PREPARATION OF POWDERED PANCREATIC EXTRACT WITH DIFFERENT METHODS

BY

Sania M. Abdou; Dawood, A.H.; Abd El-Hady, S.M. and El-Alfy, M.B.

Food Sci. Dept., Fac. of Agric., Moshtohor, Zagazig University.

ABSTRACT

Some methods for preparation and characters during storage at room temperature of powdered pancreatic extract were studied. The obtained results could be summarised as follow:

* The yield of pancreatic powder preparations represents: 40.6, 28.6, 15.0 and 12.4% for salt dried powder, lyophilized powder, actone extraction powder and precipitated dried powder/100 mg fresh glands respectively.

* The salting method for pancreas powder preparation achieved the highest proteolytic activity giving 65.0 µ/mg fresh gland while the other methods by lyophilization, precipitation and acetone methods revealed 38.4, 35.4 and 33.6 µ/mg respectively.

* The proteolytic and lipolytic activity of the different forms of powder showed low percentages of decrease during storing at room temperature for 3 months.

* The bacteriological quality of the different prepared powders showed low counts for the tested types of bacteria representing total bacterial count, aerobic sporeformers, coliform and the proteolytic and saccharolytic anaerobic sporeformers oposite to those of lyophilized powder during storage.

INTRODUCTION

In previous paper it was stated that liquid pancreatic extract decreased greatly in its proteolytic and lipolytic activity during storage inspite of the presence of different preservatives Dawood et al., (1988). These facts conducted

the research to prepare the extract in a powder form. However, enzyme powder preparations are prefered because of the advanctages in case of handling, much less storage spaces and better stability during preservation and storage (Amer, 1963). Kushner and Hugo (1971), explained the preservative effect of sodium chloride through lowering the water activity. Naguib et al., (1979) and Girgis et al. (1983), investigated the bacteriological quality and the proteolytic activity of rennet extract and cleared that the sporeformers counts were very small, while the coliforms, moulds and yeasts were absent during the six months of storage.

The present work was carried out to prepare pancreatic powder with different methods of drying and to study its ploteolytic and liplytic activity and examines its bacteriological properties during storage at room temperature.

MATERIAL AND METHDOS

Powdered panctreatic extract was prepared by different methods as follows:

Direct salting method:

Fats and connective tissues were removed from the fresh pancreas glands then they were cut up to slices and sprinkled with dried sodium chloride, drying step at atmospheric condition was carried out for about 7 days as recommended by Fahmi and Amer (1962). After drying the excess of sodium chloride was removed followed by milling for the dried salted tissues and packed in polyethylene bags.

Precipitation drying method:

The defatted fresh pancreas glands were minced, frozen and enzymes extraction was carried out with dist. water. Ammonium sulphate was added to the extraction so as to bring the concentration of $(NH_4)_2SO_4$ to 60%, then the pH was adjusted to 4. The precipitate was separated by centrifugation. The resultant precipitate was adjusted to pH 7.5 using 1 N KOH, then 20 g lactose powder + 30 g Na_2SO_4 were added to each 50 g from the precipitate. The mixture was air dried and milled then packed in polyethylene bags.

Acetone drying method:

The method described by Keller et al. (1956), was used to prepare the acetone powder of pancreas.

Lyophilization drying method:

The commen method of enzymes and drugs for drying was used. Defatted fresh glands were minced and frozen, then lyophilized by Edwards. High Vaccuum Freeze Drier, England for 24 hours, then milled and packed in polyethylene bags.

The obtained powder of pancreas preparations were subjected for the following determinations: The proteolytic activity of the powder according to the method of Kunitz (1947) and the lipolytic activity on tributrin as a substrate using the modified method of Tarassuk and Frankel (1964). The total protein was estimated according to the method recommended by Lowry et al., (1951) and the specific units for pancreatic enzymes were determined.

The total bacterial count was determined according to the Standard Method for the examination of Dairy Products (1960). The coliform count was carried out as described by Harrigan and Margaret (1966). The aerobic sporeformers were determined as spore counts on Bacto Nutrient Agar after Difco manual (1965), while the anaerobic sporeformers were carried out by the dilution frequency method on a medium prepared according to El-Sadek and Mahmoud (1958).

Each expriment was conducted for 3 times and each determination was made in duplicates.

RESULTS AND DISCUSSION

The yield:

Results in Table (1) showed the amount of the powdered pancreatic exatract prepared by the different methods, their proteolytic and lipolytic activities and the total activities were calculated as untis/100 mg fresh pancreas glands.

It is obvious from the results that the salt dired powder gave the highest yield followed by the lyophilized powder then the acetone method and finally the precipitated dried powder. The percentages of the protein in these powders were 35%, 43%, 28% and 11% in the same order. The results also revealed that the salt dried powder gave the highest total proteolytic and lipolytic activities when expressed as specific activity, then comes the lyophilized dried powder in the second order, followed by the acetone powder. The precipitated dried method was characterised by its lower yield in both specific proteolytic and lipolytic activity than the other methods.

Table (1.): Average yield of pancreatic powder and enzymes activity/100 mg fresh glands dried by different methods.

Drying method	Yield %	Proteolytic activity		Lipolytic activity		Total activity/100 mg fresh tissues			
						Proteolytic activity		Lipolytic activity	
		Units/ mg.	Specific activity	Units/ mg.	Specific activity	Units/ mg.	Specific activity	Units/ mg.	Specific activity
Salt dried powder	40.63	65.00	185.71	179.55	513.00	2640.95	7545.60	7295.20	20843.4
Acetone powder	15.00	33.60	120.00	254.37	908.45	504.00	1800.00	3815.48	13626.7
Precipitated dried powder	12.40	35.40	321.82	97.26	884.15	438.96	3990.50	1205.99	10963.5
Lyophilized dried powder	28.57	38.40	89.30	269.33	626.34	1097.09	2551.40	7694.70	17894.7

Specific activity (units/mg protein).

Effect of storage on the different pancreatic powder preparations:

A- Effect on the enzymes activity:

Table (2) represents the effect of storage at room temperature on the proteolytic and lipolytic activities of pancreatic powder prepared with various methods. It can be noticed that the proteolytic activity of salt dried powder showed slight decrease during the storage period to be; 1.38, 4.31 and 6.92% after 1, 2 and 3 months respectively. The lipolytic activity of the pancreatic powder prepared with the same method decreased also with storage progress being; 1.98, 4.53 and 7.1% durign the storage period in the same sequence.

The proteolytic activity of the acetone method showed somewhat reduction in proteolytic and lipolytic activity during storage having decrease of 3.2, 7.14 and 9.87% for proteolytic activity and 5,2, 8.75 and 14.7% for lipolytic activity after 1, 2 and 3 months storing successively.

In the case of precipitated dried powder, the loss of both proteolytic and lipolytic activities during storage at room temperature as tested after one months interval seemes to be higher than in the two previous methods giving 4.52, 9.04 and 11.40% decrease in proteolytic activity and 7.22, 11.13 and 15.69% loss in the lipolytic activity after testing periodically every month during the storage period.

Regarding the lyophilized powder, the proteolytic and the lipolytic activities showed the highest percentage of decrease during the storage for three months being 5.73, 10.5 and 14.7% for proteolytic activity and 7.8, 13.10 and 17.32% for lipolytic activity in the same order.

From the obtained results, it can be noticed that in general, the proteolytic and lipolytic activities slightly decreased as the storage period progressed. The effect of storage at room temperature on the pancreatic powder was very low when compered with the loss occurred during the storage of liquid extract. This agreed with the results of Bagdy and Banga (1957) for pancreatic extract and Amer (1963), for rennet extract.

Data also cleared that the method of salt dried powder seemed to be the best method for preparing pancreatic powder as the loss in both proteolytic and lipolytic activities were the lowest one allover the storage period as explained by Kusher and Hugo (1971).

Table (2): Effect of storage at room temperature on proteolytic and lipolytic activities enzymes of pancreatic powder dried by different methods.

	Proteolytic activity units/mg				Specific activity (units/mg protein)				% decrease		
Storage period(months)	0	1	2	3	0	1	2	3	1	2	3
Drying method:											
Salt dried powder	65.00	64.10	62.20	60.500	185.7	183.1	177.7	172.9	1.38	4.31	6.92
Acetone powder	33.60	32.50	31.20	30.284	120.0	116.1	111.4	108.2	3.20	7.14	9.87
Precipitated dried powder	35.40	33.80	32.20	31.364	321.8	307.3	292.7	285.1	4.52	9.04	11.40
Lyophilized powder	38.40	36.20	34.37	32.754	89.3	84.2	79.9	76.2	5.73	10.5	14.7

	Lipolytic activity (units/mg)				Specific activity (units/mg protein)				% decrease in units/mg		
Storage period (months)	0	1	2	3	0	1	2	3	1	2	3
Drying method:											
Salt dried powder	179.55	176.00	171.41	166.80	513.0	502.86	489.7	476.6	1.98	4.53	7.1
Acetone powder	254.37	241.14	232.10	216.95	908.4	861.20	828.9	774.8	5.20	8.75	14.7
Precipitated dried powder	97.26	90.24	86.43	82.00	884.2	820.30	785.7	745.5	7.22	11.13	15.69
Lyophilized powder	269.33	248.32	234.05	222.68	626.3	577.50	544.3	517.9	7.80	13.10	17.32

B- **Effect of storage on the bacterial counts:**

Table (3) illustrates the bacteriological analysis of pancreatic powder dried by the different methods during storage at room temperature.

The results showed that the total bacterial counts of the prepared pancreatic powders with different methods varied from one method to another. The acetone method gave the lowest number per-gram powder followed by the precipitated and dried powder to be in the second order, while the salting drying method came in the third order. The lyophilized powder contained the highest counts.

Concerning the aerobic sporeformers, the lyophilized powder illustrated also the highest load for this type of bacteria during the storage period. The other three methods produced powders with much lower counts.

The increase in the total plate counts and aerobic sporeformers during storage at the room temperature showed gradual increase within the first and the second month of storage then decreased in the third month. These results agree with Naguib et al., (1979) and Girgis et al., (1983).

In respect with coliform group counts for the four types of pancreatic powder preparations, the picture was changed. The coliform group of bacteria was not detected (Nil) in the acetone and precipitated dried powders. The lyophilized powder showed the highest numbers, while the salt dried powder cleared much less counts throughout the storage period.

Regarding the anaerobic sporeformers both proteolytic and saccharolytic types of bacteria present in the different forms of pancreatic powders prepared with the four drying methods. The results revealed that lyophilized powder showed the highest counts of proteolytic type. The most probable number (MPN) of proteolytic anaerobic sporeformers were slightly increased during storing at room temperature specially in the case of lyophilized powder.

The saccharolytic number of anaerobic sporeformers were absent in salt dired powder. In the other forms of powder their presence were rare and never exceeded 4 in counts at the initial time and throughtout storage then they decreased and was not detected after the second and the third month of storage. These results are in accordance with those of Naguib et al. (1979), who worked on liquid rennet.

Table (3): Bacteriological analysis of pancreatic powder dried by different methods during storage at room temperature.

Storage period (months)	Bacteriological analysis																			
	Total bacterial count/g x 10^2				Aerobic spore formers / g				Coliform / g				Anaerobic proteolytic M.P.N.				Spore formers saccharolytic counts / g			
	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3
Method of drying																				
Salt dried powder	5	17	21	9	33	50	60	10	20	10	Nil	Nil	4	4	4.5	4.5	Nil	Nil	Nil	Nil
Acetone powder	9	12	23	15	1	20	40	Nil	Nil	Nil	Nil	Nil	4	4.5	5.5	5.5	2	Nil	Nil	Nil
Precipitated and dried powder	11	17	30	17	2	50	80	60	Nil	Nil	Nil	Nil	6	7.8	8.1	8.3	2	2	Nil	Nil
Lyophilized powder	57	88	160	120	170	450	680	440	140	170	150	80	10	13	15	17	4	2	Nil	Nil

In general the bacterial picture of the pancreatic powders prepared with different methods during storage for 3 months at room temperature; cleared that salt dried powder, acetone and precipitated dried powders showed low counts for all tested types of bacteria. This can be attributed to the deleterious effect of salt on the initial count according to Kushner and Hugo (1971) in the first method, while due to the effect of acetone, diethyl ether and ammonium sulphate in denaturating the protoplasm of the microorganisms in the powder prepared with the other two methods.

The highest counts of bacteria in lyophilized powder may be due that there was no preservatives or inhibitory factors but the tissues of the pancreas glands were lyophilized as such.

REFERENCES

Amer, S.N. (1963): A study in drying liquid rennet extract. Ph.D. Thesis, Cairo University, Egypt.

American Public Health Association (1960): Standard mehtods for the examination of dairy products. Inc. 11th ed., New York.

Bagdy, D. and Banga, I. (1957): Pancreatic elastase. Acta Physiol. Aced. Sci. Hung. 11: 371. C.F. Method in enzym (1962) V.

Dawood, A.H.; Abd El-Hady, S.M.; Sania, M. Abou, and El-Alfy, M.B. (1988): A study on preparation of pancreatic extract and its stability during storage. Under Publication.

Difco Manual of Dehydrated Culture Medium Reagents, Ninth Ed. (1965): Difco Laboratories, Detroit, 1, Michigen, USA.

El-Sadek, G.M. and Mahmoud, S.Z. (1958): Practical and applied Microbiology in Arabic, Anglo-Publisher, Cairo, Egypt.

Fahmi, A.H. and Amer, S.N. (1962): A study on the preparation of liquid rennet extraction. In Arabic Megallet El-Eloom El-Zeraeia, Cairo, Univ. 15: 10.

Girgis, E.S.; Amer, S.N.; Nagmoush, M.R.; Anis, M.K. and El-Batawy, M.A. (1983): A study on some of the properties of sheep's rennet during extraction. Egyptian J. Dairy Sci. 11, 223-231.

Harrigan, W. and Margaret, E. (1966): Laboratory methods in microbiology. Academic Press, London and New York.

Keller, P.; Cohen, E. and Neurath, H. (1956): Purification and properties of procarboxy-peptidase. J. of Biol. Chem., 223: 457.

Kunitz, M. (1947): Proteolytic activity towards casein and hemoglobin. J. Gen. Physiology. 30: 291.

Kushner, D. and Hugo, W. (1971): Inhibition and destruction of microbial cell. London-New York: Academic Press, P. 255-283.

Lowry, O.H.; Rasebrough, N.J.; Farr, A.L. and Randell, R.J. (1951): Protein measurement with the folin phenol reagent. J. of Biological Chemistry 193: 265-275.

Naguib, M.M.; Naguib, Kh.; El-Erian, A.F. and El-Shenawy, M. (1979): Effect of storage temperature on the bacteriological properties of liquid rennet. Egyptian, J. Dairy Sci., 7:209-214.

Tarassuk, N.P. and Frankel, E.N. (1964): The mechanics of activation of lipolysis and the stability of lipase system of normal milk. J. Dairy Sci. 38: 438.

تحضير خلاصة البنكرياس المجففة بطرق مختلفـــــــة

سنية محمود عبده احمد حسن داوود صلاح مخيمر عبد الهادى محمد بدير الالفى

درست بعض طرق تحضير مسحوق خلاصة البنكرياس وتتبع بعض خواصها اثناء التخزين على درجـــة حرارة الغرفة ويمكن تلخيص النتائج المتحصل عليها فيما يلـــــــــى :

- اعطت غدد البنكرياس الطازجة الخالية من الانسجة الاخرى حوالى النسب التاليـــــــــــــــة : ٤٥٪ ، ٣٩٪ ، ١٥٪ ، ١٢٪ مسحوق مجفف بطرق الملح وطريقة التجفيد وطريقـــــــــــة الاسيتون وطريقة الترسيب على الترتيب •
- اظهرت مسحوق خلاصة البنكرياس النتائج بطريقة الملح اعلى نشاط فى التحليل البروتين حيث أعطـــى ٦٥ وحدة / ملليجرام ثم أعطت المساحيق المحضرة بطريقة التجفيد والاسيتون والترسيب حوالـــى ٣٨ ، ٣٥ ، ٣٤ وحدة / ملليجرام على الترتيب •
- أظهرت الصور المختلفة من مسحوق خلاصة البنكرياس نسب منخفضة من النقص فى نشاطها للتحليـل البروتينى والتحليل الدهنى اثناء التخزين على درجة حرارة الغرفة •
- احتوت الصور المختلفة من مسحوق خلاصة البنكرياس على أعداد منخفضة فى الاعداد الكلية للبكتريـا واعداد البكتريا الهوائية المتجرثمة وأعداد الكوليفورم والعد الاحتمالى للبكتريا المحللة للبروتيـــــن والمحللة للسكريات أثناء التخزين ماعدا المسحوق المحضر بطريقة التجفيد فكانت الاعداد بـــــــه عالية نسبيـــــــــــــــا •