"WHEYFERS": A HIGH PROTEIN SNACK FOOD MADE FROM THE YEAST KLUYVEROMYCES FRAGILIS GROWN IN COTTAGE CHEESE WHEY

By

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CHAPTER I

INTRODUCTION

The many benefits of whey fermentation by yeast in relation to waste treatment has been researched and documented in recent years (8, 9, 17, 19). Practical applications of this process have been prompted by the Environmental Protection Agency and its latest standards for dairy wastes that enter city sewage treatment facilities. Only 0.1% of the milk solids that enter the dairy can be drained into the sewer as waste to be processed by local sewage treatment facilities (21). Whey, a by-product of cheese manufacturing, contains 4.5 - 5% solids as the disaccharide, lactose. The original intent of the whey fermentation process was to reduce the Biological Oxygen Demand (BOD) in the most economic method so that local sewage treatment facilities could handle this cheese plant effluent. However, the benefits of this fermentation are twofold - not only has the BOD been reduced to an acceptable level, but the yeast yields, upon its recovery, a valuable protein source.

A variety of industrial by-products such as sulfite liquor, hydrocarbons and whey are proving to be worthwhile substrates for various microorganisms (2). The protein produced by these microorganisms, which include bacteria, algae and fungi as well as yeasts, is termed single-cell-protein, SCP (12).

Researchers at Oklahoma State University have implemented the use of the yeast, Kluyveromyces fragilis, previously called Saccharomyces fragilis (10), in the fermentation of cottage cheese whey to provide an economical method of BOD reduction for small dairy plants and extended the process by harvesting the yeast to be utilized as a single-cell-protein (8). The initial use for this yeast-whey protein in a dried form was as an animal feed, but to maximize the economics of the process it would be advantageous to explore its potential as a supplement in human foods. The assets here could include a variety of food products with improved nutritional values as well as a step toward solving world food problems.

There are a number of social and psychological factors which affect food preference and food acceptance in any society. The acceptability of new protein sources from materials not previously regarded as food will likely meet initial objection. Therefore, one of the best solutions would be to include the new protein sources as supplements in foods already accepted by the society. A popular food among American consumers, often with little nutritional value, are those products considered "snack foods" composed of potato and corn chips of various flavors, pretzels and crackers in a variety of shapes and tastes. These snack foods are consumed in large amounts by the American teenager, whose nutritional status is often poor (13). Protein fortification of these snack foods would have the dual purpose of improving their nutritional value and supplementing the diet of their consumers. Thus, the purpose of this study was to formulate an acceptable high protein snack food using the yeast-whey protein supplied by Kluyveromyces fragilis grown in cottage cheese whey as a protein supplement and product base.

CHAPTER II

REVIEW OF LITERATURE

The production of single-cell-protein and its value may be noted in numerous publications (1, 2, 4, 16). Protein fortification of foods has been the topic of a great number of discussions concerned with nutrition (20). However, very little information on the supplementation of foods with single-cell-protein has made its way into publication at this time. Presently a low calorie salad dressing may be the first product to reach the supermarket shelves containing a petroleum derived ingredient--the derivative being a torula yeast grown in petroleum. Another foods company is experimenting with a high protein macaroni in a federally funded school lunch program using the same petroleum grown yeast. Predictions for the end of 1976 are that 25 to 30 meat and bakery products containing yeast grown from petroleum will make their way to the market (6).

Earlier investigations in 1964 show that dairy researchers capitalized on yeast fermentation by growing K. fragilis on cottage cheese whey. The resulting product, stated by Robe (18) in "Wheast", is described as, ". . . a whey-grown yeast fortified with milk albumin" (p. 18). Wheast made its way into pet foods and poultry rations based on good experimental feeding results and an impressive nutrient profile as can be seen in Table I. No literature is available on any attempts to use Wheast in human foods, however, K. fragilis is an FDA (Food and Drug Administration) approved food yeast.

TABLE I

"WHEAST" COMPOSITION (18)

Crude Protein	Not Less Than 54.0%
Fat	Not Less Than 1.0%
Nitrogen Free Extract	Not Less Than 24.0%
Ash	Not More Than 10.0%
Crude Fiber	Not More Than 5.0%

The food grade K. fragilis NRRL Y-1196 (Northern Regional Research Laboratory) was of particular interest to dairy researchers for its ability to produce the enzyme, lactase, (B-galactosidase). This enzyme breaks the disaccharide, lactose, into its two monosaccharide components, glucose and galactose. The significance here can be seen when one realizes that a large percentage of the world's population is deficient in lactase and therefore suffers unpleasant effects after ingesting lactose containing dairy products. Thus, they are missing a valuable nutrient source. However, dairy products pretreated with lactase could eliminate these ill effects. Lactase produced by K. fragilis could also be used to decrease the gritty texture in concentrated milk products caused by the insolubility of lactose. It is this lactase producing ability that allows the K. fragilis to be effective in whey fermentation (11).

It should be noted here that there exists an abundance of information on the incorporation of whey in various forms in the food industry

(22). This should not be confused with the product resulting from the fermentation of whey by yeast. Hundreds of foods contain a variety of concentrated whey products, and fermented wheys exist which are the result of mixed culture fermentations. Products such as whey drinks, soups, cheese and cheese foods, bakery products, candies, spirit vinegar, food acidulants and whey butter contain whey but are not including it to increase protein levels. The use of whey in these products is due to Whey's qualities as an emulsifier, flavor enhancer, for browning characteristics or other traits (22). Such food products are examples of the variety of alternatives for whey utilization but each has its own composition and qualities. In single-cell-protein production whey is the substrate and it is the yeasts' growth that supplies the majority of the protein. The other characteristics associated with whey additives may or may not be present in the yeast fermented whey.

Protein fortification has been a subject of interest to nutritionists for a number of years. A few of the advantages of protein fortification include a reduction in sugar concentration by increasing the protein concentration, better protein utilization when its concentration is higher than that of carbohydrates and fats and a buffering action on acid produced by oral bacteria. The high phosphorus levels in high protein foods also has the effect of making teeth less acid soluble. These specific advantages are being weighed by candy manufacturers considering protein fortification (20). Soft drink companies are feeling pressure from concerned consumers as the popularity of these beverages grows and valuable nutrients supplied by milk and fruit juices are replaced with water, sugar, flavoring and carbon dioxide. Fortification of soft

drinks with cheese whey protein has been studied and successful results obtained; however, wholesale production in the United States depends on whether the cheese whey protein can be concentrated without any undesirable effects and at a reasonable price (7). Experiments in the fortification of snack foods have been carried out in which whey protein concentrate was used to supplement the foods' protein content. Again the advantages of consumer appeal for this food type and the low nutritional value of unfortified snack foods are mentioned in support of this area of research (3).

CHAPTER III

EXPERIMENTAL PROCEDURES

The purpose of this experiment was to formulate an acceptable snack food with an elevated protein content. This protein increase was to be the result of the addition of yeast-whey protein. The K. fragilis yeast was grown in cottage cheese whey at a pH of 4.7 ± 0.1 and a temperature of 35 ± 2 C. Fermentation was terminated by heating the yeast-whey to 88 C after the available lactose had been depleted as determined with "Clinitest" tablets (Ames Company). After cooling to 67 ± 5 C the liquid portion was syphoned off and any excess liquid was removed from the yeast-whey protein portion by placing it on cloths (stretched over frames) to drain. Specific details of this procedure are described in a previous publication (14). Two to four hours later the yeast-whey was collected and refrigerated for use. The yeast-whey protein as it comes off the cloths has a pH of 4.9 ± 0.1. Its composition at this point is given in Table II. Standard laboratory procedures of analysis for protein and ash (5) and solids and fats (15) were used.

The equipment used in the preparation of this snack food included a triple beam balance, mixing bowl, electric mixer, wax paper, deep fat fryer, paper towels, draining racks, oven and a "Radarange" (Amana microwave oven). All samples, both prepared and purchased, were packaged in clear plastic pouches and sealed approximately 24 hours prior to taste testing.

TABLE II

YEAST-WHEY COMPOSITION*

	Percent
Moisture	84.2
Protein	11.2
Fat	0.6
Ash	0.5
Nitrogen Free Extract	3.5

*Kluyveromyces fragilis grown in cottage cheese whey

Preliminary work was conducted in which the ingredient ratios were altered and various cooking times, temperatures and methods employed. The two recipes selected were chosen for their acceptability by the laboratory personnel and are given in Table III.

After being refrigerated at 4 C for a day the yeast-whey in its paste-like consistency was weighed and placed in a mixing bowl. The corn starch, egg white and salt were then weighed and added to the bowl and the mixture beaten with an electric mixer at medium speed for 30 to 45 seconds until it appeared smooth. The only variation in the second recipe was the addition of baking powder along with the other dry ingredients in an attempt to reduce the acid flavor suspected of causing an aftertaste. Half of the eight judging sessions were conducted with this variation. At this point dehydrated potato flakes (Hungry

TABLE III

WHEYFER RECIPES

Ingredient	Weight in Grams	
	Recipe I	Recipe II*
Yeast-whey	180.0	180.0
Corn Starch	3.0	3.0
Egg White	1.9	1.9
Salt	4.5	4.5
Potato Flakes	54.0	54.0
Baking Powder	---	7.5

*Used in the last four taste panels

Jack, Pillsbury) were weighed, added and mixed with the electric mixer for 1 - 1½ minutes. The final dough had a clay-like consistency and was molded by hand into large balls. Half of this dough was extruded through a narrow arc shaped die by packing the dough in a plastic cylinder with the die at one end and placing the cylinder in a caulking gun. The extruded dough resembled a long narrow ribbon. The ribbon was then cut into short segments. The second half of the dough was rolled between sheets of waxed paper to the desired thickness and cut into crescent and elliptical shapes. Each shape was fried separately in liquid cooking oil at 204 C (400 F) for 20 to 30 seconds, or until slightly brown in appearance. The frying also caused the dough to puff, giving the product a hollow center. Products contained in the frying basket were then drained and the contents emptied on a wire rack covered by paper towels. Continuing from here the fried product was placed in an oven to remove more moisture and improve the crispness. It was noted that when the most desirable texture was obtained, the product had browned considerably and often carried a slightly burned flavor. An effective attempt was made to counter this additional browning and flavor change by utilizing a microwave oven; that is, an increased moisture loss could be achieved in this way with a minimal increase in browning. The chips were subjected to three, 30 second heatings in the microwave oven with approximately one minute intervals. The last four taste panels were conducted with products prepared under microwave cooking. The prepared snack foods were then ready to be packaged. Two commercial snack foods whose shapes were similar to the yeast-whey snacks were repackaged so that no packaging differences could be detected.

The taste panel consisted of 12 college students enrolled in an

experimental cookery class. Evaluations of the products were made once a week on the same day at approximately the same time. Each panelist was seated at a table and separated from other panelists by partitions. At each taste session, all judges received eight packages of samples which had been randomized and coded: this determined the order in which they were tasted. The eight samples were actually two sets of the four different chips; the yeast-whey fortified "Wheyfers" in the two shapes previously described and two potato based commercial products whose shapes resembled the short narrow ribbon segments and the crescent or elliptical shapes. Instructions were given orally and in written form on the score sheet (Table IV) for the first two sessions, and then only the score sheets were supplied. Judges were given a glass of water and asked to rinse their mouths' between samples.

Composition of the yeast-whey fortified Wheyfers and of similar unfortified commercial products are summarized in Table V.

TABLE IV

SCORE SHEET FOR SNACK FOODS

JUDGE_____ #_____ DATE_____

EXPLANATION: Both commercial and experimental snack foods (e. g., potato chips) have been packaged for you to sample. The experimental foods have an elevated protein content.

DIRECTIONS: Grade each product by itself using the scale of 1-5 listed below. Assume prices of all products are equal. (products can have the same grade).

PROCEDURE: -Taste sample and allow some time (approximately 30 seconds) before scoring (in case of an aftertaste).

-Rinse mouth with water and when ready, proceed to next sample.

-Sample slowly and thoughtfully. Keep in mind there is no "right" answer and that each individual's samples are different.

1 - EXCELLENT - Like very much, would buy and eat regularly.

2 - GOOD - Like, would buy and eat often.

3 - AVERAGE - Neither like nor dislike, would buy but other products would be preferable.

4 - POOR - Dislike, would only buy and eat if no similar product available.

5 - NOT EDIBLE - Would not buy or eat.

SAMPLE #	SCORE	COMMENTS
A 1		
A 2		
A 3		
A 4		
A 5		
A 6		
A 7		
A 8		

TABLE V

COMPOSITION OF WHEYFERS AND OTHER SNACK FOODS

	WHEYFER	POTATO CHIP[a]	POTATO STICK[a]
	%	%	%
Moisture	1.1[b]	1.8	1.5
Protein	18.5[b]	5.3	6.4
Fat	31.4[b]	39.8	36.4
Ash	6.7[b]	3.1	4.9
Carbohydrate	42.3[c]	50.0	50.8

[a]Watt, B. K., A. L. Merrill, et al. 1963, Composition of Foods, Agricultural Handbook No. 8, United States Department of Agriculture, Washington, D.C.

[b]Laboratory analyses

[c]Calculated by difference.

CHAPTER IV

RESULTS AND DISCUSSION

Eight taste panels were conducted in which all products were scored to determine whether an acceptable chip had been developed. These scores were then subjected to statistical analysis and the results of this analysis interpreted.

In relating the product score averages (Table VII) to the scores' verbal descriptions, it may be discerned that Wheyfers fall in the "Average" range, as neither like nor dislike, while the commercial products range between the "Average" and "Good" categories. Two of the four falling closer to average and the other two approaching good. An overall evaluation of the commercial products versus Wheyfers showed a significant difference in the scores of these products with the commercial products being preferred. However, a least significant difference test (LSD) reveals that the differences between the two least preferred commercial snacks and all of the Wheyfer variations (the two shapes and two recipes) is not significant at the 0.05 level (Table VIII). The difference between the most acceptable Wheyfer and the most acceptable commercial product is also not significant suggesting that Wheyfers have the potential to be competitive with products already available on the market. No significant differences in score averages were indicated among the three different commercial products or among the different Wheyfer recipes and shapes.

TABLE VI

ANALYSIS OF VARIANCE FOR VARIABLE SCORE

SOURCE	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F	P
Chip	7	164.93	23.56	6.38	.001
Day (Chip)	24	88.66	3.69	4.61	.001
Judge	11	110.45	10.04	12.53	.001
Judge*Chip	77	178.12	2.31	2.89	.001
Day*Judge (Chip)	264	211.47	0.80	1.97	.001
Rep (Day Judge Chip)	384	156.50	0.41		
Corrected Total	767	910.12	1.19		

TABLE VII

WHEYFER AND COMMERCIAL CHIP TASTE PANEL SCORES[a]

SNACK FOOD	AVERAGE
Commercial 1	2.14
Commercial 1	2.39
Commercial 2	2.74
Commercial 3	2.90
Wheyfer Ribbon A[b]	3.11
Wheyfer Ribbon B[c]	3.30
Wheyfer Ellipse A[b]	3.46
Wheyfer Ellipse B[c]	3.48

[a]Average of 12 judges tasting each sample a total of 16 times

[b]Recipe without baking powder

[c]Recipe with baking powder

TABLE VIII

LEAST SIGNIFICANT DIFFERENCE VALUES

	Commercial 1	Commercial 1	Commercial 2	Commercial 3	Wheyfer Ribbon A[a]	Wheyfer Ribbon B[b]	Wheyfer Ellipse A[a]	Wheyfer Ellipse B[b]
Averages:	2.14	2.39	2.74	3.90	3.11	3.30	3.46	3.48
Commercial 1		0.25	0.60	0.76	0.98[d]	1.17[c]	1.32[c]	1.34[c]
Commercial 1			0.35	0.51	0.73	0.92[d]	1.07[d]	1.09[d]
Commercial 2				0.16	0.38	0.56	0.72	0.74
Commercial 3					0.22	0.41	0.56	0.58
Wheyfer Ribbon A						0.19	0.34	0.36
Wheyfer Ribbon B							0.16	0.18
Wheyfer Ellipse A								0.02
Wheyfer Ellipse B								

[a] Wheyfers without baking powder

[b] Wheyfers with baking powder

[c] LSD value 1.10 ($p < 0.01$)

[d] LSD value 0.81 ($p < 0.05$)

A look at the deviation in averages for each of the judges suggests that panelists had diverse opinions on foods of this type (Table IX). In reviewing the daily averages it appears that all products are becoming more acceptable as the judges become more familiar with them (Table X). Since Wheyfers are a new and different snack food product, one could expect an initial reluctance in accepting them. The improvement in acceptability over time would tend to substantiate this suspicion.

TABLE IX

AVERAGES FOR EACH JUDGE OF ALL CHIPS SAMPLED

JUDGE	AVERAGE
1	3.11
2	3.69
3	3.02
4	2.31
5	2.78
6	2.78
7	2.47
8	2.66
9	3.31
10	3.27
11	2.67
12	3.20

TABLE X

DAILY TASTE PANEL AVERAGES[a]

DAY	AVERAGE
1	3.10
2	3.15
3	2.95
4	2.66
5	2.96
6	2.85
7	2.94
8	2.91

[a]Average of all judges and all chips each day

CHAPTER V

SUMMARY AND CONCLUSIONS

The purpose of this study was to fabricate a high protein snack food using the yeast, Kluyveromyces fragilis, grown in cottage cheese whey as a base. After preliminary testing, two recipes were selected and consumer acceptability was determined by a taste panel. Each yeast-whey product was sampled and scored along with commercial snack foods for four weeks. The levels of protein, moisture, fat and ash were determined and carbohydrate calculated by difference.

Taste panel scores were interpreted after statistical analysis. Least Significant Difference (LSD) testing showed that the only significant differences were between the most acceptable commercial chip and the Wheyfers - leaving no significant differences between the remaining commercial chips and Wheyfers. This would suggest that Wheyfers could be competitive on an acceptability basis with commercial products now available.

Composition analysis revealed the Wheyfers contained more than three times as much protein, less carbohydrate, and less fat than some snack food products now on the market.

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