective when diluted with water. Glycerol has been found to be the most promising additive to date serving the dual purpose of binding water to reduce activity and to impart moist appearance and texture to the intermediate moisture food. In the six items listed, glycerol was included at levels generally up to 30.0% except for carrot dices and peas and potatoes in the beef stew which were higher. Propylene glycol was generally used at a level of 2.0% and potassium sorbate was used as an antimycotic at a level of 0.3%. Salt and sugar were added where flavor would permit. Flavors and colors could also be included.

For every item except apple pie filling, product for storage evaluation was prepared by equilibrating raw normal moisture food pieces in a soaking solution in which the food pieces were also cooked. This "cook-soak equilibration" method is considered to be the most economically feasible, eliminating the need to pre-dry the food pieces. Although this method results in excess solution, it is assumed that this can be reused.

I. Specific Item Formulation and Method of Preparation Follow

A. Diced Chicken White Meat Only

Shelf stable intermediate moisture diced white meat of chicken, $\frac{1}{2}$ -1" pieces were prepared by soak infusion of pre-cooked freeze-dried pieces and by soak equilibration of raw fresh pieces in combination with cooking. Composition of solutions used and of the products resulting from these methods are shown in Tables I and II in the Appendix.

After soaking freeze-dried chicken pieces overnight in excess solution and draining, weight percentage of solution infused (65.7%) was found to be close to percentage of original water content (62). Lesser amounts of liquid can be infused (short of saturation) by regulating soaking ratio of freeze-dried chicken to solution. A higher chicken solid product might be more desirable as a compact, concentrated field ration.

The "cook-soak equilibration" method was applied to preparing product for use in two storage studies (Table II). The later storage study product represents an improvement in texture (more tender) due to cooking at 96-99 °C. for

fifteen minutes, instead of at 234°F. for fifteen minutes, in flavor due to 3.3% salt in place of 2.2% and due to less surface drying after draining. Samples were prepared by immersing raw chicken pieces in solution, heating to cooking temperature and cooking for fifteen minutes, cooling, soaking in same solution overnight under refrigeration, draining and drying on paper towels. The final products were palatable and had acceptable water activities and moisture contents.

B. Coarse Ground Beef

Shelf stable intermediate moisture coarse ground beef was prepared for storage evaluation by applying the cook-soak equilibration method to fresh raw chuck hamburger. Hamburger was immersed, heated to cooking temperature and cooked at approximately 99°C. for fifteen minutes in a solution calculated to infuse the proper amount of additives. The mixture was then cooled, soaked overnight under refrigeration and reheated. The cooked intermediate moisture coarse ground beef was drained and dried on paper towels. The final product was palatable and had acceptable moisture content and water activity. Ingredients used are shown in Table III.

C. Diced Carrots

Shelf stable intermediate moisture diced carrots (3/8") were prepared by soak infusion of precooked freeze-dried pieces and puff-dried pieces, by soak equilibration of normal moisture cooked carrot pieces and by soak equilibration of raw, fresh pieces in combination with cooking. The latter "cook-soak equilibration" method was used to prepare product for use in storage evaluation. Raw carrot pieces were immersed in solution, heated to approximately 99°C., cooked at this temperature for fifteen minutes, cooled, soaked in same solution overnight under refrigeration, drained and dried on paper towels. The resulting product was palatable and had acceptable moisture content and water activity. Ingredients used in infusion and equilibration methods are shown in Tables IV, V and VI.

After soaking overnight in excess solution and draining, freeze dried carrot pieces contained appreciably more liquid than puffed dried pieces. It is of interest to note that the weight percentage of solution infused (93.7) into freeze-dried pieces is close to the percentage of original water content (92) of carrot. Also of interest is the high carrot

solids content (34.2%) of the "ready to eat" infused puffed-dried carrot dices. This food form could possibly be more desirable for field conditions requiring compact, concentrated food rations.

D. Beef Stew

Shelf stable intermediate moisture beef stew was in retrospect the most difficult item to make. The vegetable and meat component and the gravy component were developed separately and combined. Interactions between the components were considered in formulating. The "ready to eat" gravy was difficult to make because of the large amount of water to be removed and difficulties in emulsion stability.

Early beef stew feasibility prototype work covered various unsatisfactory techniques. An early formulation (#5699-74, 23% moisture, 5% glycerol, .84 target Aw) was prepared using dehydrated vegetables and cooked beef dices (approx. 3/8") containing 40% moisture. The mechanism sought was the penetration of vegetables and meat by water activity lowering additives infused as moisture equilibrated between meat and vegetables. Moist meat was blended with the water activity lowering additives sodium chloride, glycerol, propylene glycol, potassium sorbate and then with dehydrated vegetables. This technique proved unsatisfactory due to poor moisture equalization. At two week refrigerated storage, diced potatoes were 18% moisture and diced beef was 30% moisture.

In an attempt to accelerate moisture equalization, another formulation (#5699-82, 20% overall moisture, 0.80 measured Aw) was prepared. To accelerate moisture equalization, an intermediate moisture (18% moisture, 5% glycerol) gravy was blended with the moist meat and dehydrated vegetables. It was thought the gravy would act as a moisture exchange medium to equilibrate the food pieces. The effectiveness of the moisture equalization and degree of infusion was inconclusive. This system in retrospect was inferior in texture and flavor qualities to the later product (prepared by the cook-soak equilibration method) which contains substantially more water and glycerol.

In an attempt to infuse water activity lowering additives more uniformly and effectively, formula #5699-84, 20% moisture, 5.0% glycerol, 0.77 measured Aw, was prepared using freeze-dried diced beef in place of 40% moisture diced beef.

A solution including the water activity lowering additives was sprayed onto the surfaces of the dried vegetable-meat blend. This type of preparation appeared promising but involved a pre-drying step while the cook-soak equilibration method does not.

1. Meat and Vegetables

The shelf stable intermediate moisture meat and vegetables were prepared for the beef stew item storage study by the cook-soak equilibration method and stored with and without intermediate moisture gravy.

The individual fresh, raw meat and vegetable components (cubed chuck, diced potatoes, diced carrots, and peas) were immersed in solution, heated to approximately 99°C. and cooked at this temperature for 10-15 minutes, cooled, refrigerated overnight in the same liquid, drained and surfaced dried on paper towels. At this point, the meat and vegetables were blended and part was mixed with an intermediate moisture gravy. The ingredients used are shown in Tables VII, VIII, IX, and X.

2. Gravy & Combination

Prior to serving, a gravy can be added to the intermediate moisture beef; vegetable blend made without gravy.

The second half of the beef stew storage study included an intermediate moisture gravy in combination with the intermediate moisture beef, vegetable blend.

Commercial gravy powder when reconstituted as per package directions (1 oz. powder and 1 cup water) contains 90% water and 10% solids. In order to formulate an intermediate moisture "ready to eat" gravy, most of the water was replaced by combinations of solids, fat, oil and glycerol. Initial feasibility formulations containing 67% vegetable oil, 23% water and 10% gravy powder had thin consistencies, poor emulsion stability with no improvement from starches, CMC, Tween 60 or Span 60. The use of a spray-dried coffee lightener improved emulsion stability in a low glycerol (5%), 19% moisture system having a pasty appearing consistency. A gravy system considered promising consisted of thickened starch with most of the water replaced by Frodex, Dri Fri and glycerol. The formula for this gravy is given in Table XI.

Gravy was prepared by combining Frodex, soup base flavor, starch and water and mixing in a blender, adding melted myverol, glycerol and propylene glycol, mixing, adding Dri Fri and mixing. The gravy was combined with meat and vegetables for the storage study evaluation. The formula is shown in Table XII.

E. Barbecued Pork

Shelf stable intermediate moisture barbecued pork pieces ($\frac{1}{2}$ - 1") were prepared for storage evaluation by applying the cook-soak equilibration method.

1. Pork Pieces

Fresh, raw pork loin pieces, trimmed of fat were immersed, heated to cooking temperature and cooked at approximately 99°C for fifteen minutes in a solution calculated to infuse the proper amount of additives. The mixture was then cooled, soaked overnight under refrigeration and reheated. The cooked intermediate moisture pork pieces were drained and dried on paper towels. The formula for the equilibration solution is shown in Table XIII.

2. Sauce & Combination

To complete the intermediate moisture pork pieces into a barbecued pork, an intermediate moisture sauce was mixed with the pieces in a ratio of 85% pork and 15% sauce. The sauce was formulated to a calculated water activity of .815. Actual water activity measurement by instrument (hygrometer) was not possible due to volatilization of acetic acid contained in sauce. The combined sauce and pork had 25% moisture (vacuum oven method) and was palatable. The formula for the sauce is shown in Table XIV.

F. Apple Pie Filling

Shelf stable intermediate moisture apple pie filling formulations were developed separately for apple slices and for apple pie matrix.

1. Apple Slices

Dried apple slices are known to be stable at 24% moisture but are dry and hard for eating as is. Water activities of 24, 30, 35% moisture apple pastes were measured as

.73, .81 and .84, respectively. These results show that apples are naturally high enough in soluble solids so that at intermediate moisture levels the water activities are in a range low enough to inhibit microbiological growth. To improve the eating quality, a solution of glycerol and sucrose was infused into the 35% moisture pieces along with propylene glycol and potassium sorbate and sodium meta-bisulfite to reduce browning. The formula shown in Table XV was used for preparation of intermediate moisture apple pieces as used in the storage study evaluation.

2. Apple Pie Matrix

Ingredients and levels in previous General Foods project formulations on dehydrated apple pie exploration were referred to for background information.

The water activity, percent moisture and additive levels of the intermediate moisture matrix were made similar to that of the apple slices. It was thought that this would minimize water activity changes and ingredient transfer between blended, stored components with the probable end result of improved storage stability.

Accordingly the water, glycerol, propylene glycol and potassium sorbate levels were made close to the levels in the intermediate moisture apple slices. The addition of sucrose as the main ingredient did not lower the water activity sufficiently, necessitating use of lower molecular weight dextrose. Starches were added as thickeners, Frodex and sucrose as fillers and to extend sweetness, spices and salt were added for flavoring. The formula shown in Table XVI was used in the storage study evaluation.

Matrix was prepared by blending dry ingredients, adding water, mixing, heating to 71°C. for two minutes, adding glycerol and propylene glycol and mixing.

3. Intermediate Moisture Combined Apple Pie Filling and Recipe

The intermediate moisture apple pieces and matrix were blended for the storage study evaluation in the proportions shown in Table XVII.

The pie filling had good eating qualities, acceptable water activity and moisture content. The pie filling was intended to be eaten "as is" or to be diluted with water and baked in a pie. For the latter, 382 grams of intermediate moisture filling and one cup of water were blended and heated in a saucepan to gelatinize the starch to give filling for one 8-or 9-inch pie having the following liquid composition:

	<u>%</u> <u>Water</u>	<u>%</u> <u>Glycerol</u>
Before recipe water addition	26	14
After recipe water addition	66	6

4. Browning in Storage

Intermediate moisture apple pie filling #5850-54 browned at 38°C. storage. Subsequent formulation changes and storage evaluation showed that browning is intensified by addition of ascorbic acid and/or EDTA and can be reduced by removing sodium chloride and dextrose from the matrix formula.

A cook-soak infusion of fresh and dried apple pieces to achieve complete saturation was found to be effective in completely eliminating browning. Resulting product had undesirable tastes attributed to the higher amount of glycerol infused. Total pie filling formula contained 43.0% moisture and 27% glycerol.

The product having the formula shown in Table XVIII has been in storage at 38°C. for two months with no browning. Taste is not satisfactory but indicates that browning can be controlled.

Product was prepared by cooking 15 minutes at 88-93 °C a combination of apple pieces and a solution of water, glycerol, propylene glycol, potassium sorbate and sodium meta bisulfite. Drained excess solution was then blended with formulation pre-mix of sugar, starch and spices to form matrix which was combined with drained apple pieces to form complete pie filling.

II. Storage Study

For the storage test, each food sample was filled into a size

211 x 400 tinplate can which was hermetically sealed under a nitrogen atmosphere after evacuation of the air in the can. Canned samples were stored at 0°C. and at 38°C. for four months. After four months, the samples were removed from storage and evaluated. Following are the results of the evaluations:

A. Moisture, Aw and Lipid Content

Moisture (4,5), Aw (6) and lipid (7,8) contents for the six items are shown in Table XIX.

There were no overall moisture or water activity changes in any of the items. In a multi-component system there seems to occur an equilibration of water activities between the components such as in the beef stew without gravy item. A water activity equilibration to 0.75 with corresponding moisture decrease or increase occurred in beef, carrot and potato pieces having initial water activities of .77, .71 and .80. Peas, the exception, remained at .80 water activity possibly due to the outer surface skin.

B. Microbiological Stability

Initial counts and counts after four months storage (at 38°C.) for bacteria, mold and yeast are shown in Table XX. The methods employed to evaluate microbiological stability are given in the Appendix.

From the data in Table XX, it can be concluded that all six items were resistant to the growth of bacteria, mold and yeast. Also, there was no detection of thermophiles, coliforms, E. coli, staph, strep, fecal strep and salmonella in any of the items.

C. Gross Examination

1. Diced Chicken

First storage study - Formula #5850-56; dry, tough and sweet. 38°C. sample slightly redder.

Second storage study (3 Mos.) - Formula #5856-53; retention of moist, tender, reduced sweetness characteristics. 38°C. sample slightly redder.

2. Coarse Ground Beef

Little or no change. 38°C. sample slightly ^{redder} ~~redder~~ color.

3. Diced Carrots

Little or no change. 38°C. sample slightly darker color.

4. Beef Stew

At 38°C., gravy emulsion unstable, meat slightly redder, carrots and peas slightly lighter color.

5. Barbecued Pork

Little or no change. 38°C. sample slightly redder.

6. Apple Pie Filling

Browning at 38°C., appearing at 2 weeks' storage and intensifying with longer storage.

D. Sensory Evaluation

Sensory evaluation for palatability was conducted to compare differences in flavor and texture between the intermediate moisture stored items at both 0°C. and 38°C. and commercial products (grocery store canned items or freshly prepared full moisture items). Intermediate moisture samples were evaluated "as is" and after rehydration. Sensory evaluation has been completed on all items except the second storage study samples of chicken, which have only been in storage for two months.

The procedure was to have a panel of four judges, trained in and experienced with the flavor and texture evaluations of food products, evaluate the intermediate moisture items on a 10-point rating scale of difference. The commercial products were considered as controls and assigned a 10 rating. The panel compared rehydrated 0°C. samples and both unrehydrated and rehydrated samples at 38°C. to the commercial product controls and to the "as is" items stored at 0°C. in order to determine the relative difference among the samples within each product type. In addition to the difference in ratings, flavor, texture and mouth feel characteristics were described.

The sensory evaluation ratings for all items are summarized in Table XXI. All the stored samples of the intermediate

moisture items received scores of 5 or better. For all products, rehydration improved the ratings in the direction of the commercial products because of decreased sweet and bitter notes and more moist and softer textures.

Relative differences among samples within each product type follow:

1. Chicken Pieces

Of the chicken formulations (unrehydrated, 0°C.), formula 5850-56 (storage study 1,4 months old) was rated at 5.1 and formula 5856-53 (storage study 2,2 months old) was rated 6.6 because of texture differences. Formula 5850-56 was drier, harder and more rubbery. Upon rehydration, both of these samples rated closer to the commercial chicken because of softer, more moist texture (formula 5850-56 rated 7.4, formula 5856-53, 7.7).

Commercial control was the white meat part of a Swift's Premium canned whole chicken. Rehydrated intermediate moisture pieces were prepared by soaking in excess hot water for 8 minutes and draining.

2. Coarse Ground Beef

The 0°C. unrehydrated beef was rated fairly close to the commercial beef (8.1). Rehydration further improved the ratings to 9.1 and 9.6 for the 38°C. and 0°C. samples respectively. The 38°C. unrehydrated beef was rated most different from the commercial beef (7.1).

Full moisture control was a freshly prepared butter sauteed ground chuck hamburger. Rehydrated intermediate moisture ground beef was prepared by soaking in excess hot water for 8 minutes and draining.

3. Diced Carrots

The 0°C. unrehydrated diced carrots were rated 6.6. Rehydration improved the ratings to 7.9 and 8.0 for the 0°C. and 38°C. samples respectively. The 38°C. unrehydrated diced carrots were rated most different from the commercial canned carrots (6.1).

Commercial control was Richmond canned diced carrots distributed by First National Stores, Inc. Rehydrated intermediate moisture pieces were prepared by soaking in excess

hot water for 8 minutes and draining.

4. Beef Stew

Two samples of beef stew were stored and evaluated. Sample #5492-73 was a beef stew with an intermediate moisture gravy. It was evaluated "as is", and was rated 6.3 and 5.8 for the 0°C. and 38°C. samples, respectively. Sample #5492-74 was a beef stew without gravy, and it was evaluated two ways. Rehydration method I used a reconstituted dry mix gravy added to the beef stew. This product was rated 7.1 and 6.9 for the 0°C. and 38°C. storage samples, respectively. Rehydration method II consisted of soaking the beef stew in hot water for 8 minutes, draining and then adding the reconstituted dry mix gravy. This sample was rated higher than method I, having 7.7 and 7.3 for the 0°C. and 38°C. samples respectively.

The commercial control sample was Dinty Moore canned beef stew.

5. Barbecued Pork

The 0°C. unrehydrated pork rated 7.9 while the 38°C. un-rehydrated pork rated 7.2 because of its harder texture.

Two rehydration methods were used and both had some improvement in the 38°C. sample ratings. Rehydration method I, soaking in excess hot water for 8 minutes, draining and mixing with 15% level of Open Pit Barbecue Sauce, had no effect on the 0°C. sample rating. Rehydration method II, soaking 7 parts intermediate moisture barbecued pork with 3 parts warm water with no draining improved the 0°C. sample rating (8.3).

6. Apple Pie Filling

The 0°C. unrehydrated apple pie filling was rated 5.9. Rehydration improved the ratings to 6.2 and 7.6 for the 38°C. and 0°C. samples, respectively. The 38°C. unrehydrated apple pie filling was rated most different from the commercial apple pie filling (5.1).

Commercial control was Comstock canned apple pie filling. Rehydrated intermediate moisture pieces were prepared by combining 6.2 parts apple pie filling and 3.8 parts warm tap water and heating to gelatinize starch.

Discussion

The soak-infusion of dried foods and cook-soak-equilibration of raw foods as methods of preparing intermediate moisture products which were worked out on this project and are described in the Results Section of this report, were used successfully to prepare the six items specified. The work on this project was exploratory and the emphasis was on preparing the six food items rather than on perfecting the method and its parameters.

Two problem areas that need further work were the browning encountered in the stored samples of the apple pie filling and the instability of the gravy emulsion in the beef stew. As Reported in the Experimental Procedures and Results Section, possible solutions to these problems were indicated and additional work could provide satisfactory products.

Although the results show that the products were microbiologically stable and fairly acceptable to the sensory panel, there is room for improvement in quality of the products. One area of improvement is in the optimum ratio of water and glycerol content as related to water activity. There is the possibility in some items of having more water and less glycerol, with a resulting higher A_w , but still not so high as to risk microbiological spoilage. There is also the possibility of having less water and glycerol with the same A_w . The reduction in glycerol might improve the flavor, but the resulting increase in food solids might adversely affect texture.

Another area for improvement is in the levels of propylene glycol and potassium sorbate. We used levels of 2.0% propylene glycol and 0.3% sorbate in most of the products, because our experience was that such levels would prevent spoilage. With the generally low A_w 's we obtained, it is possible that lower levels of glycol and sorbate would be effective, with a resulting improvement in flavor.

Conclusions and Recommendations

The following six items, as specified in the contract, were prepared as intermediate moisture products: (a) diced chicken; (b) coarse ground beef; (c) diced carrots; (d) beef stew; (e) barbecued pork; and (f) apple pie filling.

Two methods of distributing water activity lowering additives and controlling moisture uniformly throughout food pieces were developed in the course of the project and used to prepare the six items.

Samples of the six items were stored for four months at 38°C. On evaluation after storage, all items were found to be microbiologically stable under non-sterile conditions and to require no commercial sterilization. Sensory evaluation of the stored samples showed them to be palatable when eaten "as is" and closer to commercial products when rehydrated. Browning of the apple product and gravy emulsion instability were the two main technological problems, and they were not completely solved.

It is recommended that Phase II of the contract be authorized. Phase II calls for extending the procedures developed in Phase I to a series of representative casserole-type items. Such work would involve solution of the gravy emulsion instability problem, because gravies would be required in all the casserole items. Along with formulation of the casseroles, many of the parameters discussed above would be studied in order to obtain products with higher quality.

Summary

The objective of this project was to develop and demonstrate procedures for preparing a variety of potential ration components with a reduced moisture range corresponding to water activities from 0.75 to 0.90 that would assure wholesomeness and preserve essential physical, chemical, nutritional and sensory characteristics during storage.

The following six items specified in the contract were investigated:

- a. Diced chicken; white meat only
- b. Coarse ground beef
- c. Diced carrots
- d. Beef stew
- e. Barbecued pork
- f. Apple pie filling

All the items identified consisted of individual, distinct food pieces which did not lend themselves to the "soft, moist" pet food technology of mixing and grinding together wet and dry materials to achieve the desired final product.

Two methods of infusing food pieces with additives to adjust the water activity were identified during the project. One method consists of infusing a dehydrated food with a solution of water, glycerol and such salts, sugars, flavors and antimycotics as may be required to obtain a stable product with the desired flavor and texture. In the second method the water activity of a normally moist food mass is adjusted by diffusional exchange of the internal moisture with an external

solution having a composition similar to the infusing solution used in the first method. The diffusional process is markedly accelerated when the system is subjected to cooking temperature.

Both methods as well as other intermediate moisture technology were used to prepare satisfactory samples of the six items specified. All six items had water activities between 0.70 and 0.80 and contained approved additives to modify water activity and FDA approved anti-mycotics to suppress mold contamination.

The prepared items were stored for four months at 38°C. and then examined for moisture and lipid contents, water activity, micro-organisms and palatability. All items were microbiologically stable under non-sterile conditions and required no commercial sterilization.

The samples were evaluated after storage by a sensory panel; all samples were rated acceptable. The samples were evaluated "as is" and also after rehydration. The ratings on the rehydrated samples were higher than those for the "as is" samples.

Two technical problems encountered in the project were browning of the apple product during storage and instability of the gravy emulsion in the beef stew. Neither problem was completely solved during the time limit of the project, but progress on these problems indicates that eventual solution is feasible.

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Standard Instruments Employed in Testing and Measurements

Cenco Moisture Balance - Cenco Instruments Corp., 2600 S. Kostner Ave.,
Chicago, Illinois

Electric Hygrometer - Indicator
(Precision Humidity Instrument) - Hygro Dynamics, Inc., Silver Spring,
Maryland

Citation of the above names does not constitute an official
endorsement or approval.

TABLE I

Formula #5699-100 For Soak Infusion of Freeze-Dried Chicken

<u>Pieces ($\frac{1}{2}$-1")</u>	<u>Solution (%)</u>	<u>Est. Final Product (%)</u>
F.D. Chicken Solids	-	34.3
Glycerol	40.2	32.4
Water	42.5	27.8
Good Seasons Chicken Soup Base	6.2	4.1
Propylene Glycol	1.6	1.1
Potassium Sorbate	.5	.3
	<u>100.0</u>	<u>100.0</u>

$A_w = 0.78$

TABLE II

Formulas for Cook-Soak Equilibration of Fresh Raw Chicken
Pieces ($\frac{1}{2}$ -1") Prepared for Storage Evaluation

TABLE II-A

Analysis of Chicken Used in Test

Chicken, Raw, Trimmed White Meat	%	Grams	
		#5850-56	#5856-53
Solids	28	798	1680
Moisture	<u>72</u>	<u>2052</u>	<u>4320</u>
	100	2850	6000

TABLE II-B

Infusion Solution for Chicken Pieces #5850-56
(First Storage Study)

	%	Grams
		#5850-56
Glycerol	71.5	3344.0
Water	16.7	780.9
Good Seasons Chicken Soup Base	8.9	418.0
Propylene Glycol	2.2	104.5
Potassium Sorbate	<u>.7</u>	<u>31.4</u>
	100.0	4678.8

Moisture Content = 27.0% (Vacuum-Oven Method)

Aw = 0.73

TABLE II-C

Infusion Solution for Chicken Pieces #5856-53
(Second Storage Study*)

	<u>%</u>	<u>Grams</u> <u>#5856-53</u>
Glycerol	68.8	6774
Water	16.7	1638
Good Seasons Chicken Soup Base	8.9	876
Sodium Chloride	2.8	276
Propylene Glycol	2.2	216
Potassium Sorbate	.6	60
	<u>100.0</u>	<u>9840</u>

Moisture Content = 26.0% (Calculated)

Aw = 0.73

*The chicken prepared by this method is still in storage.

TABLE III

Formula #5850-60 for Cook-Soak Equilibration of Coarse Ground Beef
Prepared for Storage Evaluation

<u>Beef, Ground Chuck</u>	<u>%</u>	<u>Grams</u>
Solids	39.0	2655.9
Moisture	61.0	4154.1
	<u>100.0</u>	<u>6810.0</u>

Infusion Solution for Coarse Ground Beef

	<u>%</u>	<u>Grams</u>
Glycerol	65.61	5916.0
Water	15.61	1407.6
Good Seasons Beef Soup Base	11.92	1074.4
Propylene Glycol	5.96	537.2
Potassium Sorbate	0.90	81.6
	<u>100.00</u>	<u>9016.8</u>

Moisture Content = 37.00 (Vacuum - Oven Method)

Aw = 0.78

TABLE IV

Formula #5856-1 for Soak-Infusion of Freeze-Dried Carrots Dices (3/8")

	<u>Solution</u> <u>(%)</u>	<u>Est.</u> <u>Final Product</u> <u>(%)</u>
Glycerol	54.4	51.1
Water	42.0	39.3
F.D. Carrot Solids	-	6.3
Sodium Chloride	2.2	2.1
Propylene Glycol	1.1	.9
Potassium Sorbate	.3	.3
	<u>100.0</u>	<u>100.0</u>

Aw = 0.77

TABLE V

Formula #5856-4 for Soak Infusion of Puffed-Dried Carrot Dices (3/8")

	<u>Solution</u> <u>(%)</u>	<u>Est.</u> <u>Final Product</u> <u>(%)</u>
Glycerol	54.4	35.9
P.D. Carrot Solids (C.V.C.)	-	34.2
Water	42.0	27.5
Sodium Chloride	2.2	1.5
Propylene Glycol	1.1	.7
Potassium Sorbate	.3	.2
	<u>100.0</u>	<u>100.0</u>

Aw = 0.76

TABLE VI

Formula #5850-58 for Cook-Soak Equilibration of Fresh Raw Carrot
Dices (3/8") Prepared for Storage Evaluation

<u>Carrots, Peeled, Trimmed</u>	<u>%</u>	<u>Grams</u>
Solids	11.0	632.5
Moisture	<u>89.0</u>	<u>5117.5</u>
	100.0	5750.0

Infusion Solution

Glycerol	88.70	7256.5
Water	5.54	453.1
Sodium Chloride	3.66	299.0
Propylene Glycol	1.57	128.8
Potassium Sorbate	<u>0.53</u>	<u>43.7</u>
	100.00	8181.1

Moisture Content = 37.5 (Vacuum - Oven Method)

Aw = 0.74

TABLE VII

Formula #5492-67 for Cook-Soak Equilibration of Fresh Raw Carrot
Dices (1/4-1/2") Prepared for Beef Stew Storage Evaluation

<u>Carrots, Peeled, Trimmed</u>	<u>%</u>	<u>Grams</u>
Solids	11.0	275
Moisture	<u>89.0</u>	<u>2225</u>
	100.0	2500

Infusion Solution

Glycerol	88.7	3155
Water	5.5	197
Sodium Chloride	3.7	130
Propylene Glycol	1.6	56
Potassium Sorbate	<u>.5</u>	<u>19</u>
	100.0	3557

Moisture Content = 35.7 (Vacuum - Oven Method)

Aw = 0.71

TABLE VIII

Formula #5492-64 for Cook-Soak Equilibration of Fresh Raw Beef Pieces ($\frac{1}{2}$ -1") Prepared for Beef Stew Storage Evaluation

<u>Beef, Cubed Chuck</u>	<u>%</u>	<u>Grams</u>
Solids	35.0	1617.0
Moisture	<u>65.0</u>	<u>3003.0</u>
	100.0	4620.0
<u>Infusion Solution</u>		
Glycerol	56.6	2112.0
Good Seasons Beef Soup Base	19.8	739.2
Water	8.0	300.0
Sodium Chloride	7.4	277.2
Propylene Glycol	7.1	264.0
Potassium Sorbate	<u>1.1</u>	<u>39.6</u>
	100.0	3732.0
Moisture Content =	31.0 (Vacuum-Oven Method)	
Aw =	0.77	

TABLE IX

Formula #5492-66 for Cook-Soak Equilibration of Fresh Raw Potato Pieces ($\frac{1}{2}$ -3/4") Prepared for Beef Stew Storage Evaluation

<u>Potatoes, Peeled, Diced</u>	<u>%</u>	<u>Grams</u>
Solids	20.0	1000.0
Moisture	<u>80.0</u>	<u>4000.0</u>
	100.0	5000.0
<u>Infusion Solution</u>		
Glycerol	80.4	4687.5
Water	10.4	610.0
Sodium Chloride	4.3	250.0
Propylene Glycol	4.3	250.0
Potassium Sorbate	<u>.6</u>	<u>37.5</u>
	100.0	5835.0
Moisture Content =	36.8 (Vacuum-Oven Method)	
Aw =	0.79	

TABLE X

Formula #5492-68, for Cook-Soak Equilibration of Fresh Raw Peas
Prepared for Beef Stew Storage Evaluation

<u>Peas, Fresh, Shelled</u>	<u>%</u>	<u>Grams</u>
Solids	22.0	277.2
Moisture	78.0	982.8
	<u>100.0</u>	<u>1260.0</u>

Infusion Solution

Glycerol	78.4	1143.0
Water	11.0	160.5
Sodium Chloride	6.2	90.0
Propylene Glycol	4.1	60.0
Potassium Sorbate	.3	4.5
	<u>100.0</u>	<u>1458.0</u>

Moisture Content = 39.4 (Vacuum-Oven Method)
Aw = 0.80

Formula #5492-74 for Beef Stew Without Gravy
Prepared for Storage Evaluation

	<u>%</u>
I.M. Potato Pieces #5492-66	40
I.M. Beef Pieces #5492-64	30
I.M. Carrot Dices #5492-67	15
I.M. Peas #5492-68	15
	<u>100</u>

Moisture Content = 35.3 (Vacuum-Oven Method)
Aw = 0.77

TABLE XI

Formula #5492-72 for Gravy Prepared for Beef Stew
Storage Evaluation

<u>Ingredients</u>	<u>%</u>
Frodex 15 DE (Corn Products)	34.95
Dri Fri (Drew)	30.00
Water	17.00
Glycerol	10.00
Good Seasons Beef Soup Base	4.00
Propylene Glycol	2.00
Pre-gelatinized Potato Starch (Morningstar-Paisley, Redisol R-13)	1.00
Corral Beef Flavor (Pfizer)	0.50
Potassium Sorbate	0.30
Myverol 18-30 (DPI)	0.25
	<u>100.00</u>
Moisture Content =	17.4 (Vacuum-Oven Method)
Aw =	0.79

TABLE XII

Formula #5492-73 Beef Stew with Gravy Prepared for Beef
Stew Evaluation

	<u>%</u>
I.M. Gravy #5492-72	50.0
I.M. Potato Pieces #5492-66	20.0
I.M. Beef Pieces #5492-64	15.0
I.M. Carrot Dices #5492-67	7.5
I.M. Peas #5492-68	7.5
	<u>100.0</u>
Moisture Content =	26.2 (Vacuum-Oven Method)
Aw =	0.78

TABLE XIII

Formula #5850-64 for Cook-Soak Equilibration of Fresh Raw Pork Pieces ($\frac{1}{2}$ -1") Prepared for Barbecued Pork Storage Evaluation

<u>Pork, Trimmed Loin</u>	<u>%</u>	<u>Grams</u>
Solids	30.0	2017.5
Moisture	<u>70.0</u>	<u>4707.5</u>
	100.0	6725.0

Infusion Solution

Glycerol	74.8	8087.5
Water	14.4	1559.0
Salt	5.5	594.7
Propylene Glycol	5.0	540.9
Potassium Sorbate	<u>.3</u>	<u>30.2</u>
	100.0	10812.3

Moisture Content = 23.0% (Cenco)

Aw = 0.72

TABLE XIV

Formula #5856-16 for Barbecue Sauce Prepared For Barbecued Pork Storage Evaluation

	<u>%</u>
Good Seasons Open Pit Barbecue Sauce	78.0
Glycerol	20.0
Propylene Glycol	<u>2.0</u>
	100.0

TABLE XV

Formula #5850-43-I for Soak Infusion of Dried Apple Slices

	<u>Solution</u> <u>(%)</u>	<u>End</u> <u>Product</u> <u>(%)</u>
Apple Pieces, Vacu-Dry Puffed ($\frac{1}{2}$ x $1\frac{1}{2}$ " wedges)	-	44.97
Water	45.42	25.00
Glycerol	26.35	14.50
Sucrose	23.98	13.20
Propylene Glycol	3.65	2.00
Potassium Sorbate	0.55	0.30
Sodium Meta Bisulfite	0.05	0.03
	<u>100.00</u>	<u>100.00</u>

Aw = 0.72

Prepared by mixing 9 parts apple and 11 parts solution and soaking under refrigeration for approximately one day with occasional stirring.

TABLE XVI

Formula #5850-50-I for Intermediate Moisture Apple Pie Filling Matrix

	<u>%</u>
Dextrose (anhydrous)	39.24
Water	21.62
Glycerol	13.40
Cornstarch (Argo)	9.26
Frodex (24 DE)	5.19
Sucrose	4.32
Starch (Stein Hall Redisol 88)	3.09
Propylene Glycol	1.73
Sodium Chloride	1.22
Cinnamon	0.43
Potassium Sorbate	0.26
Nutmeg	0.24
	<u>100.00</u>

Aw = 0.68

TABLE XVII

Intermediate Moisture Apple Pie Filling Formula #5850-54

	<u>%</u>
Intermediate Moisture Apples Pieces #5850-43-I	57.7
Intermediate Moisture Matrix #5850-50-I	<u>42.3</u> 100.0
Moisture Content =	25.7 (Vacuum-Oven Method)
Aw =	0.70

TABLE XVIII

Apple Pie Filling Formula #5856-62

	<u>%</u>
Water	43.20
Glycerol	27.00
Sucrose	13.04
Apple Pieces, Vacu-Dry Puffed ($\frac{1}{2}$ x $1\frac{1}{2}$ " wedges)	12.00
Starch (Stein Hall Redisol 88)	2.30
Propylene Glycol	2.00
Potassium Sorbate	.30
Cinnamon	.08
Nutmeg	.05
Sodium Meta Bisulfite	<u>.03</u> 100.00
Aw =	0.83

TABLE XIX

Moisture, Aw and Lipid Content of the Six Items After Storage

<u>Item</u>	<u>% H₂O</u>	<u>Aw</u>	<u>% Lipids</u>
Chicken #5850-56	27.0	0.73	0.92
Coarse Ground Beef #5850-60	36.9	0.81	7.06
Diced Carrots #5850-58	37.5	0.74	0.13
Beef Stew with Gravy #5492-73	27.2	0.75	17.45
Beef Stew (no gravy) #5492-74	35.1	0.76	3.31
Peas	37.5	0.80	0.56
Carrots	43.3	0.75	0.33
Potatoes	35.3	0.75	0.25
Beef	29.5	0.75	10.25
Barbecued Pork #5850-64	29.0	*	6.97
Apple Pie Filling #5850-54	25.7	0.71	0.50

*Not measured due to volatile acetic acid in sauce.

TABLE XX

Microorganism Counts Before & After Storage for the Six Items

<u>Item</u>	<u>Standard Plate</u>		<u>Molds</u>		<u>Yeast</u>	
	<u>Initial</u>	<u>Mos.</u>	<u>Initial</u>	<u>Mos.</u>	<u>Initial</u>	<u>Mos.</u>
Chicken #5850-56	10	<10	<10	<10	<10	<10
Chicken #5856-53	140	*	<10	*	<10	*
Coarse Ground Beef #5850-60	<10	<10	<10	<10	<10	<10
Diced Carrots #5850-58	<10	<10	<10	<10	<10	<10
Beef Stew with Gravy #5492-73	40	60	<10	<10	<10	10
Beef Stew, No Gravy #5492-74	<10	40	<10	<10	<10	400
Barbecued Pork #5850-64	30	10	<10	<10	<10	<10
Apple Pie Filling #5850-54	20	20	10	<10	<10	<10

*Storage study to be completed

TABLE XXI

Sensory Evaluation Ratings

	Storage Time (Mos.)	Control	0°C As	38°C Is	0°C Re- hydrated	38°C Re- hydrated
<u>Chicken Pieces</u>						
Study 1	4	10	5.1	5.1	7.4	6.6
Study 2	2	10	6.6	*	7.7	*
<u>Coarse Ground Beef</u>	4	10	8.1	7.1	9.6	9.1
<u>Diced Carrots</u>	4	10	6.6	6.1	8.0	7.9
<u>Beef Stew & Gravy</u>	4	10	6.3	5.8	-	-
<u>Beef Stew - No Gravy</u>						
Rehydration I	4	10	-	-	7.1	6.9
Rehydration II	4	10	-	-	7.7	7.3
<u>Barbecued Pork</u>						
Rehydration I	4	10	7.9	7.2	7.9	7.6
Rehydration II	4	10	7.9	7.2	8.3	7.6
<u>Apple Pie Filling</u>	4	10	5.9	5.1	7.6	6.2

*Storage study to be completed.

MICROBIOLOGY METHODS

STANDARD PLATE COUNT

General

The method described here uses a pour plate procedure for determining Standard Plate Count. The dilutions to be used will depend on the nature and history of the sampling material.

A. Culture Media - Tryptone Glucose Extract Agar

Commercially available dehydrated media is used and prepared according to manufacturer's specifications.

Procedure

A. Sample Preparation and Dilution

1. For preparation of a 1:10 dilution in blenders or water bottles use: 11 grams sample to 99 ml buffered (Butterfields Phosphate Buffer) distilled water.
2. For preparation of a 1:40 dilution (for dehydrated vegetables and other difficult to disperse materials) in blenders or water bottles use:
e.g., 2.5 gms sample to 97.5 ml buffered distilled water (3 gms of 117 ml, etc.) or suitable working equivalent.
3. Open sample: Flame top of can with alcohol, open with sterile can opener.
4. Sample weighing method
 - a. Direct Weighing Method
 - (1) Tare sterile blender containing appropriate amount of tempered buffered distilled water.
 - (2) Aseptically spoon in proper aliquot of sample material representing a cross section of entire sample.
 - (3) Cover blender jar immediately.
5. Blend sample aliquot, at high speed, for 90 seconds.

B. Sample Plating

1. Pipetting procedure

Note: Sample material must be pipetted within 20 minutes after blending and plating media poured immediately.

- a. Fill a sterile pipette with freshly blended sample material.
 - b. Replace blender top immediately.
 - c. Pipette 0.1 ml into the 10^{-2} dilution plate and 1 ml into the 10^{-1} dilution plate.
 - d. If additional serial dilutions are to be used; pipette 1 ml from the blender to a sterile dilution bottle containing 99 ml sterile, buffered, distilled water; shake for 10 seconds, and pipette 0.1 ml into the 10^{-4} plate and 1 ml into the 10^{-3} plate. All further serial dilutions follow this procedure.
2. Controls
- a. Dilution controls are plated to test sterility of the diluents in blender jars and dilution bottles.
Pipette 1 ml of the diluent from one blender and dilution bottle into appropriate sterile plates.
 - b. Media controls are made by pouring 15 ml of the plating media into a sterile petri dish.
3. Media Pouring Method
- a. Remove sterile flask of media from 45°C water bath.
 - b. Wipe residual water from outside of flask.
 - c. Swirl media to insure consistent mixture.
 - d. Remove cover and flame lip over a bunsen burner, to insure sterility while pouring.
 - e. Slowly pour 10-20 ml into each plate; 4-6 plates may be poured before swirling them.
 - f. Flame lip of flask and replace cover.
 - g. Slowly swirl plate to give uniform dispersion of sample, taking care that media does not splash onto underside of petri dish covers.
 - h. Pour media control plate last to test for constant sterility throughout the procedure.
- C. Incubation
1. When media in plates has solidified, invert plates and place in incubator.
 2. Incubate at 35°C for 48 hours.
- D. Diagnosis: Plates are examined on a Colony Counter. All Colonies observed are counted from plates containing 30 to 300 colonies. Counts are determined by multiplying the number of colonies by the dilution factor.

Reporting Results

Report Standard Plate Count per gram, based on the number of colonies counted and the sample dilution. The count should be recorded to two significant digits. If the third digit is 1-4, change the third digit to zero; if the third digit is 5-9, change the third digit to zero and increase the second digit to the next higher whole number.

MOLDS AND YEASTS

General

The method described here applies to a pour plate procedure for determining Molds and Yeasts.

A. Culture Media - Malt Agar

Commercially available dehydrated media is used and prepared according to manufacturer's specifications.

B. Reagents

Lactic Acid; A 10% stock solution is made with distilled water and stored at room temperature. Amounts are added immediately before use to the sterile media, as specified by instructions on each package of media, to adjust the pH to 4.5.

Procedure

A. Sample Preparation and Dilution (see procedure outlined for STANDARD PLATE COUNT)

B. Sample Plating (see procedure outline for STANDARD PLATE COUNT)

C. Incubation

1. When media in plates has solidified, invert plates and place in incubator.
2. Incubate at 25°C for 5 days.

D. Diagnosis:

1. Yeasts: All plates exhibiting colonial growth on malt agar should be examined microscopically by preparing wet mounts from at least three colonies. Confirmation should always be microscopic not macroscopic.
 - a. Macroscopic Appearance: Most young yeast colonies are moist and somewhat slimy, but may appear mealy, and most colonies are whitish; however, cream colored, pink and other pigmented varieties do occur.

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13. ABSTRACT			
The following food items were prepared at water activities (A_w) between 0.71 and 0.81: diced chicken, ground beef, diced carrots, beef stew, barbecued pork and apple pie filling. All items were rated acceptable for consumption at reduced water activity, and acceptability was enhanced by restoration to near-normal moisture levels. No evidence of microbial deterioration developed during 4 months storage at 38°C; however, the apple pie filling exhibited excessive browning and the sauce for the beef stew underwent phase separation. Two general procedures were used to adjust foods to intermediate moisture levels, (1) infiltration of dehydrated, usually freeze dried, food with aqueous glycerol solution containing small amounts of propylene glycol, potassium sorbate (antimycotic), salt, sugar and other seasonings as appropriate and adjusted to desired A_w, and (2) a soak-cook procedure in which the raw food of normal moisture content was equilibrated against a similar but more concentrated solution to yield the desired A_w. Except for the apple pie filling, the latter procedure is preferred.			

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