INFRARED SPECTRUM OF BEEF SAUSAGE AND CHICKEN PATTIES MANUFACTURED WITH OR WITHOUT PROTEASE INHIBITOR.

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SUMMARY:

Protease inhibitor that isolated from potato tubers (alpha-variety) was used in its crude form in the processing of beef sausage and chicken patties. The processed samples that contained 0, 1, 2, 3% of protease inhibitor were stored at 4°C for two weeks through which infrared spectrum was considered. Experimental results proved the superiority of the addition of protease inhibitor through processing of beef sausage and chicken patties; a trend which based on the stability of the indentified functional groups through the storage periods.

INTRODUCTION:

Infrared analysis is based on the constant motions, vibration and rotation of the molecules which occur with fixed and specific frequencies. So, when a molecule is subjected to radiation whose frequency corresponds to its natural frequency, some of that radiation will be absorbed and used to reinforce the natural molecular motions, particularly vibrations. However, the absorbance at any given wavelength is the sum of the absorbance of the individual components at such wavelength, and the contribution of each component depends upon its concentration and absorptivity. Consequently, the spectrum of a mixture is the sum of the spectra of these components; Dillard and Goldberg (1978).

At least 13 different inhibitors have been identified from potatoes and about 10 have been purified and partially characterized by Liener and Kakada (1980). However, protease inhibitor of potato can be differentiated (by their stability in solution for 10 min at neutral pH and 80 C) into heat-stable and heat-labile proteins (Ryan et al., 1976).

Pearce et al. (1979) found that protease inhibitors which are rich in cysteine were heat stable in its pure form, but rapidly destroyed in intact tubers especially on cooking.

It was aimed through such research to use the curde protease inhibitor (1, 2 and 3%) in processing of beef sausage and chicken patties. Such trend was considered to see to that extent the protease inhibitor that isolated from potato could control the activity of protease enzymes during storage of the aforementioned products for two weeks at 4 C. In such case, infrared spectrum of the aformentioned samples was compared.

MATERIALS AND METHODS:

A. Materials:

* Beef meat, spices and chicken samples:

- Beef meat and spices were obtained from "RAMADA" refrigerator of the"GERCO"Company; related to the Ministry of supplies, Egypt. The spices mixture are composed of the following ingredients.

Ingredients	Weight/gm	Ingredients	Weight/gm
Black pepper	20	Red pepper	10
Vitamin C	20	Nut meg	10
Cardamom	10	Cubeb (powder)	10
Coriander	20	TOTAL	100

- "EL SHARK EL AWSAT" Company that located in "KALYOBIAH" governorate (EGYPT) is the source of frozen chicken samples; with an individual qweight that ranged between 850 up to 1100 gm.

** Potato tubers (alpha variety):

The Horticulture Department, Agricultural Research Centre, Ministry of Agriculture is the source of potato tubers (alpha variety) that used as a source of extraction of protease inhibitor.

B. Technological Methods:

* Extraction of protease inhibitor was performed as described by Belitz et al., (1971).

** Manufacturing of beef sausage and chicken patties:

The formula of beef sausage and chicken patties were based on using the previous meat sources which were grinded by an electrical meat mincer and remixed with spices and starch in preparing of the control sample (Free of protease inhibitor; P.I). On the other hand, other samples were manufactured in the presence of the crude P.I. to reach the level of 1, 2 and 3% of the recipe formula as follows:

Ingredients	Control (Free of P.I)	Levels of protease inhibitor (P.I) in the tested samples		
		1%	2%	3%
Meat (Beef or chicken)	90	90	90	90
Spices	1	1	1	1
Protease inhibitor	0	1	2	3
Starch	3	2	1	0
Water	6	6	6	6

With respect to the beef sausage samples, they were prepared by stuffing the responsed mixture in a natural casings (small intestine) after which they were kept in a refrigerator at 4 C for 15 days. The chicken patties were prepared by using the previous formula after shapping in patties forms and the produced samples were wrapped in a parchement paper and kept at 4°C for 15 days. Before analysis, both of the beef sausage and chicken patties samples were dried at 60°C overnight.

C. Analytical method:

Samples after being prepared in a powder form were held on a potassium promide disk (3 mg sample over 300 mg KBr) of the Philips PU 9712 Infrared Spectrophotometer available at the Central Services Laboratory of the National Research Centre. The functional groups of the investigated samples were identified according to Bellamy (1964).

RESULTS AND DISCUSSION:

Infrared spectrum of beef sausage samples:

The functional groups of the beef sausage samples containing 1, 2 and 3% of protease inhibitor as well as, that of the control sample (without inhibitor) were identified according to Bellamy (1964) and Pomeranze and Meloan (1971) as seen in Table (1).

Unstored beef sausage samples:

With respect to the unstored beef sausage sample, its infrared spectrum was given within wave numbers 200 up to 3830 cm^{-1} as seen in Fig. (1).

** The control sample is characterized by the presence of specific functional groups at the following wave numbers; 615 cm^{-1} (29 T%) of Mercaptans (CH_2-S-); 809 cm^{-1} (41.3 T%) of Ethylene group. 1170 cm^{-1} (29.2 T%); CH_2-O-CH_2 of Aliphatic ethers.

** As a result of adding the protease inhibitor, there are some functional groups that detected in the three inhibitor treatments, while others are only found in two or one of these samples. Forinstance in case of the beef sausage containing 3% protease inhibitor, the characterized functional groups are noticed at wave numbers of 797 (Ethylene), 1260 (primary alcohols; CH_2-OH) 1563 (Amino acid -NH^+_3), and 3482 cm^{-1}.

It is of importance to clarify that the beef sausage containing 3% protease inhibitor are characterized by a pronounced functional group at wave numbers ranging from 1860-1871 cm^{-1} (Cyclic anhydrides) with the following T%; 41.6% (1% P.I), 46% (2% P.I.)and 63.4%. So addition of protease inhibitor is succeded in improving the hardness of the tested samples.

Beef sausage stored for two weeks:

When the infrared spectrum of the beef sausage that stored for two weeks at 4°C was considered, it is clear from Fig. (2) the appearance of functional groups which are out of detection in the control samples. These chemical groups are sharply identified at the following wave numbers;

- 1914, 3360 cm^{-1} for the beef sausage samples containing 1% protease inhibitor.
- 254/257 and 3780 cm^{-1} for the samples containing the 1 and 2% protease inhibitor.
- 1119/1121 and 1875/1876 cm^{-1} for the beef sausage samples processed with 1,2 and 3% protease inhibitor.
- 285, 627, 3426 and 3456 cm^{-1} for the samples processed with 2% protease inhibitor.
- 1563 and 3448 cm^{-1} for the samples containing 3% protease inhibitor.

From the aforementioned observations the addition of protease inhibitor during the processing of beef sausage succeeded in preventing the changes that could occure in the protein molecule during storage as a result of the activity of protease enzymes.

Infrared spectrum of chicken patties samples:

The infrared spectrum of the unstored chicken patties that given in Figs. (2 and 4) indicated the presence of sharp functional groups that appeared at specific wave numbers; i.e.

- 226 to 413 and 2962 to 2954 cm^{-1} are detected in the control as well as in the samples containing 1% and 3% portease inhibitor.
- 527 (CH_2-O-CH_3),574; 632 and 675 (Mercaptans), 1046 and 1078 (C-OH), and 1704 and 1842 of Cyclic anhydrides, 2102 (C-NH_3^+ Cl) and 3518 to 3858 cm^{-1} are identified only in the samples manufactured with 3% protease inhibotor.
- 792 (Ethylene), and 3404 cm^{-1} are found only in the samples containing 2% of protease inhibitor.
- The wave numbers of 737, 779, 804, 3460 and 3482 cm^{-1} characterizing the chicken patties samples containing 1% protease inhibitor, while 744 cm^{-1}, 1579, 1855,and 3318 cm^{-1} are clearly noticed in the control sample.
- The wave numbers of 205 up to 212; 696/698, 1121/1122, 1235 to 1246, 1543 to 1548, 1645 to 1657 cm^{-1} are predominant in the samples containing 1, 2 and 3% protease inhibitor.

As a result of storage for two weeks at 4°C, the investigated chicken patties showed a different pattern of infrared spectrum in both the responsed functional groups and their T%. Forinstance , the functional groups that was detected at 205 to 230 cm^{-1} in the unstored samples are still presented after one week of storage at 4°C; contrary to those of the sample stored for two weeks at the same temperature which lacking these groups. Other noticeable changes are found at wave numbers of 1235/1246 cm^{-1}; since after two weeks of storage such wave numbers extended from 1154 to 1398 cm^{-1}. The previous discriminations are mainly due to the presence of protease inhibitor which reshapes the balance pattern of the functional groups as a result of controlling or minimizing the activity of protease enzymes.

CONCLUSION:

Infrared spectrum of the beef sausage and chicken patties that manufactured with or without protease inhibitor assured the presence of protease inhibitor reshapes and the pattern of the functional groups as a result of controlling or minimizing the activity of protease enzymes within the tested samples.

Table (1) Nature of vibration and the corresponding functional chemical groups of specific wave numbers of the Infrared spectrum.

Wave numbers cm^{-1}	Nature of vibration	Functional chemical groups
200	-C-H-	
228	-C=N-	Amide and Imides (-C=N-)
320	-NO_2	
343	-N=N-	
577		
615	C-S Strech	Mercaptans (CH_2-S-)
616		
700		
714	R-NO_2 (medium)	
736		
793		Ethelyene
850	C-H	Thiophosphoryl chloride
932	C-N-Strech	C-CH_2
1040	-C-OH- Strech	Unsaturated Secondary alcohol
1080		
1120	R-CH_2-NH_2	Carboxylic acid
1150	-COOH	
1236	C-N-Strech	(R-CH_2)-N
1240	C-N-Strech	(R-CH_2)-N
1410	CO-NH_2- CH_3 or CO-NR_2	Amides Aliphatci hydrocarbon chain
1540	-NH_3^+	Amino acid,hydrochloride and Zwitterion
1650	Ring Vibration (Strong)	Aromatic hydrogen
1730	CH_3-CO-OH(Strong)	Acetates
1770	O=C-O-C=O (C-C)	Cyclic anhydrides
1794	(Strong)	
1831		
1854		
1871		
1914	C-NH_3^+ Cl	Amine hydrocloride
1980		
2850	-CH_2-(C=O)-	
2920	CH_3-C-H	Saturated hydrocarbon
2960	(Strong)	
3440	Free-NH_2	Primary Amine CH_2-NH_2
3460	sym.stretch	and O-H
3542		
3676		

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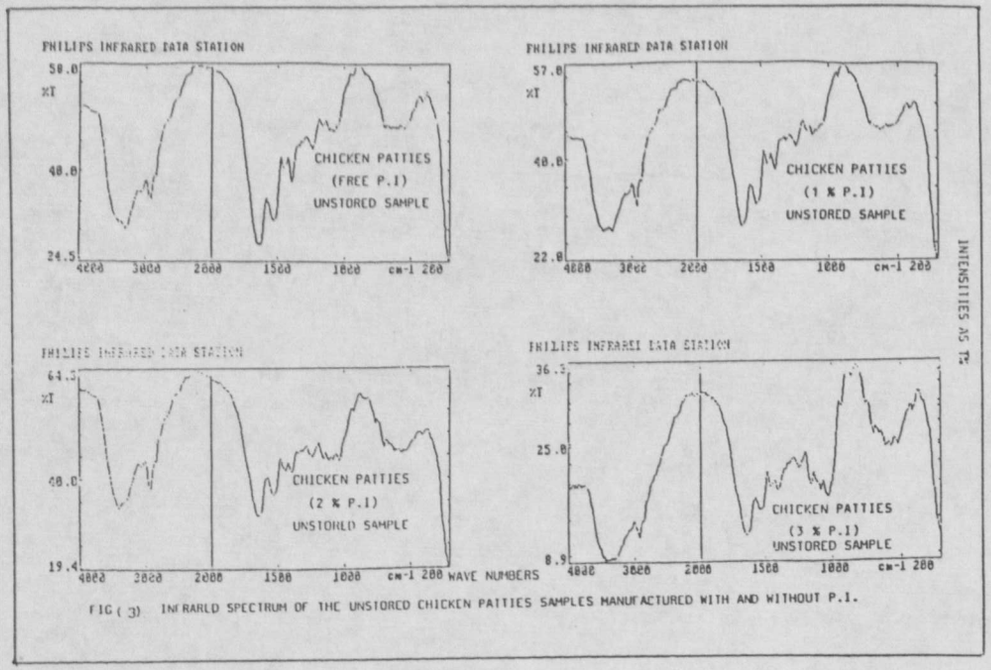

FIG (3) INFRARLD SPECTRUM OF THE UNSTORED CHICKEN PATTIES SAMPLES MANUFACTURED WITH AND WITHOUT P.I.

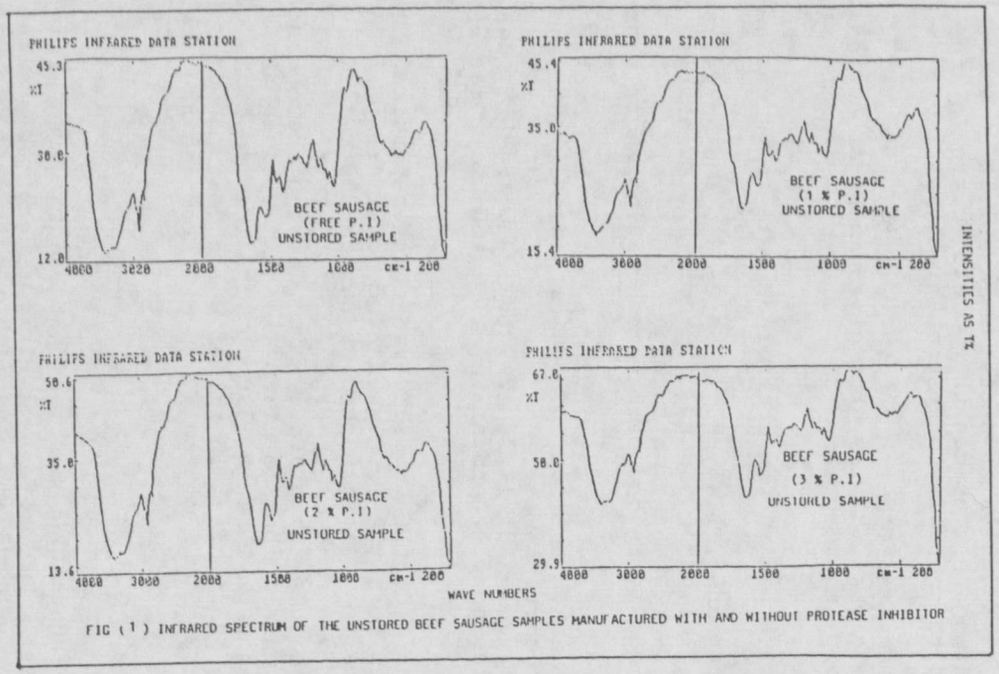

FIG (1) INFRARED SPECTRUM OF THE UNSTORED BEEF SAUSAGE SAMPLES MANUFACTURED WITH AND WITHOUT PROTEASE INHIBITOR

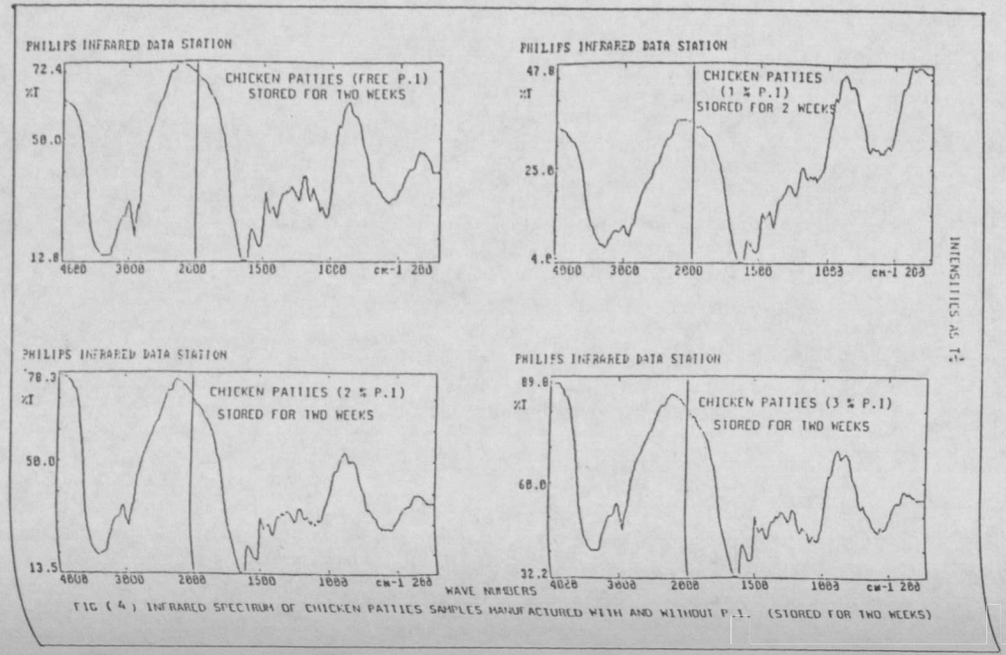

FIG (4) INFRARLD SPECTRUM OF CHICKEN PATTIES SAMPLES MANUFACTURED WITH AND WITHOUT P.I. (STORED FOR TWO WEEKS)

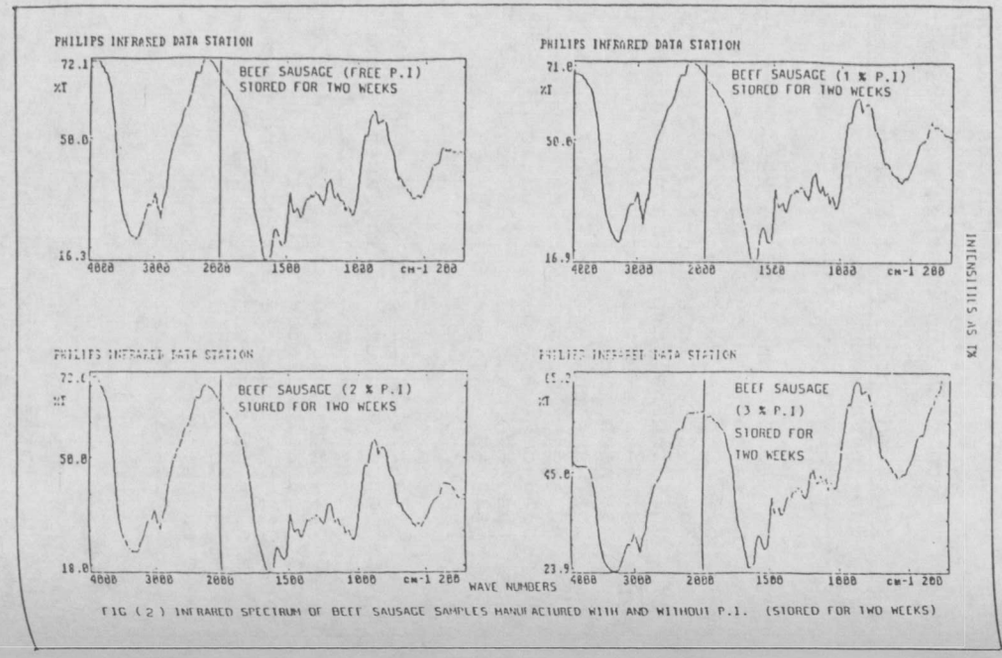

FIG (2) INFRARED SPECTRUM OF BEEF SAUSAGE SAMPLES MANUFACTURED WITH AND WITHOUT P.I. (STORED FOR TWO WEEKS)