PHYSICAL , CHEMICAL , SENSORY AND MICROBIOLOGICAL PROPERTIES OF PORK SAUSAGE EXTENDED WITH PEA PROTEIN ISOLATES

A Thesis
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of
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by

Pascal J. Delaquis

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Requirements for the Degree of
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And finally to my wife Thora, who should rightfully co-author this work, thanks for the patience, support, typing and our wonderful daughter.

IN MEMORY OF BJARKI B. JAKOBSON MD.

Abstract

A pea protein isolate prepared by protein micellization (PMM) was substituted for meat in raw pork sausage at levels of 10, 20, 30 and 40% by whole weight. The isolate was incorporated both in the spray dried state (SDP) and as extruded protein fibre (TP) of .15 mm. diameter. The substitution of the vegetable protein for meat led to higher protein contents in the substituted products and they are, therefore, referred to as extended products and the pea PMM preparations as extenders.

The microbiological shelf life of the extended products was similar to the unextended. Mesophillic and psychrophillic total plate counts (SPC), coliforms on violet red bile agar (VRB) and yeasts and molds on potato dextrose agar (PDa) were not statistically different between levels of extension after five days under refrigerated storage.

Sensory analysis of the products revealed that the TP extender had less effect on firmness, chewiness, flavour or overall acceptability of the sausage than SDP. No effect on juiciness was observed using either extender but a decrease in greasiness scores was found where TP was used as the extender. The use of both extenders decreased cooking losses as measured by weight and liquid loss during cooking.

Objective measurements of texture were obtained using the Ottawa Texture Measuring System (OTMS). Single point measurements such as Warner-Bratzler shear values proved more useful than a multiple texture profile analysis in establishing a comparison with data from the sensory taste panel.

Results indicate that extension of pure pork sausage up to levels of 20% with pea PMM that has been spun into fibres does not significantly alter the microbiological, physical or sensory properties of the product.

Table of Contents

List of Figures

List of Tables

A. INTRODUCTION

Vegetable proteins make a significant contribution to the world's food supply by replacing more expensive animal proteins. In the past three decades a tremendous amount of research time has been devoted to the development of new sources of vegetable proteins for a variety of uses. Economic considerations were certainly the impetus behind this activity as well as prospective improvement of the nutrition of mankind. The relatively high cost and limited availability of animal proteins make it necessary for most nations in the developing world to supplement their largely cereal-based diets with legumes and oil seeds such as is the case with soy in Asia (Scrimshaw, 1981).

As energy and thus animal protein costs rise in the Western world, the dependence on animal sources for high quality food proteins is bound to shift to some extent to vegetable sources. Eating habits and food systems as a whole are difficult to change. Hence, isolated and refined vegetable proteins are being used increasingly in a wide variety of food systems to improve nutritive values and functional properties without displacing the system itself (Hardin, 1979). Before a new protein product can be introduced as an ingredient in a consumer product, the full effect of its use on the quality and safety of the target system must be evaluated.

Among the most important aspects of vegetable proteins are their functional properties. These will determine the field of application as well as whether a new protein ingredient will be competitive on the market (Hermansson, 1979). The challenge for protein chemists at present is to understand and thus be able to exploit structure-function relationship in vegetable proteins so that functional requirements in food systems can be met (Kinsella, 1979). Until more information about specific food proteins from vegetable sources can be compiled, researchers today are faced with little recourse but to incorporate these products into target foods and to study them as a whole (Comer and Dempster, 1981).

The following study was performed with the aim of determining the effect of substitution of vegetable protein for meat protein in a comminuted meat product with the following objectives:

1) to determine the effect of substitution on the sensory characteristics, texture, composition, chemical characteristics and microbiology of the final product.
2) to assess the influence of texturing of the extender on these same properties.

B. LITERATURE REVIEW

I. Vegetable Proteins

The first source of vegetable protein to become widespread in use was that derived from soy. Soy based protein still accounts for the majority of the market in the North American food industry (Langsdorf, 1981). Food grade soy protein flours and isolates made their appearance on the market in 1959 (Wolf and Cowan, 1971). It was not until the mid 1960's, however, that soy isolates and concentrates gained an advantage over non-fat dry milk which had been the traditional source of protein for use in many food products. This was due largely to an increase in the price of the latter (Wolf and Cowan, 1971).

Regions adapted to growing soybeans are limited on the earth (Lischenko, 1979). In Canada they are at present limited to areas in southern Ontario although work is being conducted to develop strains adapted to the short Western summers in the prairies (Anonymous, 1979). Most of our requirements for vegetable proteins thus are filled by expensive imported soy products (Vaisey et al., 1975). For these reasons other sources of vegetable protein are being considered not only in this country but in other parts of the globe where the situation is similar.

Many vegetable sources of protein have been studied and considered either to replace or supplement those derived from soy. They include the glandless cottonseed (Rosenblum, 1981), poppy seed (Srinivas and Narasinga, 1981), sunflower (Wills and Kabirullah, 1981), potato (Neg, 1979), peanut (Lusas, 1979), canola (Ohlson and Anjou, 1979), and field beans (Marquez and Lajolo, 1981) among others. In Canada special attention has been focussed on fababeans, field peas and lentils for the prairie regions while mung beans, chick peas, lupine and lima beans may be adapted to warmer parts of the country (Fan and Sosulski, 1974). Field peas, in particular, have received much interest in Manitoba. In 1981 approximately 70,000 acres were devoted to their cultivation making the province the leading producer and exporter of this special crop in Canada (Anonymous, 1981).

II. Uses in the Meat Industry

a) Soy-based Proteins

The economic advantages inherent in direct utilization of vegetable proteins in meat products were recognized in the early 1950's (Thulin and Kuramoto, 1967). At this time patents for such products began appearing in the U.S.A. (Boyer, 1954). In 1959, as previously mentioned, the first commercial preparations for use in processed foods made their appearance on the market. These were all derived from soy and fell into three categories which are still recognized today: flours and grits containing up to 50% protein, concentrates up to 90% protein and isolates upwards of 90% protein (Ohren, 1981). The use of these materials depends to a large extent on the requirements of the products to which they are applied.

The majority of the protein used in the meat industry is destined for comminuted or chopped meat products which may or may not be processed. Numerous studies have demonstrated the potential use of soy proteins as an extender for beef hamburger (Judge et al., 1974; Drake et al., 1975; Seideman et al., 1977; Twigg et al., 1977; Gadze et al., 1979.) The so-called Soy-burger has received some acceptance on U.S. markets (Twigg et al., 1977). Soy protein preparations are used in such other products as fermented or unfermented salami (Joseph etal., 1978; Berry et al., 1979), fresh beef and pork sausage (Craven et al., 1978), meat balls (Hermansson, 1975), wieners (Smith et al., 1970; Randall and Voisey, 1974), and meat loaves (Carlin et al., 1978; Sokolsky, 1979). Currently they are also used in canned stews, luncheon meats, pet foods, reformed meats and hams (Nowacki, 1979), as well as various processed seafood products (Sipos et al., 1979).

b) Other Vegetable Sources

Vegetable proteins from other sources are beginning to enter the market very slowly. Cottonseed protein extenders are available (Rosenblum, 1981) as well as a pea protein based meat-analog (Murray, 1982). Lin et al. (1975) and Wills and Karibullah (1981) have shown that sunflower flour and protein isolates can be used in wieners and beef sausage without loss of quality or acceptability. Vaisey et al. (1975) extended ground beef with fababean and pea protein concentrates at levels of 30% on a fresh weight basis.

The full contribution that such vegetable proteins have to offer has still to be realized. As the price of soy protein product rises there is little doubt that their potential will be considered in more detail in the future.

III. Recovery of Vegetable Proteins

a) Recovery from Traditional Sources

A variety of technologies are employed in the processing of flours, concentrates and isolates of vegetable proteins. The nature of the source influences the type of process employed for their recovery. The processing of oil seeds such as soy and canola into protein preparations requires initial solvent extraction to yield defatted flakes (Milligan et al., 1981). These are subsequently desolventized, usually using heat resulting in some denaturation of the proteins (Kinsella, 1979).

Flours and grits are obtained by milling of the flakes followed by separation of the particles employing such techniques as air classification and cycloning (Potter, 1978). Three basic processes are currently employed to produce commercially available concentrates : i) extraction in 60 - 80% alcohol; ii) extraction of solubles with water acidified to pH 4.5 and iii) water extraction preceded by heating or toasting to denature the protein before extraction of solubles (Ohren, 1981). The objective in each of these methods is to immobilize the major protein fractions while extracting soluble carbohydrates, nitrogenous materials and other soluble minor constituents. Other techniques exist but have not as yet become employed to the same extent.

The commercial process employed in the production of isolates includes aqueous extraction of protein from defatted flakes at neutral or elevated pH, separation of the protein containing extract from the insoluble residue, acidification of the protein extract to precipitate the protein, separation and drying at the isoelectric point or after neutralization with alkali (Wolf and Cowan, 1971; Ohren, 1981). The cost of production and wholesale price of these products increases from flour, concentrate to isolate (Langsdorf, 1981).

b) Recovery from New Vegetable Sources

Legume seed proteins such as the field pea and fababean have a distinct advantage over the oilseed proteins in that no defatting is required and relatively simple methods such as air classification and ultrafiltration can be used for separation (Bramsnaes and Olsen, 1979). Currently, the Prairie Regional Laboratory in Saskatchewan is producing pea and fababean protein concentrates using a combination of pinmilling and air-classification. Isolates are usually obtained by acid precipitation and ultrafiltration.

Murray et al., (1978) have developed a technique for making isolates which uses no extremes of alkali, acid or organic solvents but which is based on an extraction of the protein with a salt solution of .2 to .8 ionic strength followed by dilution with water to allow precipitation. Proteins settle out in compact spheres as the result of hydrophobic interaction forces. Isolates of up to 95% purity are obtained using this technique and have been called protein micellar mass (PMM). New and efficient techniques such as this will undoubtedly lead to increased production and utilization of new sources of vegetable proteins.

IV. Functional Requirements

a) Functional Requirements in Meat Products

Numerous studies pertaining to the functional properties and effects of proteins in meat products have been published. The first uses of soy proteins in food systems were undertaken to exploit these properties rather than to act as a source of dietary protein. Table 1 shows some of the uses of various soy products in food systems (de Buckle, 1981).

There are no standard methods or tests for measuring the functional properties of proteins (Comer, 1979). The

TABLE 1 . Functional properties performed by soy protein preparations in food systems (de Buckle, 1981).

Functional property	Food system	Preparation Used
Solubility	Beverages	F,C,I,H[a]
Water absorption and binding	Meats, sausages, breads, cakes	F,C
Viscosity	Soups, gravies, gruels, "coladas"	F,C,I
Gelation	Meats, sausages, baked goods, pasta products	F,C,I
Elasticity	Meats, bakery	I
Emulsification	Sausages, bologna, soup, cakes	F,C,I
Fat adsorption	Meats, sausages, donuts	F,C,I
Flavor-binding	Simulated meats, bakery	C,I,H
Foaming	Whipped toppings, chiffon desserts, angel cakes	I,W,H
Color control, staling control	Breads, "arepas," "tortillas"	F

[a] F,C,I,H,W: soy flour - concentrate, isolate, hydrolysate and whey, respectively.

best test for the functional properties of vegetable proteins is often direct incorporation into a product (Wolf and Cowan, 1971; Comer and Dempster, 1981).

By controlling processing conditions, soy products are fabricated with different functional properties that are useful in a variety of meat products (Bressani, 1981). They include emulsification, fat and water absorption, texture, adhesion, cohesion and elasticity, foaming, flavour and colour control.

b) Textured Proteins

In recent years textured soy protein products that simulate the eating characteristics of meats have gained increasing acceptability and use in meat products both as fillers and extenders. Many processes exist for the manufacture of such products but only a few are used commercially at present.

The most widely used to date has been thermoplastic extrusion which uses soy flour as a starting material. The flour is formed into a dough prior to feeding into an extruder where it is subjected to shear mixing, high temperatures and pressures in order to plasticize the protein matrix. This plastic mass is forced through a die and allowed to expand to yield a porous structured soy product (TSP) (Campbell, 1981).

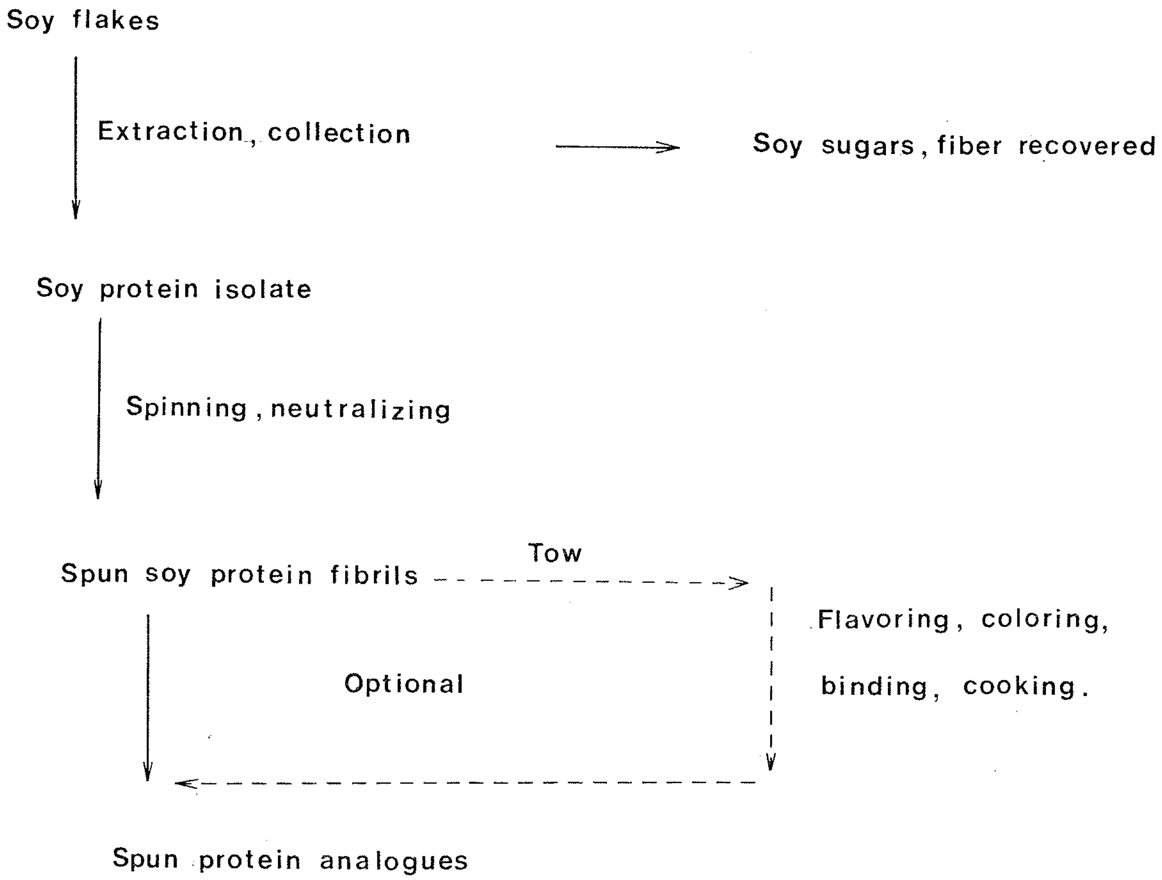
Other processes have attempted to duplicate the fibrous nature of meat. These involve solubilizing the protein in alkali followed by extrusion through a spinneret into an acid bath where coagulation occurs. Many variations on this basic process exist. In particular, additives such as binders, fats, flavourings and colors can be incorporated with the fibers into a matrix to form true meat analogs. A schematic flow diagram of such a process is shown in Figure 1. Murray et al., (1978) have developed a procedure for the production of protein fibers using the previously described PMM. It involves the extrusion of rehydrated PMM through a die or spinneret into water of pH 5.5 to 7.5 at temperatures from 90 to 95° C. Variation of pH and temperature leads to formation of fibers of differing strengths and elasticities. These are essentially bland in flavour and can be stored in the frozen or dehydrated state.

In the future vegetable proteins from sources other than soy should provide similar functional advantages for use in meat products. The best means for determining the validity of this statement is still to incorporate such new vegetable proteins into a target meat product and to study the system as a whole.

V. Texture

The measurement of the texture of meat and meat products has been the subject of numerous investigations but still remains a relatively difficult goal to achieve (Stanley, 1976). The most widely used approach consists

Fig 1 . Flow diagram for the production of spun protein analogues. (Quass, 1979)



of comparing a subjective response such as obtained through use of a sensory taste panel to objective measurements derived from physical methods (Larmond and Petrasovits, 1972; Kapsalis et al., 1973).

Objective methodology has led to the development of a wide variety of instruments and procedures for physical determinations. The Warner-Bratzler shear has been used extensively in the assessment of tenderness (Stanley, 1976). The Kramer shear press is another traditional instrument in the field of meat research (Szczesniak et al., 1970). Instruments capable of performing multiple assays by interchanging various test cells have gained acceptance due to their versatility. Such an instrument is the Ottawa Texture Measuring System which was devised by Voisey (1971). Using this system tests for compression, shearing, extension and others can be performed.

Determination of the effect of protein substitution on the texture of meat products has been approached using these instruments and procedures. Studies on the use of soy protein preparations of various types have reported no deleterious effects on texture at low levels of extension in ground beef (Drake et al., 1975; Twigg et al., 1977; Gadze et al., 1979) and meat loaves (Carlin et al., 1978; Sokolsky, 1979). None of these studies has been very detailed, however, usually being limited to a direct comparison between single instrumental measurements and relatively undiscriminating sensory methods of analysis.

Some investigators have attempted to predict the functional effects of nonmeat ingredients in comminuted meat systems using empirical approaches. Hermansson (1975) and Hermansson and Åkesson (1975 a,b) established a correlation between performance and a group of functional properties such as viscosity and gelation which was derived from complex statistical models in an ideal system. Using similar approaches others have reported that untextured soy protein fillers and extenders contribute to texture by virtue of their heat setting or gelation properties in meat products (Comer and Dempster, 1981; Siegef and Schmidt, 1979). None of these studies has been followed by application to complete meat systems. Hence a need exists to further investigate the effect of protein substitution on the texture of comminuted products by applying more thorough methods in sensory and textural evaluation.

VI. Microbiology

a) Microbiology of Extended Meat Products

Consumer interest in the quality and safety of foods has increased dramatically in the last decade (Kramer *et al.*, 1976.). Hence a need exists to ensure that the shelf life of extended products, off-flavours caused by microbial growth and the possibility of food related illness be carefully studied (Draughton, 1980).

Conflicting reports are found in the literature with respect to the shelf life of soy extended ground beef. Judge et al. (1974) found increases in numbers of aerobic bacteria at the time of formulation but no differences with controls after 7 days of storage at 4° C. Foster et al. (1978) determined that beef-soy patties held at an identical temperature did have a lower keeping quality than unextended product. Thompson et al. (1978) observed increases in standard plate counts during storage at 3° C which were accompanied by a parallel increase in number of proteolytics. *Staphylococcus aureus* numbers also increased although coliforms did not. Bell and Shelef (1978) also reported such an increase in Staphylococcus as well as *Streptococcus* although they could detect no difference in aerobic plate counts. Craven et al. (1978), and Kocet et al. (1978), observed unchanged bacterial populations in beef and pork sausage extended with soy protein. Such conflicting reports can be attributed to the use of different soy extenders resulting in differences in nutrient values, solubilities and interactions with other proteins. It is also unfortunate that researchers use varying methods for determining bacterial numbers (Draughton, 1980).

b) Microbiology of Vegetable Proteins

Protein extenders themselves have been subjected to microbiological studies. Commercially available soy extenders usually have such low bacterial values that they may actually decrease total numbers of bacteria in a mixture by a dilution effect (Foster et al, 1978). No cases of food-borne bacterial infections have been reported which could be traced directly to a soy-protein extender. There is a lack of literature available on the microbiology of other textured vegetable protein products, but it is likely that no differences will be found with those derived from soy.

A review of the literature in the area of meat substitution with vegetable proteins illustrates the difficulties inherent in the development of such products. The best method for evaluating the effects and desirability of extension remains to study the substituted products once the substitution has been made.

C. MATERIALS AND METHODS

I. Experimental Design

The experiments were conducted with the following objectives:

1. to substitute pea protein into fresh pork sausage at levels of 10, 20, 30 and 40% on a whole weight basis.
2. to determine the effect of the type of extender employed on parameters described below.
3. to evaluate changes in fat and water binding abilities and hence cooking losses in the product at the different levels of extension.
4. to compare sensory qualities of the products using a semi-trained taste panel.
5. to evaluate textural differences of the product at varying levels of extension.
6. to compare the chemical composition of the products.
7. to conduct a microbiological shelf life study of the extended sausage.

II. Raw Materials

Spray dried field pea (Pisum sativum) PMM was obtained courtesy of General Foods in Cobourg, Ontario. It shall henceforth be referred to as SDP. Sixty percent lean pork trimmings (25 kg.) were purchased at Canada Packers in Winnipeg, Manitoba and frozen until used. Spices used in the formulation were obtained from Western Canada Compound in Winnipeg, Manitoba.

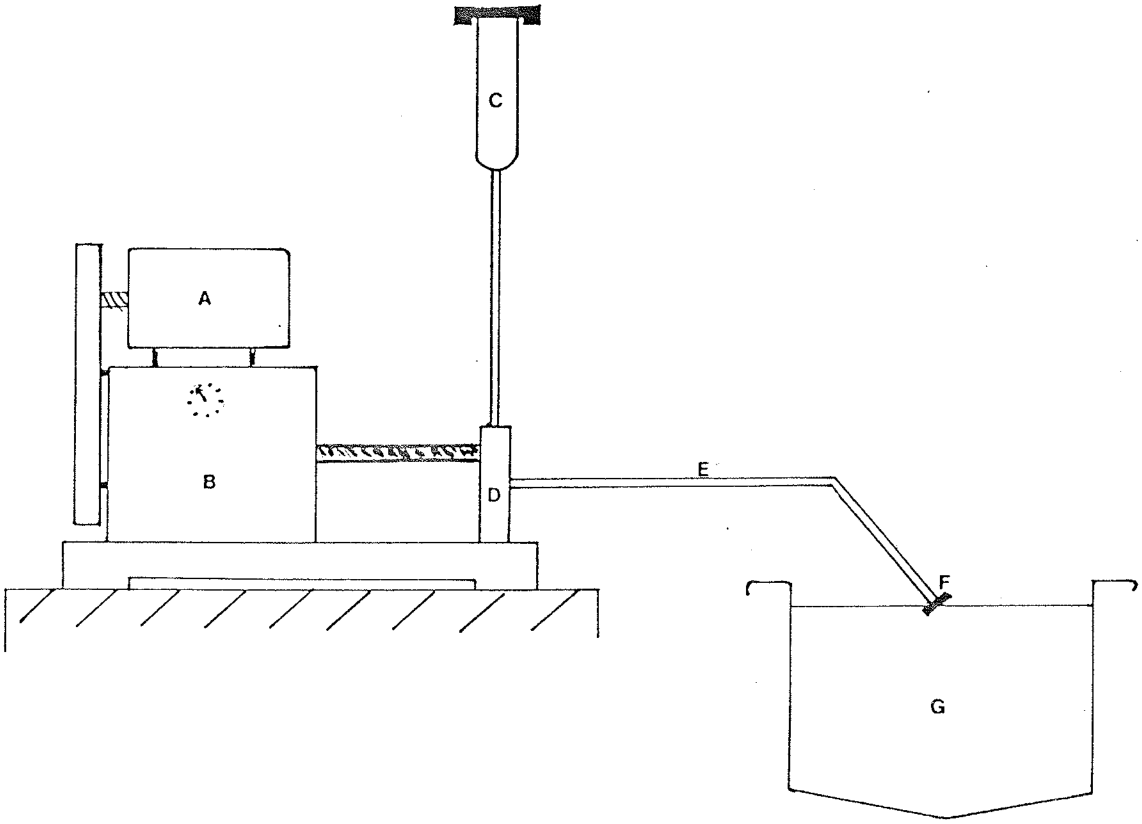
III. Preparation of the Fiber Extender

Fibers were prepared according to the process described by Murray *et al*. (1978). Wet pea PMM was pumped through a die into hot water as shown in Figure 2. A variable speed gear driven pump was obtained courtesy of Dr. M. King of the Faculty of Human Ecology, University of Manitoba. The die or extruder was prepared by filling holes in a metal screen with epoxy resulting in a symmetrical arrangement of pores of .15 mm. diameter. The wet PMM was pumped through the extruder into hot water (95° C.) at pH 7.0 in a thermostat controlled steam kettle (Gröen). The fibers were allowed to heat set for approximately one minute, drained and immediately frozen in heat sealed plastic bags in a blast freezer at -40° C. Prior to processing of the product they were thawed at room temperature. This protein preparation is referred to as TP (textured protein).

IV. Sausage Formulation

A simple formulation was obtained from Kramlich *et al*.

Fig 2 . Apparatus used in production of protein fibres.



A: motor

B: differential gear box

C: sample reservoir

D: pump head

E: extrusion feed

F: extruder plate

G: temp. controlled water bath

(1973). The basic formulation is shown in Table 2. After grinding through 95 mm. and 47 mm. plates in a Hobart grinder the batter was stuffed into 21 mm. diameter casings (Devro TM) using a F. Dick stuffer. All processing was performed in a cold room at 4° C.

Approximately 4 kg. batches of sausage extended at 0, 10, 20, 30 and 40% green weight with spray dried and textured pea PMM were processed. The water content was kept constant at approximately 60% by addition of cold (1° C.) water. Amounts were calculated following moisture determination of the raw materials and final formulations are shown in Table 3. The products were hermetically sealed in freezer bags and held at -20° C. until used.

V. Proximate Analysis

Moisture, fat, ash and protein were all determined according to A.O.A.C. Fat, ash and protein assays were performed on three freeze-dried samples of sausage batter. Moisture determinations were also done in triplicate.

VI. Microbiological Shelf Life Study

Five hundred gram samples of sausage at each level of extension were placed in styrofoam trays and wrapped in polyethylene film. These were stored in a walk-in cooler held at 4° C.

Microbiological Assays:

Eleven grams of aseptically sampled batter from each level of protein addition were weighed in a stomacher bag

TABLE 2 . Formulation of the Sausage.

Ingredients	% of Total
60% lean Pork trimmings	97.59%
Salt	1.68%
White pepper	.25%
Sugar	.27%
Sage	.14%
Mace	.07%

TABLE 3 . Formulation of batches of sausage at all levels of extension.

Protein		SDP				TP			
Level of extension (green weight)	0	10	20	30	40	10	20	30	40
g. of meat	1500	1350	1200	1050	900	1350	1200	1050	900
g. meat protein	225	203	180	157	135	203	180	157	135
g. vegetable protein	0	31	61	92	122	30	60	90	120
% of total protein as vegetable protein	0	13.2	25.3	36.9	47.5	12.8	25.0	36.4	47.0
g. H_2O	210	275	341	407	472	157	105	52	0
% total protein (calculated)	13.2	14.1	15.0	16.0	17.1	13.2	14.1	15.0	17.0

together with 99 ml. of peptone water (1 g. peptone (Difco) in 1000 ml. of deionized distilled water). The samples were blended in a stomacher for one minute. Serial dilutions from 10^1 to 10^8 were prepared for counts.

Standard plate counts, aerobic mesophilic count at 32°, 48 hours incubation and 4° C., aerobic psychrophillic at 7 days incubation were performed. Coliforms were determined on Violet Red Bile (VRB) agar according to the method reported by Keeton and Melton (1978). Incubation was performed at 35° C. for 24 hours. Yeast and mold counts were obtained on Potato Dextrose (PDa) agar (pH 3.5) according to the method reported by Koburger and Farhat (1975). Incubation was at 25° C. for 5 days. These counts were performed after 1, 2, 3 and 5 days of storage.

Friedman's two-way analysis of variance based upon rank sums was applied to the data obtained by the enumeration procedures (Hollander and Wolfe, 1973).

VII. Sensory Evaluation

Sensory evaluation of the products was carried out by a panel of 7 members of the Department of Food Science, University of Manitoba. Cooking of the samples was achieved in aluminum trays in a conventional oven at 175° C. for 25 minutes. For individual evaluations, 2 cm. long sections of sausage were presented to the panelists.

A series of preliminary panels were held during which the panelists were familiarized with the product. A series of round table discussions led to the identification of 6 sensory parameters associated with the sausage. These consisted of:

Chewiness: referring to the degree of mastication required to reduce the sample to the state ready for swallowing. An estimate of degree of chewiness was obtained by counting numbers of chews necessary for sample destruction.

Firmness: this parameter was found to be similar to chewiness yet quite distinct. It refers to the resistance to "bite" that the sample imparts.

Juiciness and greasiness: in the sensory evaluation of meat and meat products these characteristics are particularly important in overall textural impression (Stanley, 1976). Panelists recognized this fact and readily agreed to include them as parameters to be evaluated.

Flavour: deviation from characteristic pork sausage flavour as found in the unextended product was considered to be an important parameter in the evaluation.

Overall Impression: panelists were given an opportunity to pool all their observations into one. This parameter was felt to be a statement of acceptability for the product.

A structured line scale was used for individual evaluations. Ballots were prepared as shown in Figure 3

Figure 3. Score sheet used in sensory evaluation.

Evaluation of Pork Sausages -- 1982

Name ____________________________ Date ________________________

For each "descriptor"(ie. chewiness, firmness,etc.) draw a vertical line through the horizontal scale at the point which best describes your coded sample. So that you can use the same line for several coded samples write the code number of your sample at the top of each vertical line. For example:

```
      Code B   Ref       Code C          Code A
         |       |          |               |
    |___|_______|__________|_______________|__________________________|
         |       |          |               |
```

USE THE SAMPLE CODED "R" AS A REFERENCE FOR EACH EVALUATION.

PLEASE EVALUATE THE SAMPLES IN THE FOLLOWING ORDER:

_______ _______ _______ _______ _______

1. Chewiness: Evaluate the number of chews required to reduce the sample to the state ready for swallowing.

```
                                                                 |HIGH
LOW|_____________________________________________________________
    0000000000111111111122222222223333333333444444444455555555556
    0123456789012345678901234567890123456789012345678901234567890
```

2. Firmness: Evaluate the amount of force required to compress the sample between the molars.

```
                                                                 |HIGH
LOW|_____________________________________________________________
    0000000000111111111122222222223333333333444444444455555555556
    0123456789012345678901234567890123456789012345678901234567890
```

3. Juiciness: Evaluate the amount of moisture released in the mouth after 3 chews.

```
                                                                 |HIGH
LOW|_____________________________________________________________
    0000000000111111111122222222223333333333444444444455555555556
    0123456789012345678901234567890123456789012345678901234567890
```

4. Greasiness: Evaluate the amount of grease in the mouth after 3 chews.

```
                                                                 |HIGH
LOW|_____________________________________________________________
    0000000000111111111122222222223333333333444444444455555555556
    0123456789012345678901234567890123456789012345678901234567890
```

5. Flavour:

```
                                                                 |HIGH
LOW|_____________________________________________________________
    0000000000111111111122222222223333333333444444444455555555556
    0123456789012345678901234567890123456789012345678901234567890
```

6. Overall Impression:

```
                                                                 |HIGH
LOW|_____________________________________________________________
    0000000000111111111122222222223333333333444444444455555555556
    0123456789012345678901234567890123456789012345678901234567890
```

7. List any comments regarding the samples.

____

____

____

Thank-you very much for your time. Pascal.

(Larmond, 1977). The panel facilities in the Department of Food Science consist of individual booths with red lights. All evaluations were performed there.

After a series of four "dry" runs the panelists assessed each sample (level of extension) twice. Samples were randomly coded with three digit numbers and presented to the panelists in random order, three samples at a time. The serving order is given in Table 4 . The unextended product served as a reference and its location on the line was determined through agreement among the panelists. Datum was decoded and recorded on a master list.

VIII. Cooking Losses

Determination of cooking losses was performed along with preparation of the samples for sensory evaluations. Samples were weighed prior to and immediately following baking and all juices remaining in the pans were collected in 50 ml. graduated cylinders and measured.

IX. Fat and Water Binding Studies

Binding capacities of the meat-pea protein sausage batters were determined using the method described by Brown and Toledo (1975). Fifty g. samples of raw batter were placed in tared 500 ml. Erlenmeyer flasks. The flasks were covered with aluminum foil and placed in a water bath at

Table 4. Serving order used in taste panels.

Panel	Samples evaluated
1	TP 10% , SDP 20%
2	SDP 40% , SDP 30%
3	TP 30% , SDP 10%
4	TP 20% , TP 40%
5	SDP 40% , TP 30%
6	TP 10% , SDP 20%
7	SDP 30% , SDP 10%
8	TP 20% , TP 40%

80° C. for one hour. At the end of cooking, the liquids released were poured into 50 ml. graduated cylinders and volumes of fat and water lost were measured. Triplicate evaluations were performed on each level of extension.

X. Instrumental Measurements of Texture

Two experiments were performed to determine the effect of protein substitution on sausage texture. The second was carried out after evaluation of the data obtained from the first did not reveal enough information on these effects.

Experiment 1

The experiment was conducted according to the procedure described by Voisey *et al*. (1975) with a few variations. Warner Bratzler (WB) shear values were determined using a double blade WB attachment on the Ottawa Texture Measuring System. This system was selected despite criticism (Voisey and Larmond, 1977) because of its continued use in meat texture evaluation. Samples consisting of one sausage were assessed individually.

Compression values were determined on the same machine using a compression test cell consisting of two circular flat surfaces 10 cm. in diameter. Two cm. long sections of sausage were placed between the surfaces and compressed to complete rupture of the sample. Relaxation characteristics were obtained using the compression cell. Two cm. samples were compressed to a force of about 1.2 kg. and allowed to relax over a period of 4 minutes.

All samples were prepared in the same fashion as for sensory analysis. They were wrapped in aluminum foil immediately after cooking in order to limit temperature and moisture losses until analysis. Triplicate determinations were performed for each level of extension with both SDP and TP as well as on control (0% pea protein) samples.

Experiment 2

Single point measurements are limited in their usefulness in evaluating texture. Multiple texture profiles are more useful in that they can provide indices of several textural characteristics and thus more information about food behavior (Bourne, 1976). An instrumental multiple texture profile analysis of the products was produced according to the method of Voisey and Larmond (1977). The sausage batters were tested in the 10 $cm.^2$ wire extrusion cell of the OTMS using a deformation rate of 5 $cm. min.^{-1}$ Two hundred gram patties were formed using a K-Tel (TM) Patty Stacker and broiled in a conventional oven set at 160° C. for 20 minutes. The samples were cooled to room temperature prior to assaying.

Weighed samples of approximately 25 g. were put into the cells after being cut into 1 $cm.^2$ pieces. They were tested through the cell, collected and recycled a total of six times. Duplicate samples were taken from patties made with batter from sausages extended with SDP and TP at all levels as well as control (0% pea protein). In both experiments, maximum or peak force was recorded from the digital readout on the machine. A strip chart recording of force over time was obtained for all samples. In experiment 2, a Hewlett-Packard integrator was hooked up to the OTMS in order to calculate the areas under these curves.

D. RESULTS AND DISCUSSION.

I. Microbiological Shelf Life Study

The counts observed on SPC, VRB and PDA over a 5 day storage period at 4° C. are shown graphically in Figures 4 to 11 . The points on each graph represent an average of two replicate platings performed on each sample. It is apparent from these figures that in all cases the addition of proteins led to reduced total counts initially. This observation was also made by Judge *et al.* (1974) when ground beef was extended with soy protein and is due to very low bacterial loads on the extenders.

Statistical interpretation of the data revealed that there is no significant difference between counts observed during storage at levels of extension studied in any of the bacteriological assays performed. (See Table 5 . For detailed statistical work see Appendix 1).

It appears, therefore, that either of the extenders may be used in pork sausage without altering mesophilic, psychrophillic, coliform and yeast and mold growth patterns and thus without altering the refrigerated shelf life of the product. These findings are in agreement with some published reports in the literature dealing with extended meat products (Craven *et al*, 1978, Kocet *et al*, 1978).

One cannot conclude from these articles that addition of vegetable proteins to meat products will not lead to increased bacterial loads and therefore, reduced shelf-life in all cases. Different products, extenders, storage conditions, initial bacterial loads and microbiological assays are observed in each study, thus making it impossible to define any effects of extension accurately.

In most of these studies, however, the possible influence of available water on the microbiology of the products was not discussed. This factor is of primary importance in affecting the growth of microorganisms in food (Frazier and Westhoff, 1977). In the products prepared for this study the water levels were kept constant. As will be shown later, the water holding capacity of the sausage increased with accrued level of extension with both proteins. It is possible that available water thus remained the same or actually decreased over level of extension whereas this was not the case in studies where significant effects on microbiology were observed (Judge et al, 1974; Foster et al, 1978). Other factors which may have led to the discrepancies observed would include specificity of some bacteria for utilization of certain proteins (Sikes and Maxcy, 1979), and the presence of extraneous materials from the extenders such as carbohydrates, minerals and amino acids (Draughton, 1980).

FIG. 4 . SHELF LIFE STUDY OF SAUSAGE EXTENDED WITH TP.

COUNTS ON Spc, 32°.

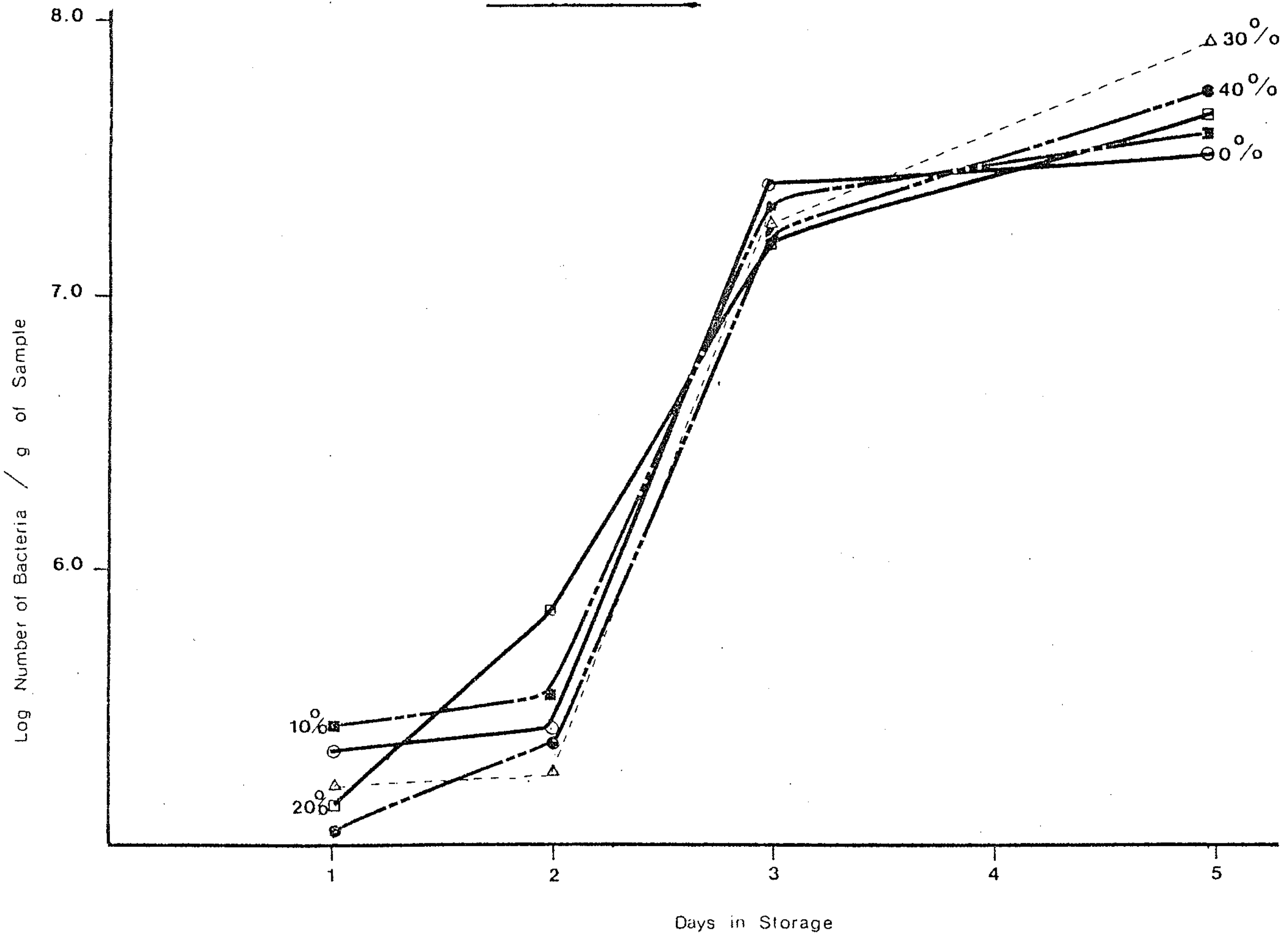


FIG. 5. SHELF LIFE STUDY OF SAUSAGE EXTENDED WITH TP.

COUNTS ON Spc, 4°.

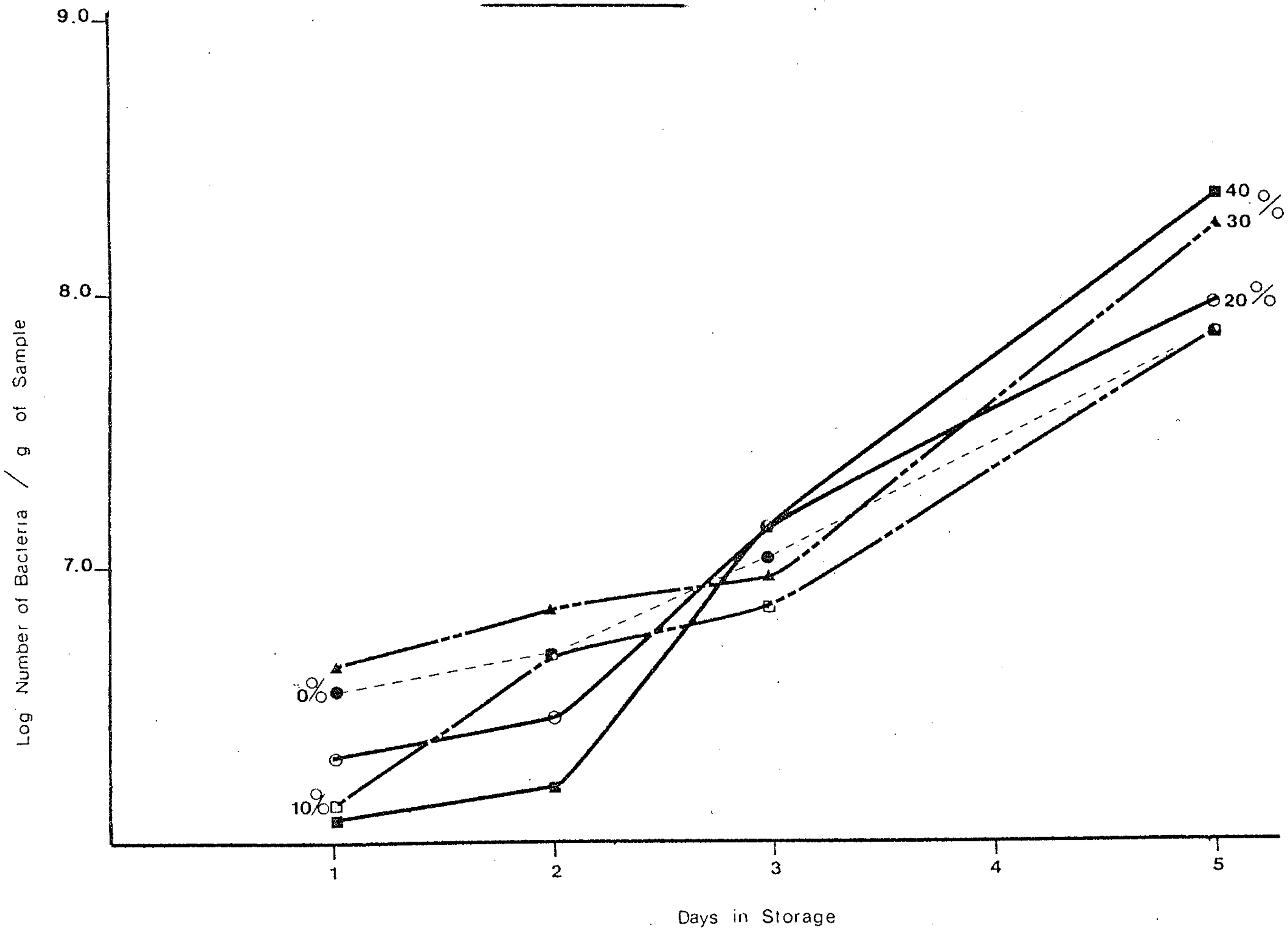


FIG. 6 . SHELF LIFE STUDY OF SAUSAGE EXTENDED WITH TP.

COUNTS ON Vrb.

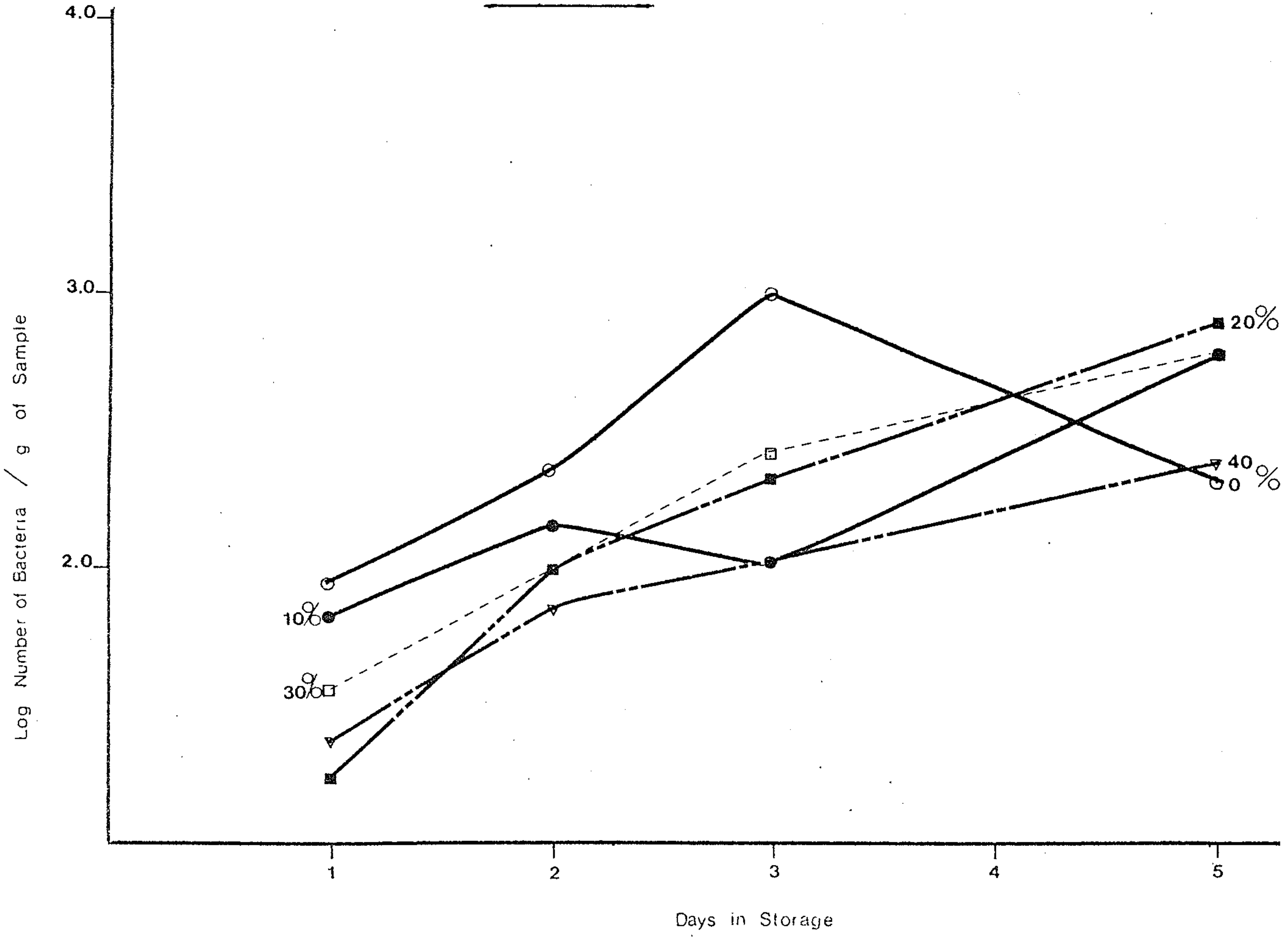


FIG. 7. SHELF LIFE STUDY OF SAUSAGE EXTENDED WITH TP.

COUNTS ON Pda.

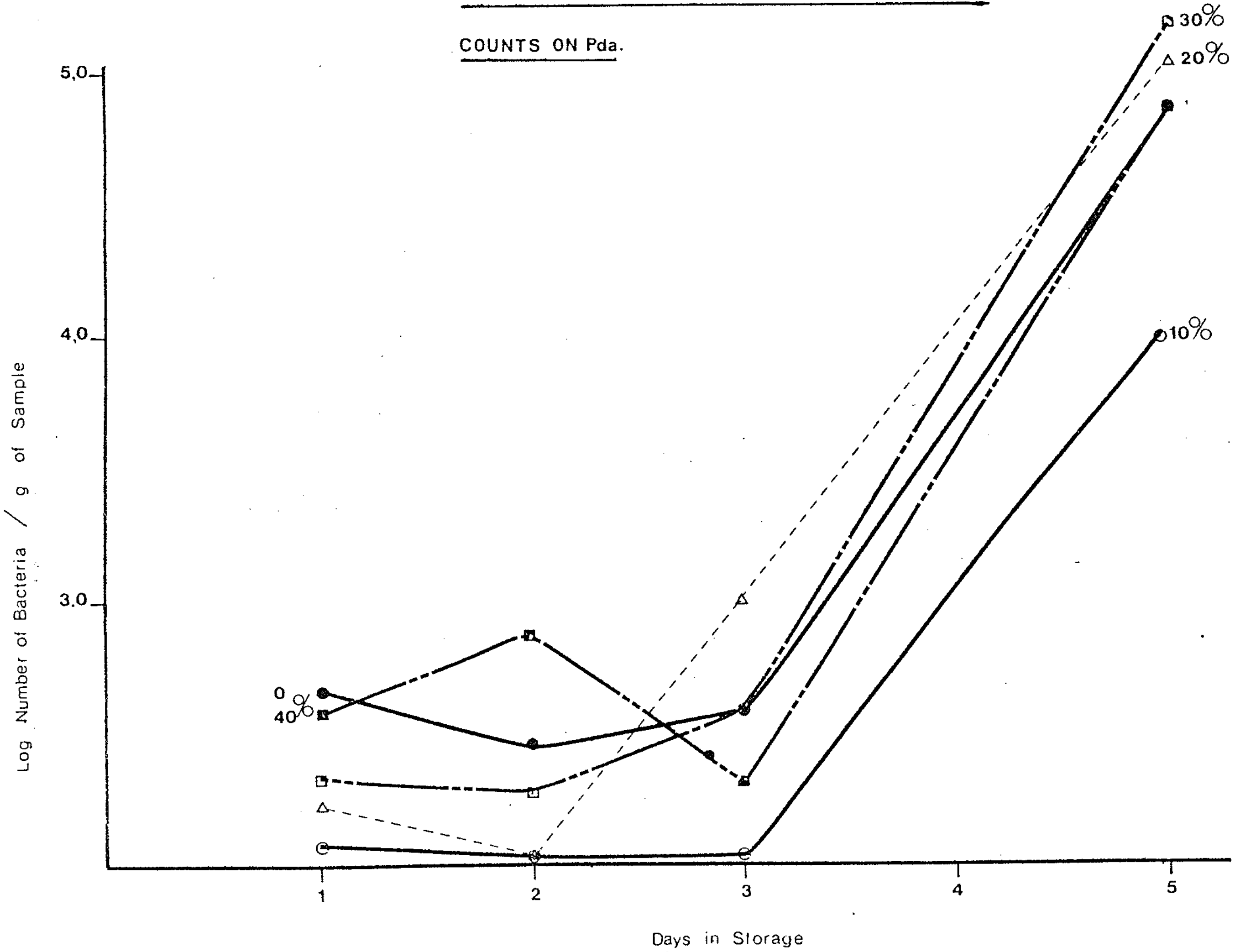


FIG. 8. SHELF LIFE STUDY OF SAUSAGE EXTENDED WITH SDP.

COUNTS ON Spc, 32°.

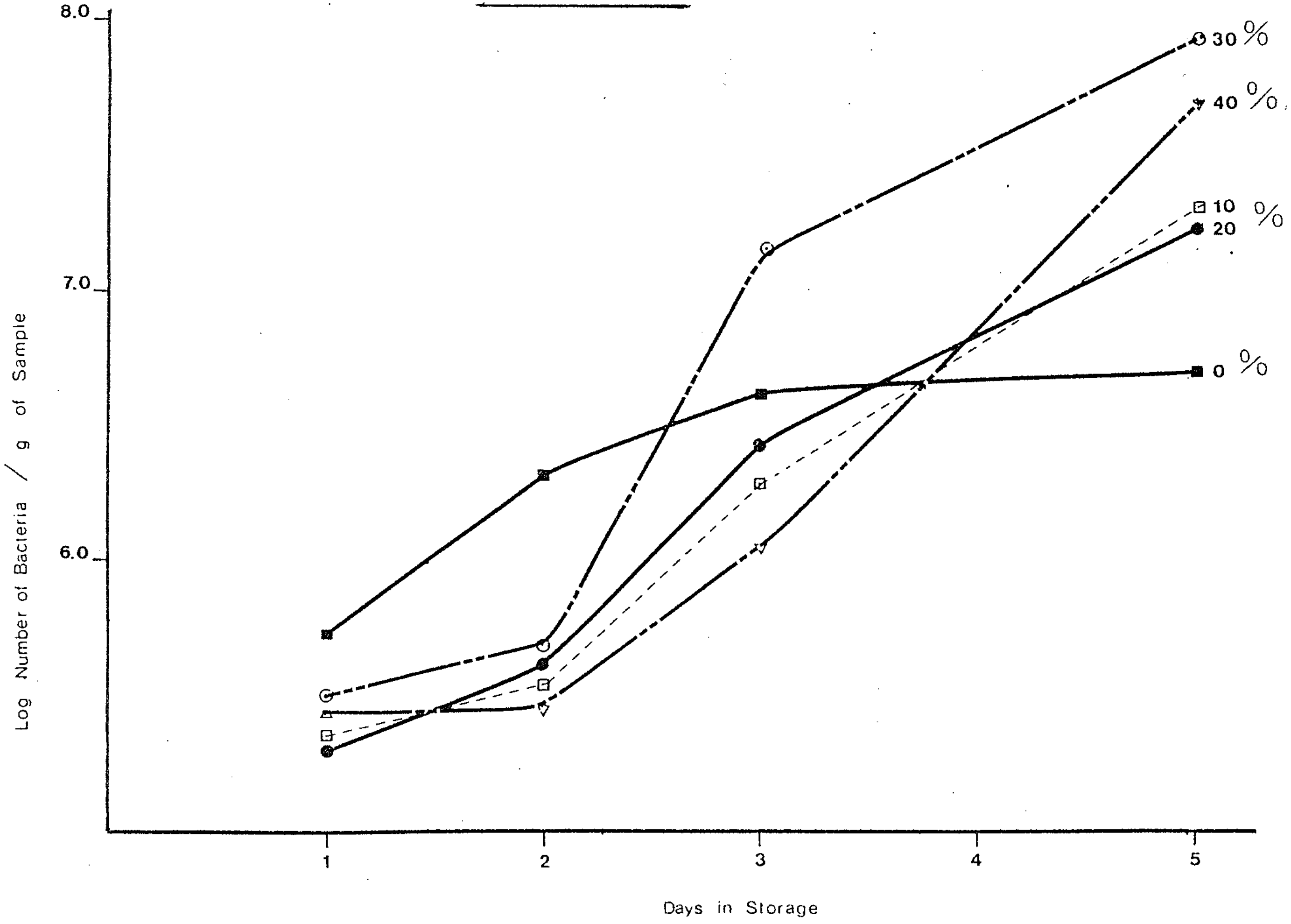


FIG. 9 . SHELF LIFE STUDY OF SAUSAGE EXTENDED WITH SDP.

COUNTS ON Spc, 4°.

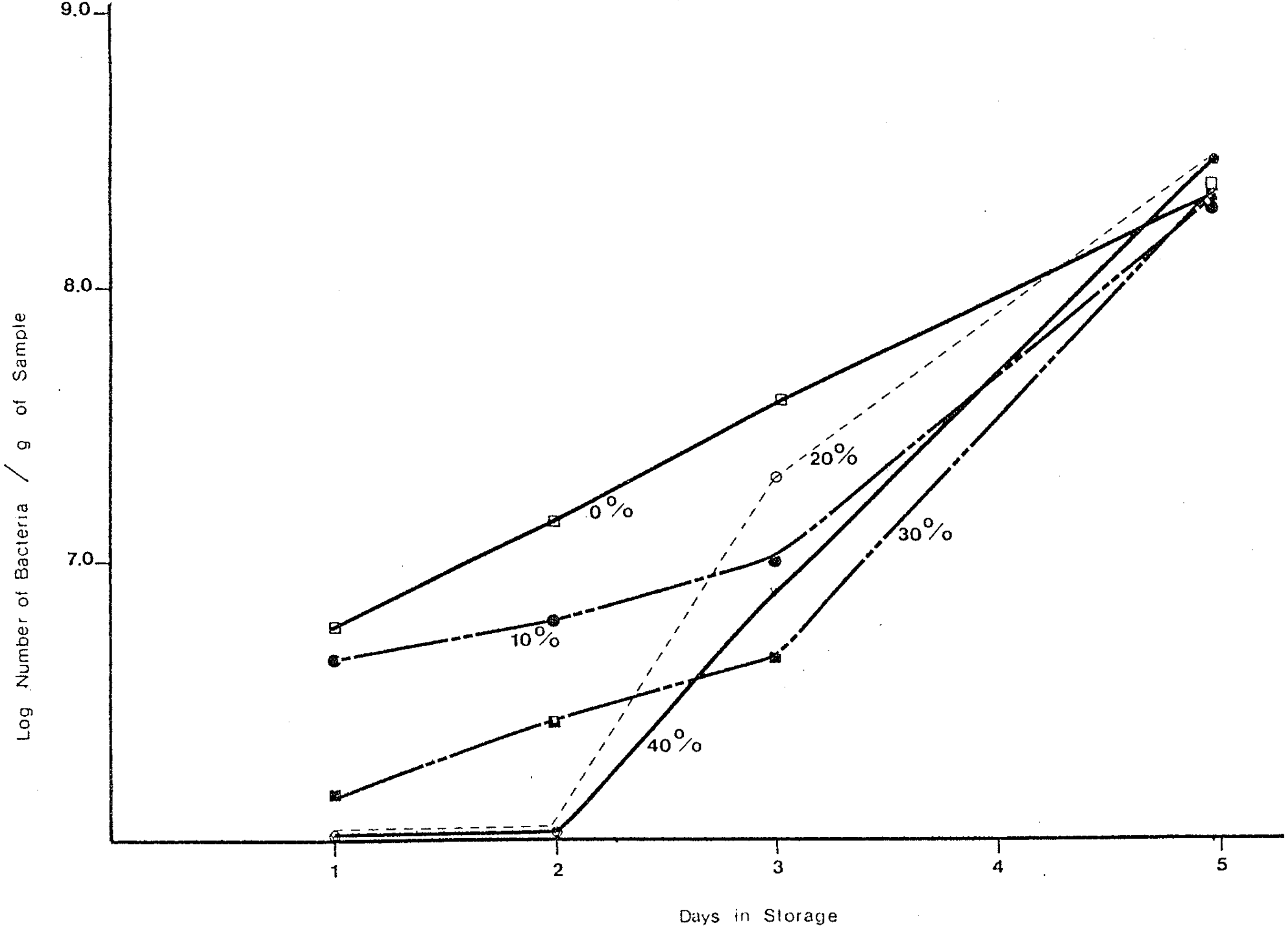


FIG.10. SHELF LIFE STUDY OF SAUSAGE EXTENDED WITH SDP.

COUNTS ON Vrb.

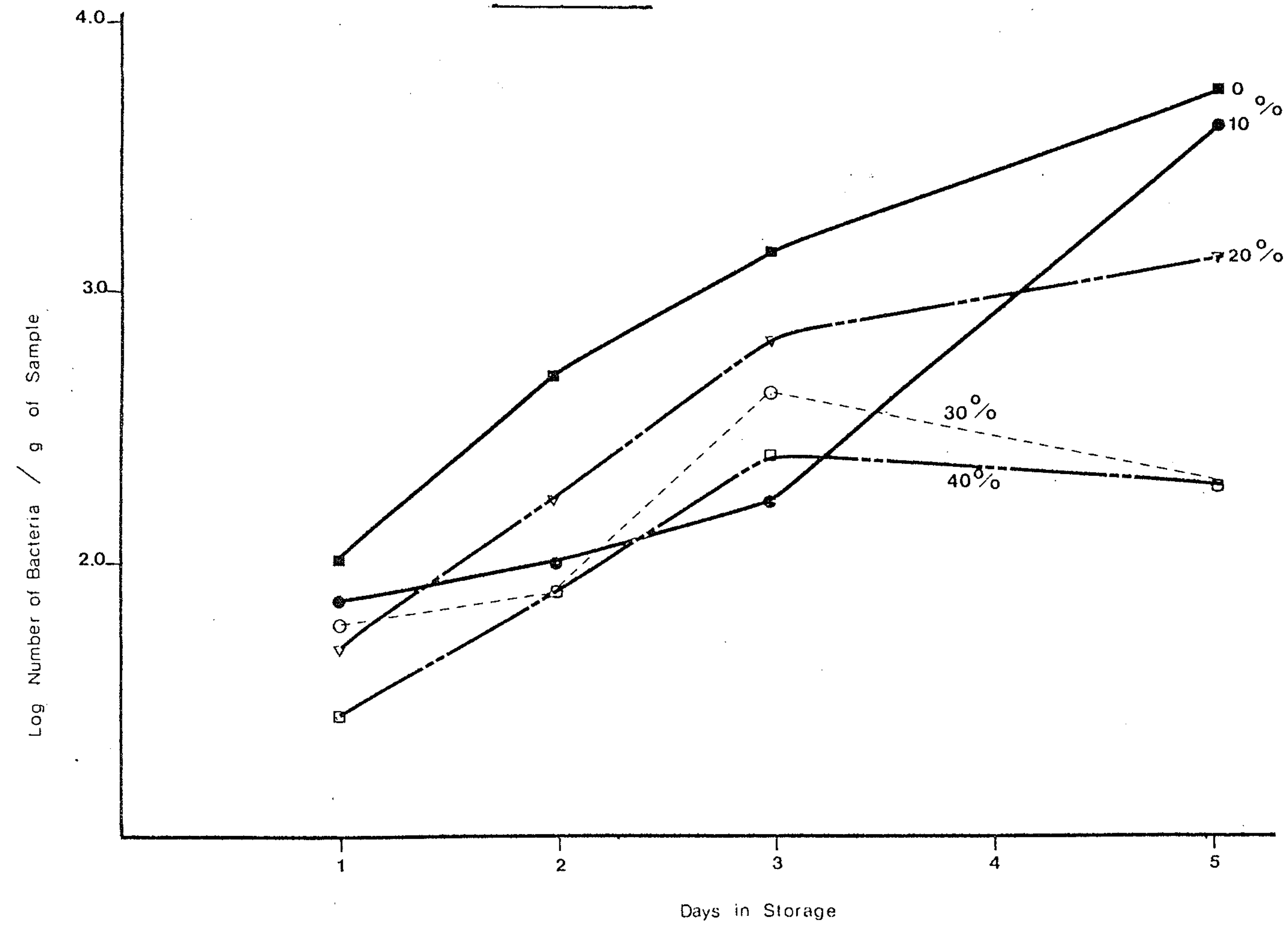


FIG.11 . SHELF LIFE STUDY OF SAUSAGE EXTENDED WITH SDP.

COUNTS ON Pda.

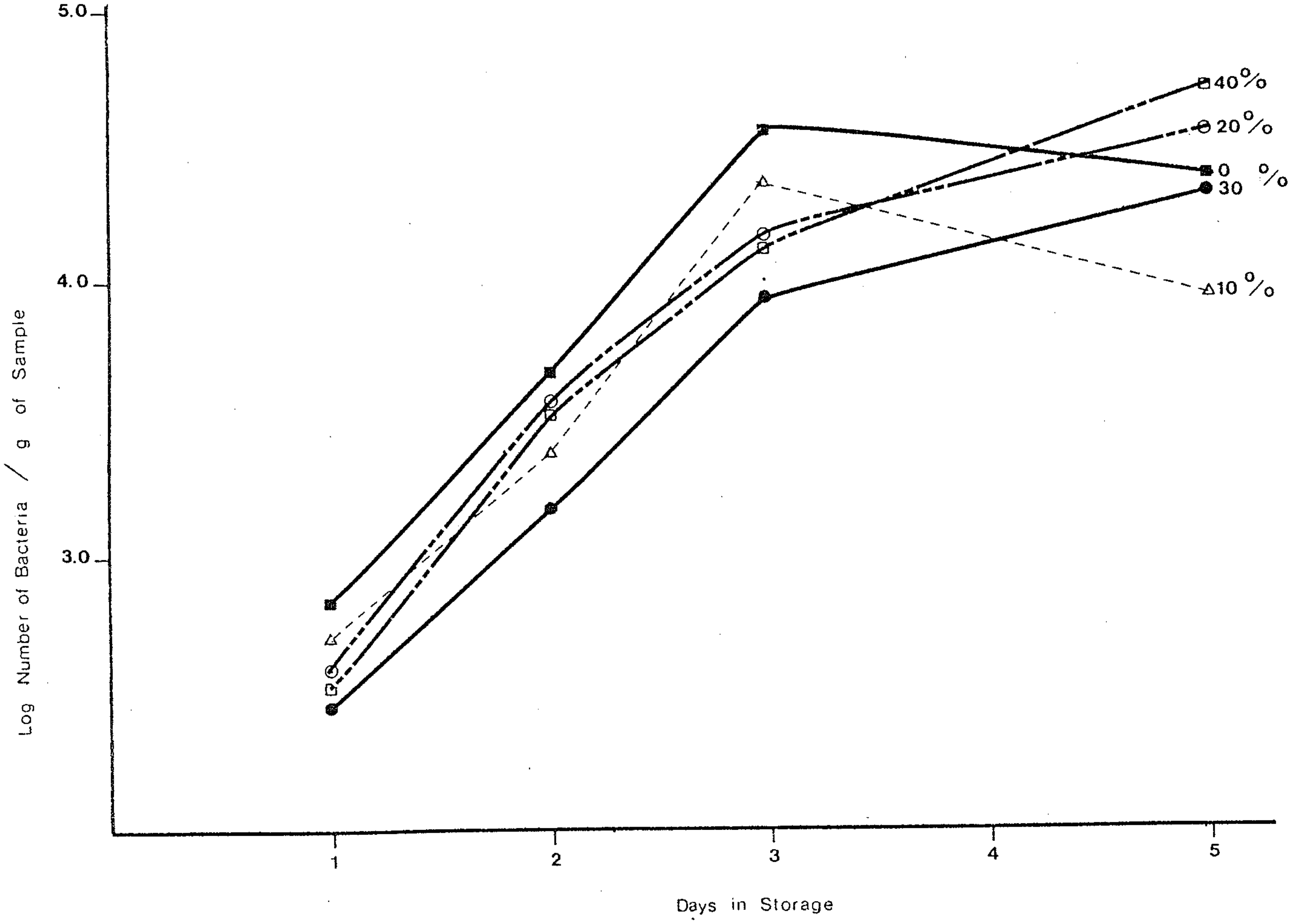


TABLE 5 . Results of Friedman's Test statistic on counts observed or sausage extended with SDP and TP at 4 levels.

Protein	Microbiological Assay	S value
SDP	Spc 30	2.93 NS
SDP	Spc 4	5.52 NS
SDP	VRB	3.12 NS
SDP	PDA	6.70 NS
TP	Spc 30	.80 NS
TP	Spc 4	2.28 NS
TP	VRB	3.67 NS
TP	PDA	8.16 NS

NS = not significant at $\alpha = .05$

$s (.05, 5, 3) = 8.4$

II. Proximate Analysis

Proximate analyses of the sausages substituted with both spray dried pea protein (SDP) and textured pea protein (TP) are shown in Table 6. The level of protein increased slightly with increasing levels of protein substitution. Therefore, it can be said that the products with added protein concentrates were true extended meat products (Judge *et al*., 1974). As a result of these higher protein levels a decrease in total fat was observed at all levels. Water content also varied slightly. It had been hoped this would not be the case as this may have had an influence on sensory juiciness response and texture. Fortunately, this variation was not large and may have been due to variations within samples rather than error in formulation.

TABLE 6. Proximate analyses of the sausage in the uncooked state.

Protein extender and Level	% H_2O	% Fat	% Protein	% Ash
Control	57.50	28.59	12.60	1.13
SDP 10%	58.25	26.74	13.51	0.99
SDP 20%	59.78	23.39	13.68	1.08
SDP 30%	61.34	20.30	14.83	1.01
SDP 40%	62.95	18.23	15.95	0.97
TP 10%	58.20	25.59	13.88	1.01
TP 20%	59.94	22.35	14.34	1.07
TP 30%	61.49	19.93	15.02	1.10
TP 40%	60.17	18.58	16.38	.98

III. Sensory Analysis.

Graphical representations of results obtained from sensory analysis are shown in figures 12 to 17. Average scores were calculated using data from all panelists and replicates and plotted against level of extension for all sensory parameters. Results of statistical work are given in appendix 2.

Figures 12 and 13 indicate that addition of textured (TP) protein had little effect on sensory chewiness and firmness scores , a result reflected in a lack of statistically significant difference among levels (Appendix 2B). There was a decrease in firmness scores over increasing SDP levels, but no significant changes in chewiness (Appendix 2A). The type of protein used in extension had an effect on sensory chewiness and firmness scores as is revealed from statistical work (Appendix 2C). Mean sensory scores for chewiness and firmness were significantly lower over all levels of substitution with SDP than TP.

Figure 14 shows the effect of protein substitution on sensory juiciness response. The average scores decreased over level of protein replacement with both proteins. No effect of protein type on juiciness was observed from statistical data.The SDP protein replacement had no effect on greasiness scores (Fig. 15, Appendix 2A). Substitution with TP, on the other hand, decreased the average greasiness scores (Fig15, Appendix 2A).

The responses observed in figure 16 for products extended with SDP revealed an adverse effect on flavour scores (Appendix 2C). TP also had the effect of decreasing average scores over level of extension but to a lesser extent than SDP. These observations are supported by statistical data (Appendix 2C). Figure 17 shows that sensory results for overall acceptability were very similar to those observed for flavour,indicating that flavour probably had an overbearing influence on the acceptability of the products. A significant decrease in overall acceptability occurred with increasing levels of protein substitution, as was observed in the case of flavour (Appendix 2C).

Panelists were given the opportunity to record comments on the products they evaluated. All the comments registered concerned flavour and texture problems. Table 7 reveals the numbers of comments for each product studied. At higher levels of extrusion off flavours described as "beany" were recorded with both protein extenders. More panelists reported "soft" or "mushy" textures in product extended with SDP, again at higher levels.

The textured protein extender has less effect on sensory chewiness, firmness, flavour and overall acceptability scores than the spray-dried form. Hence,texturization of the protein concentrate has altered the sensory performance of the extender. The differences in responses observed for greasiness and juiciness are unexpected, however. Results of the cooking losses study indicate that both extenders retain water and fat to the same extent upon cooking. Since water content was kept constant in all formulations it follows that this cannot be the cause of the discrepancy. Different fat levels in the formulation were earlier described as being slight. It is possible, however, that this was perceptible by the panelists. Whether this would influence juiciness scores is unknown.

Flavour problems in extended meats have been reported often in the literature (Carlin et al., 1978). Problems usually occur at higher levels of extension and are characteristically linked to the appearance of "beany" off-flavours. The nature of the substances responsible for this occurrence has not been fully described (Ohren, 1981). Texturizing the protein concentrate had an effect on flavour in the sausage. It is possible that extrusion into the hot water may have led to a loss of factors responsible for off-flavours in the extender. Fiber formation may also be accompanied by a sequestering effect on these factors thereby reducing their flavour intensity.

In both proteins studied as extenders of pure pork sausage, the mean scores observed over all sensory parameters were not significantly affected by substitution at levels up to 20% (Appendix 2A and B). This indicates that 20% pea PMM by weight could correspond to an acceptable level of use in the commercial manufacture of pork sausage. At higher levels of substitution the characteristics of the product change significantly.

FIG 12

EFFECT OF PROTEIN EXTENDER ON SENSORY CHEWINESS SCORES

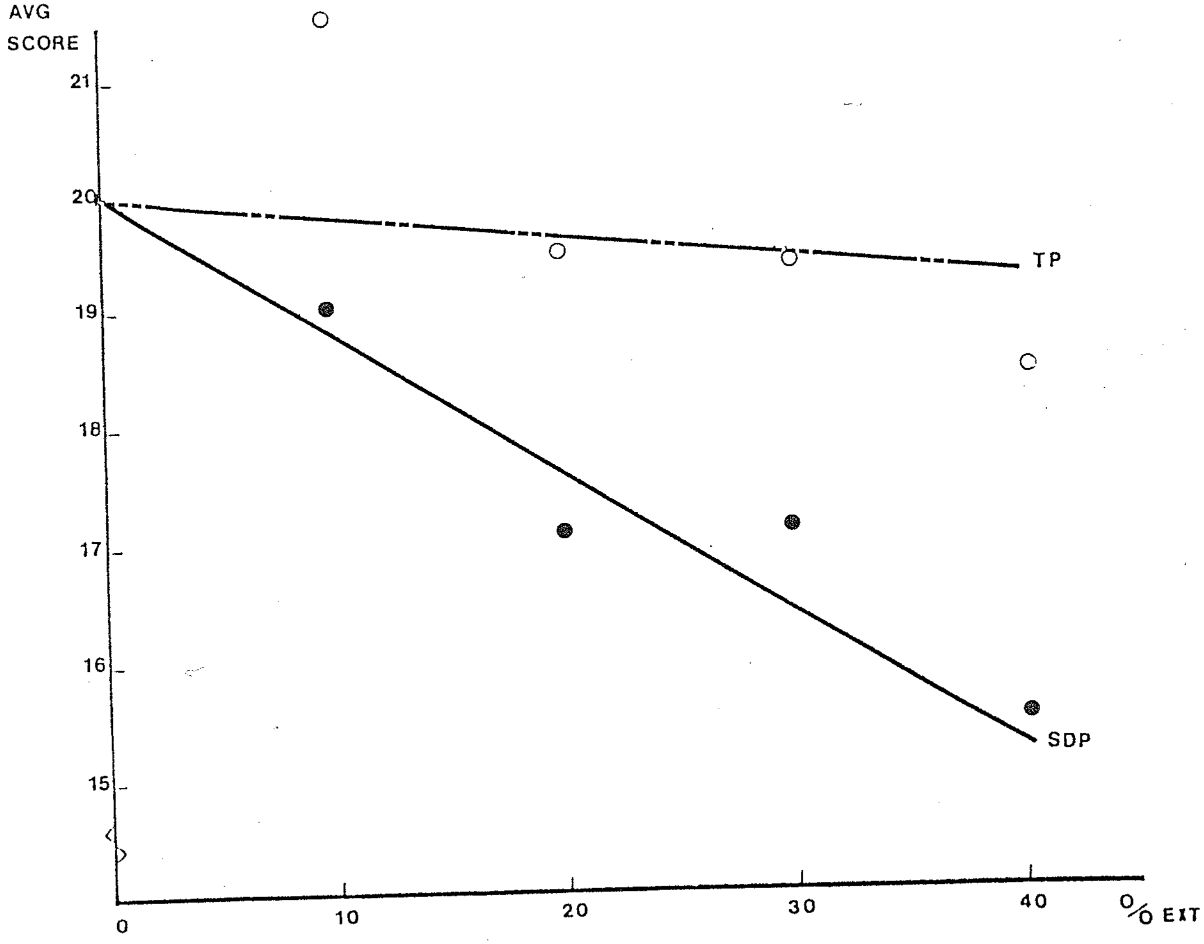


FIG 13

EFFECT OF PROTEIN EXTENDER ON SENSORY FIRMNESS SCORES

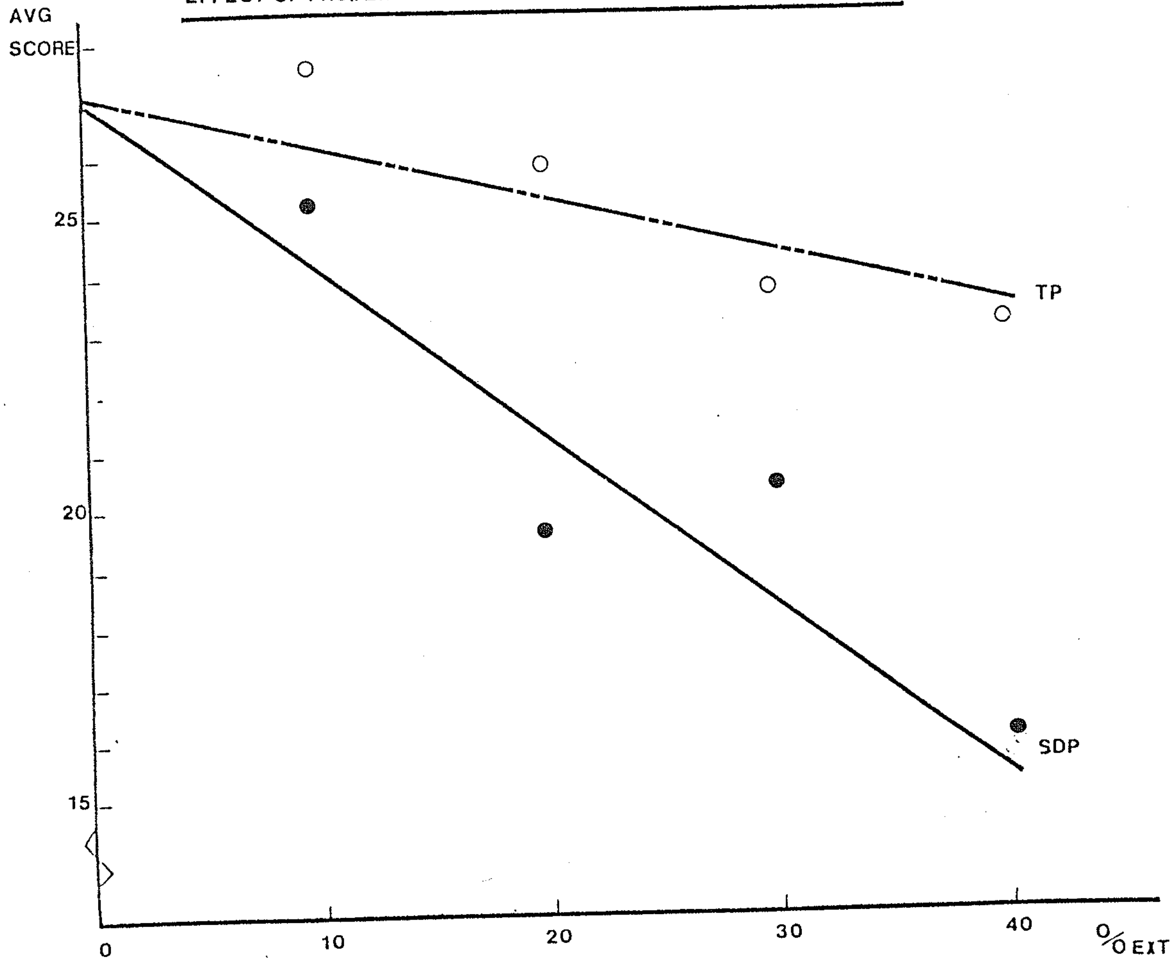


FIG 14.

EFFECT OF PROTEIN EXTENDER ON SENSORY JUICINESS SCORES

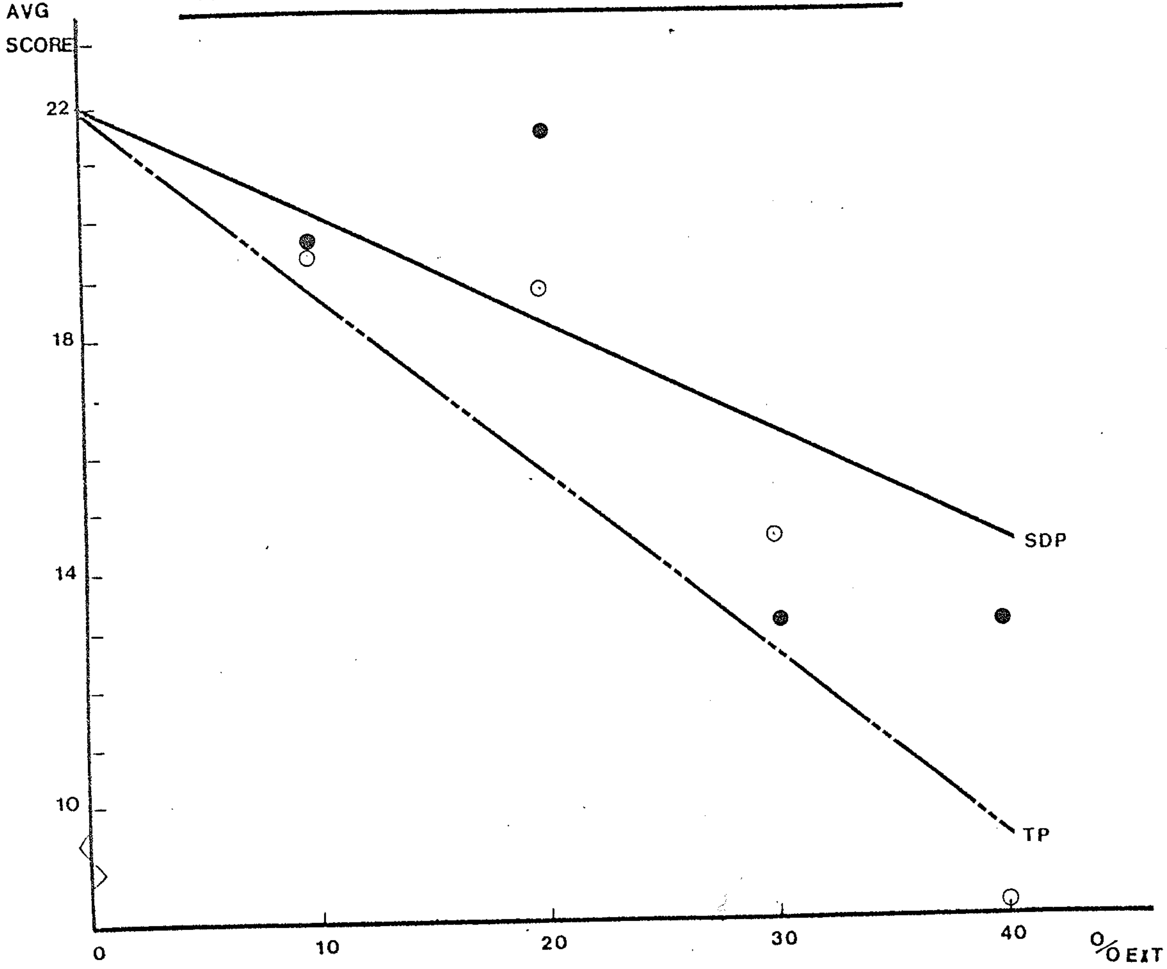


FIG 15

EFFECT OF PROTEIN EXTENDER ON SENSORY GREASINESS SCORES

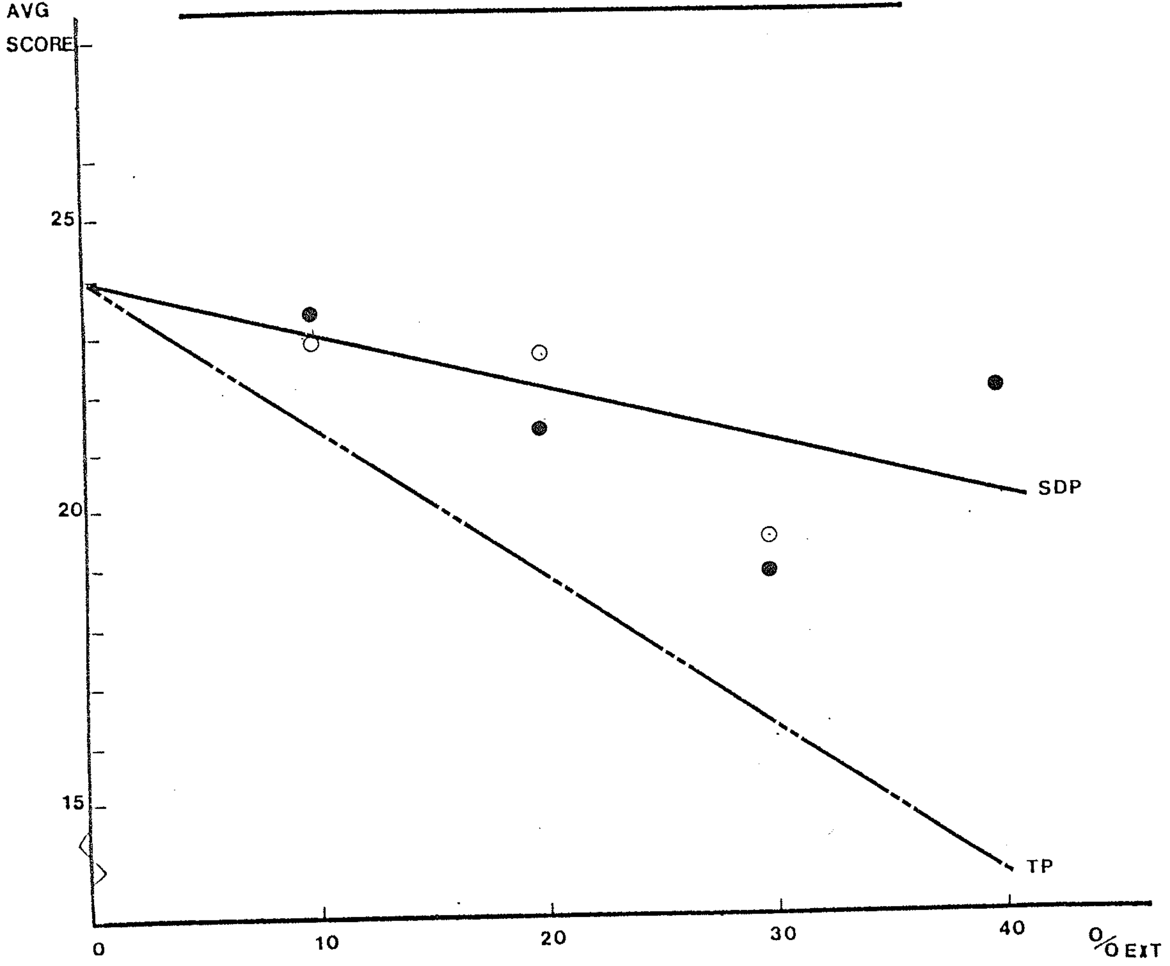


FIG 16

EFFECT OF PROTEIN EXTENDER ON SENSORY FLAVOUR SCORES

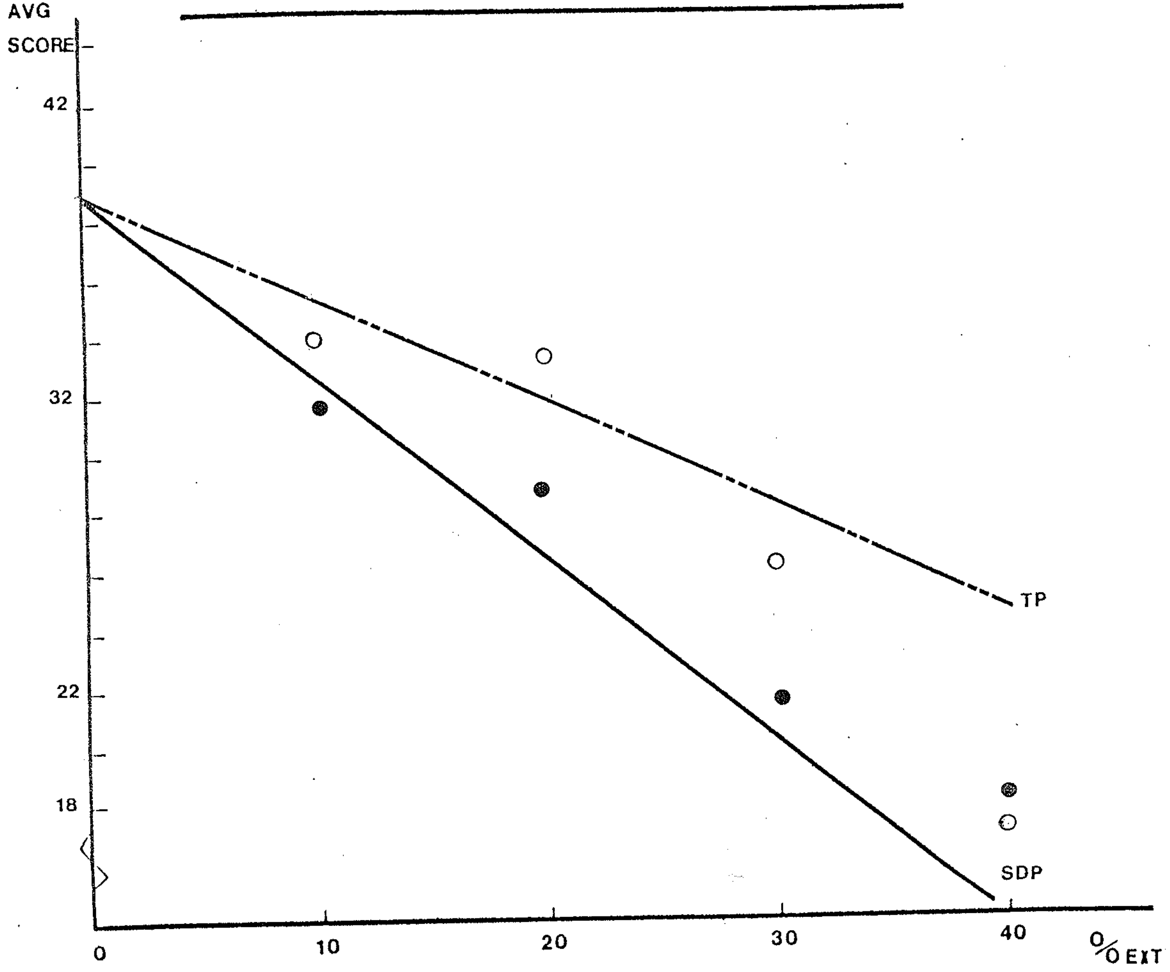


FIG 17

EFFECT OF PROTEIN EXTENDER ON SENSORY OVERALL SCORES

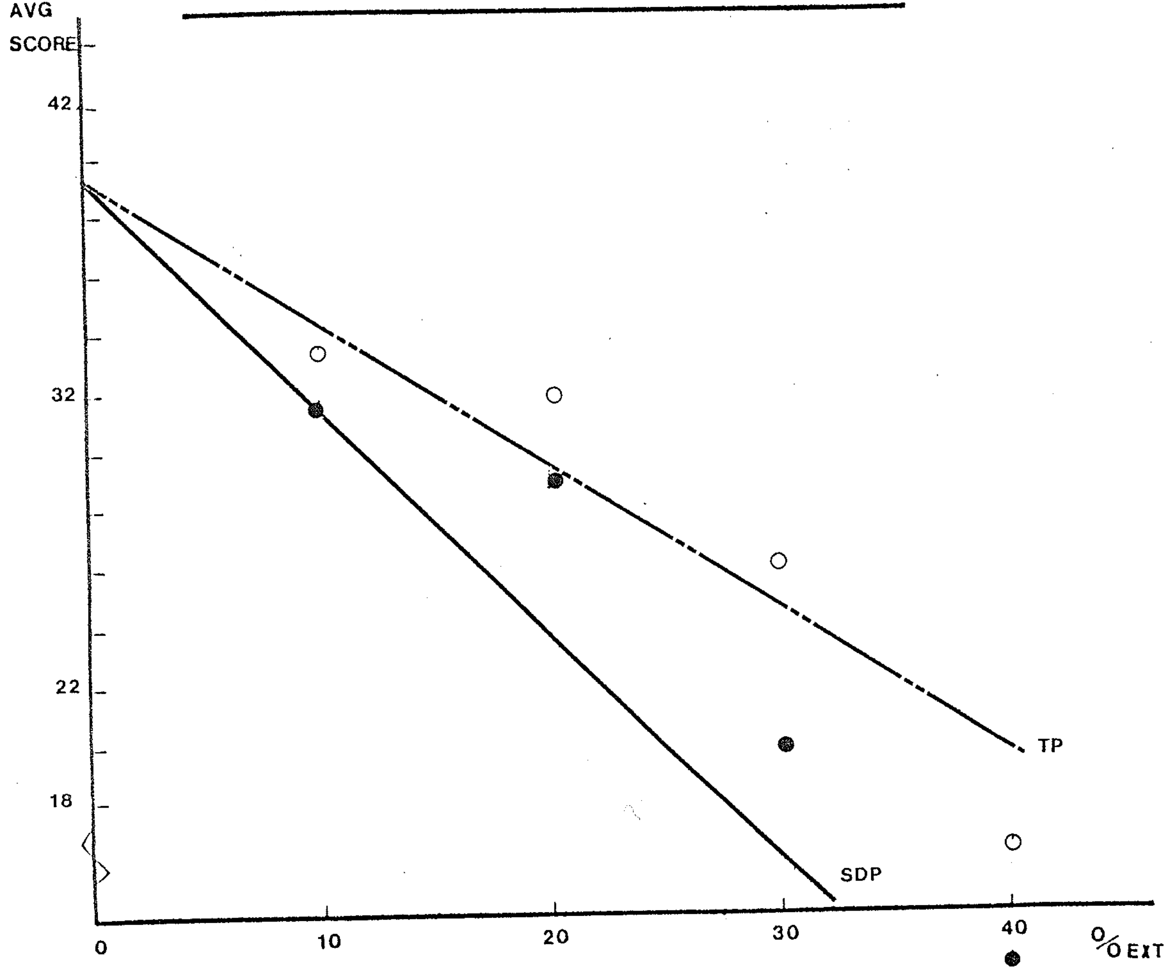


TABLE 7. Comments registered by panelists pertaining to products evaluated.

Protein extender	Flavour	Texture
SDP 10%	0	0
SDP 20%	2	1
SDP 30%	3	3
SDP 40%	4	4
TP 10%	1	0
TP 20%	1	1
TP 30%	3	1
TP 40%	5	2

- Flavour comments included: beany, floury, hay-like, dry.
- Texture comments included: soft (at higher levels of extension), mushy , crumbly (SDP at high levels of extension).

IV. Cooking Losses and Fat and Water Binding

The percentage weight loss during cooking was plotted as a function of level of extension with the two proteins in Figure 18 . The lines were drawn from regression equations as shown in Appendix 3 . Results show the effects of substitution to be similar with both TP and SDP: a reduction in weight loss of about 4% per 10% addition of protein was observed. Hence, both meat substitutes led to a substantial reduction in cooking losses in the product.

The juices left in the pan after cooking were collected and measured. It was hoped that the water and fat layers would separate in measuring cylinders so that an estimate of losses of both could be obtained. In each case, however, a thick third layer appeared which made interpretation difficult. This layer seemed to consist of water, fat and protein in emulsion. The total loss of liquid per gram of meat was plotted against level of extension in Figure 19 . Losses in liquids were similar for products extended with both proteins. A decrease occurred over level of extension which directly reflected losses in weight as reported in Figure 19.

An attempt to estimate fat and water binding capacities was made as reported in the experimental section. Figure 20 shows the results of two such experiments. The data obtained was inconclusive. There was inconsistency in data from separate experiments and wide variation in replicates within experiments. The procedure may be more effective with finely comminuted products than with coarse ground sausage because of better meat/fat particle distribution and even extraction rates. Hence, no useful information regarding fat and water binding could be obtained other than from cooking losses.

Fig 18 Percent cooking losses as a function of level of extension.

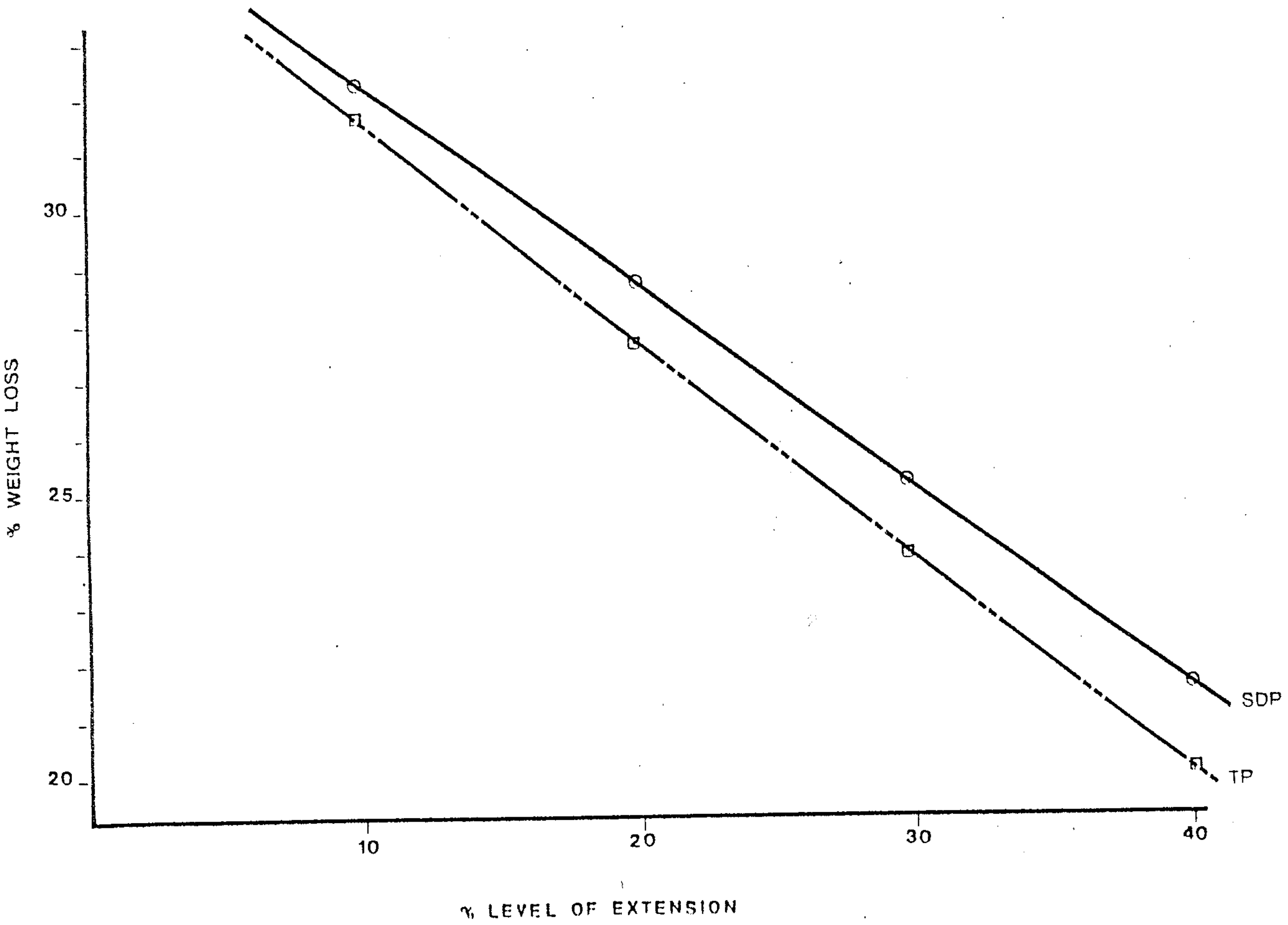


Fig 19. Losses in liquid/g of sausage meat

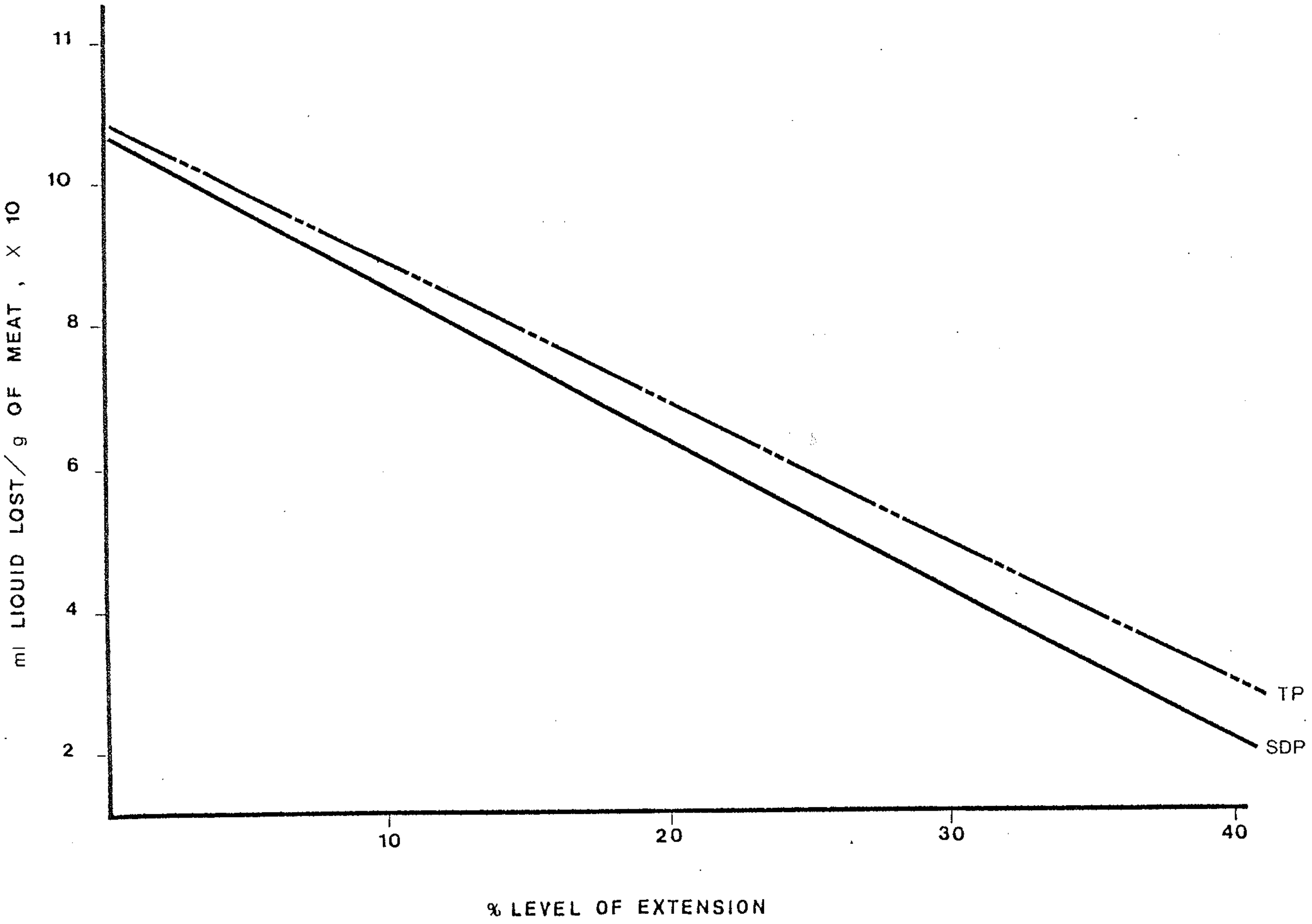
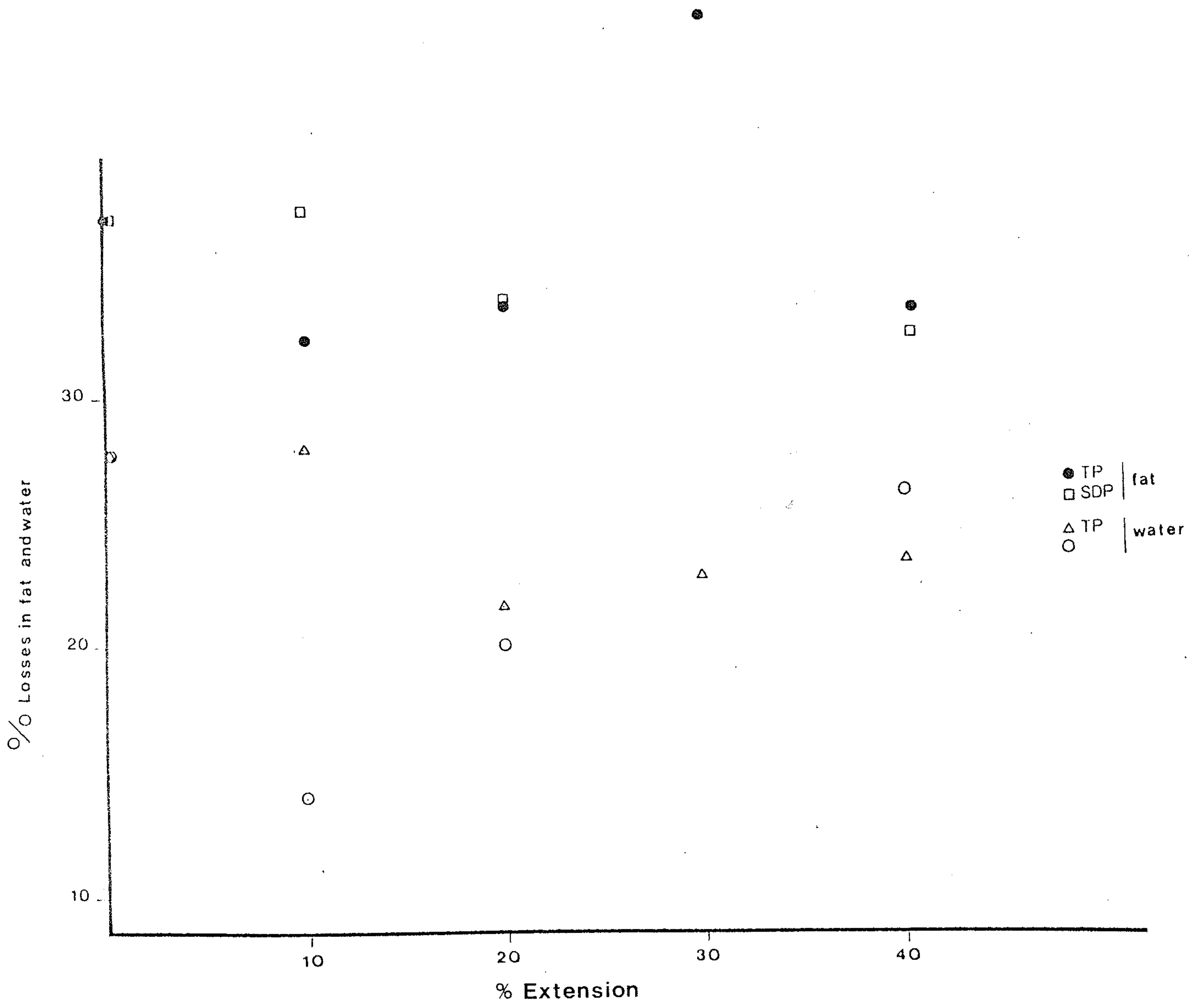


Figure 20. Cooking losses in fat and water as affected by SDP and TP.



V. Instrumental Measurements of Texture

Experiment I

Typical response curves for the three tests performed are given in figure 21. In the Warner Bratzler shear test, the forces applied built up non-linearly to a peak until failure occured due to breakage of the sample. In Figure 22, the forces were plotted against level of extension for all products. TP had no effect on the maximum force to break but substitution with SDP led to a decrease in force with increasing level of extension. The type of protein used to extend the sausage, therefore, had a marked effect on the Warner-Bratzler shear test. This result was also reflected in statistical work as reported in Appendix 4.

In the compression test the force built up to a maximum until the sample ruptured and then decreased rapidly. Maximum compression to rupture forces provide a measure of the shearing strength of the samples. These forces were plotted against level of extension in Figure 23. Extension with both proteins led to an increase in maximum rupture forces. The response was very pronounced with TP and was non-linear. The effect of SDP was not as dramatic but a slight increase in force may be observed. Statistics revealed a significant effect on compression to rupture forces due to type of protein used to extend the product.

Relaxation time effects are shown in Figure 24. Relaxation time is equated with resilience in physical terms (Voisey and Larmond, 1977). SDP had no effect on this factor while increasing levels of TP led to an increasing relaxation time. Once again there was a difference in effects due to type of protein extender which was significant statistically (Appendix 4).

Reported correlations between Warner-Bratzler readings and sensory response cover a wide range (Voisey and deMan, 1976). Whether this is due to poor experimental conditions and incorrect interpretations of results or improper application of correlation techniques still represents a very controversial subject (Korth, 1982). The same situation exists in trying to establish correlations between all objective and sensory texture measurements (Szczesniak, 1968). The main area of contention lies in difficulty in determining whether the two factors being considered are dependent, a requirement in correlation theory, or independent, thereby leading to chance correlations. In the past, most researchers have assumed dependence between experimental objective and sensory methods in texture measurement and have determined correlations from data under these erroneous conditions. The author believes this approach to be unacceptable and, therefore, no correlation data have been generated from the experiments performed under this study.

It is apparent from the data that chewiness and firmness scores as presented in Figures 12 and 13 show a response very similar to that observed for Warner-Bratzler scores in Figure 22. Hence it appears that this objective test confirms the sensory observation that extension with TP and SDP led to decreases in these sensory responses. There exists a serious drawback to readings from the Warner-Bratzler press, however, in that they combine two properties of the sample which may or may not be dependent on each other: 1) firmness as indicated by the applied force and compression area of the sample, a viscoelastic property of the sample; and 2) tensile rupturing properties of the sample (Stanley, 1976). It is unclear, therefore, whether the similarities in objective and sensory responses are a result of assessing the same textural properties. For this reason it cannot be assumed that Warner-Bratzler readings confirm the effect of extension observed in sensory chewiness and firmness scores.

Compression to rupture results were highly unexpected. With decreasing sensory firmness scores compression forces should theoretically be observed to decrease. The opposite was observed especially in products extended with TP. These results could not be explained. A similar behaviour is observed with relaxation time. Again scores rise over level of substitution, especially with TP, while sensory chewiness scores show a decrease. Sensory chewiness was expected to increase with resilience in the sample.

Because of the contradictions observed in this experiment a more accurate method for comparing mechanical food characteristics to sensory data was sought. This led to the investigation of instrumental multiple texture profile analysis methods because of advantages toward yielding more information about food behaviour (Voisey and Larmond, 1977).

FIG 21 . Typical force responces in Warner-Bratzler, compression, and relaxation tests.

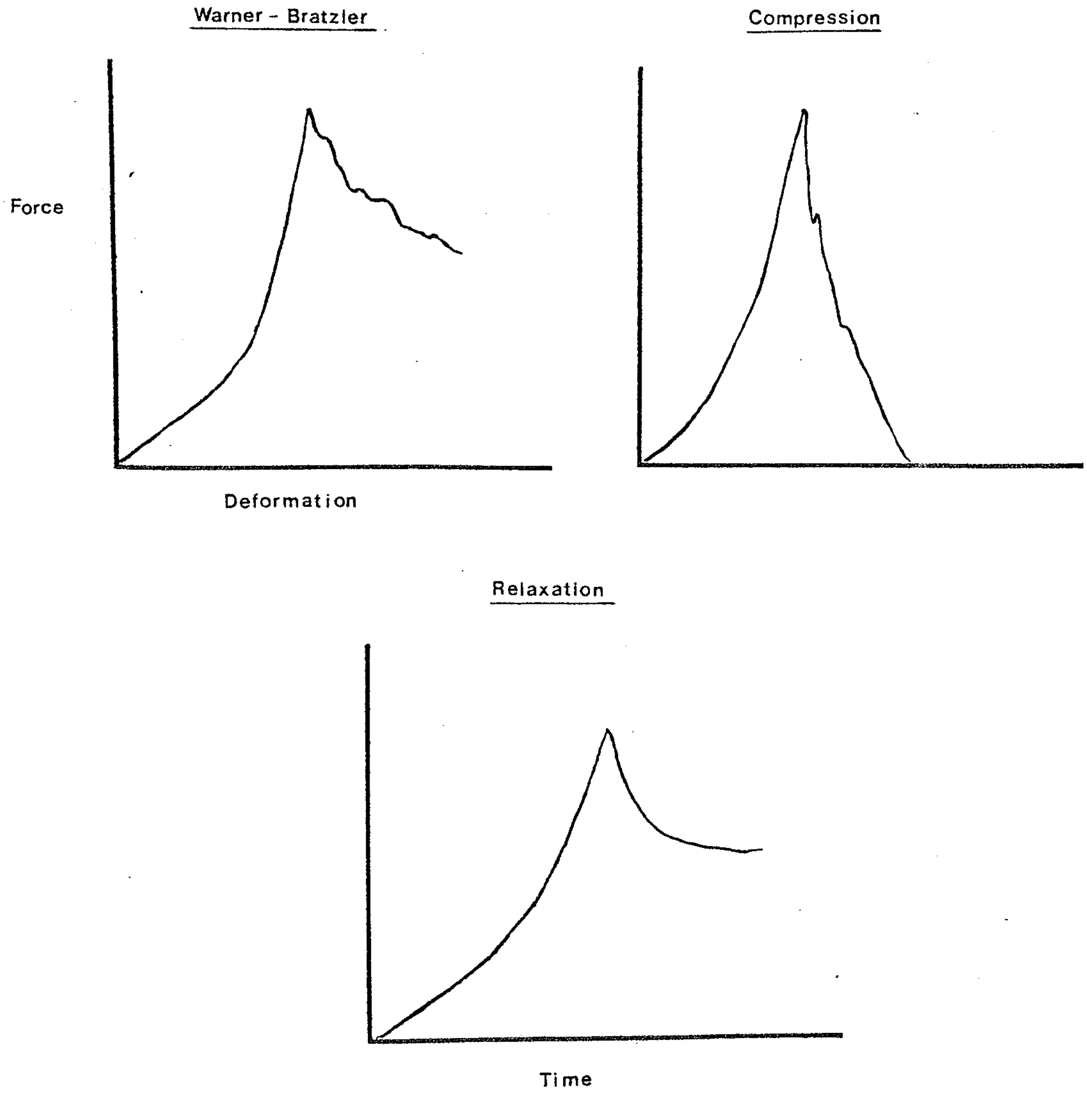


FIG 22

EFFECT OF PROTEIN EXTENDER ON WARNER–BRATZLER SHEAR VALUES

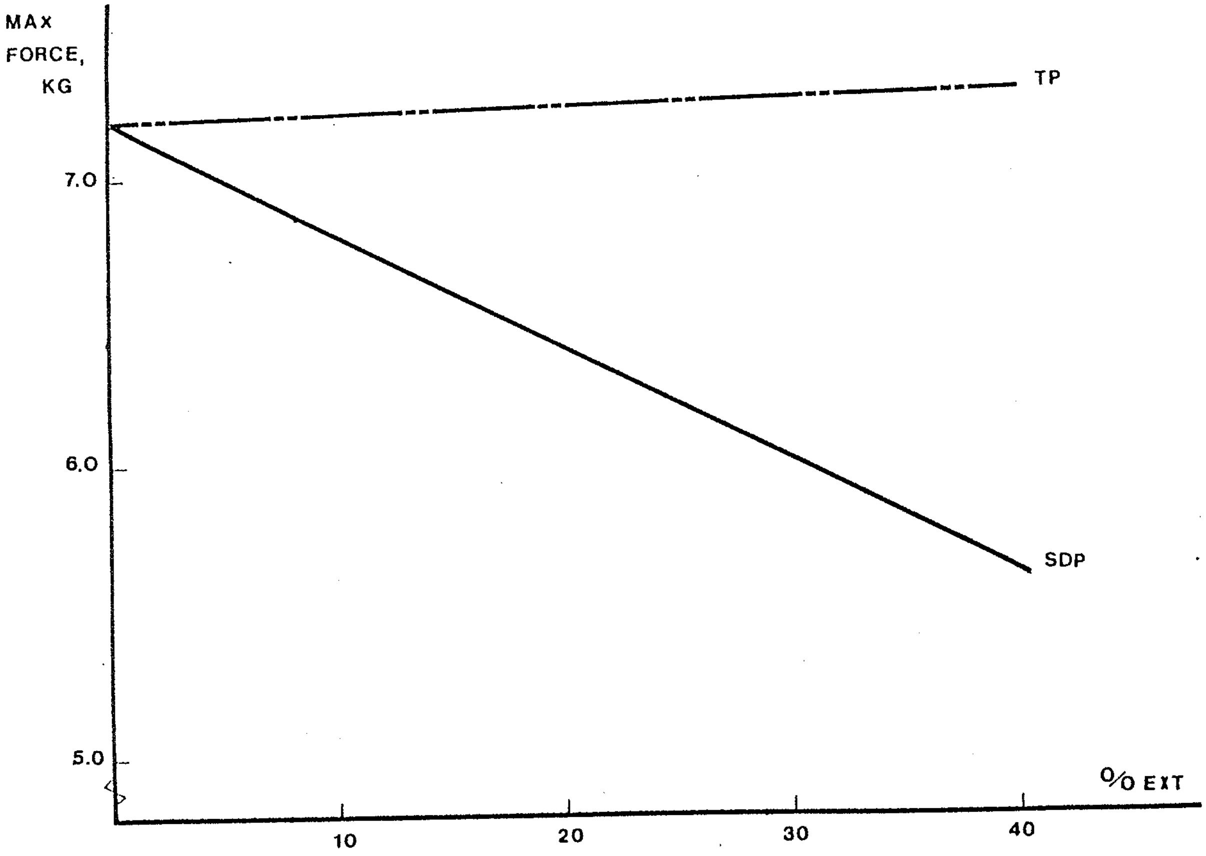
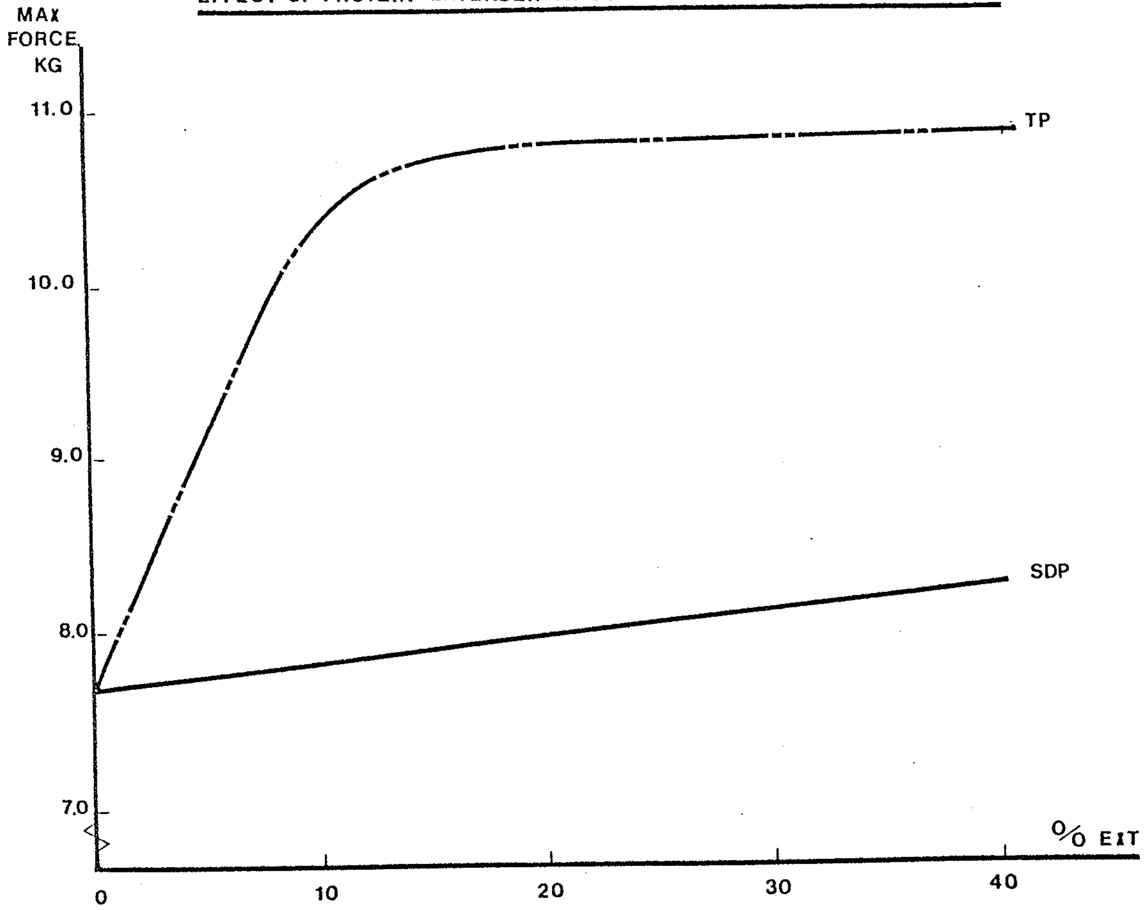


FIG 23

EFFECT OF PROTEIN EXTENDER ON COMPRESSION TO RUPTURE FORCES



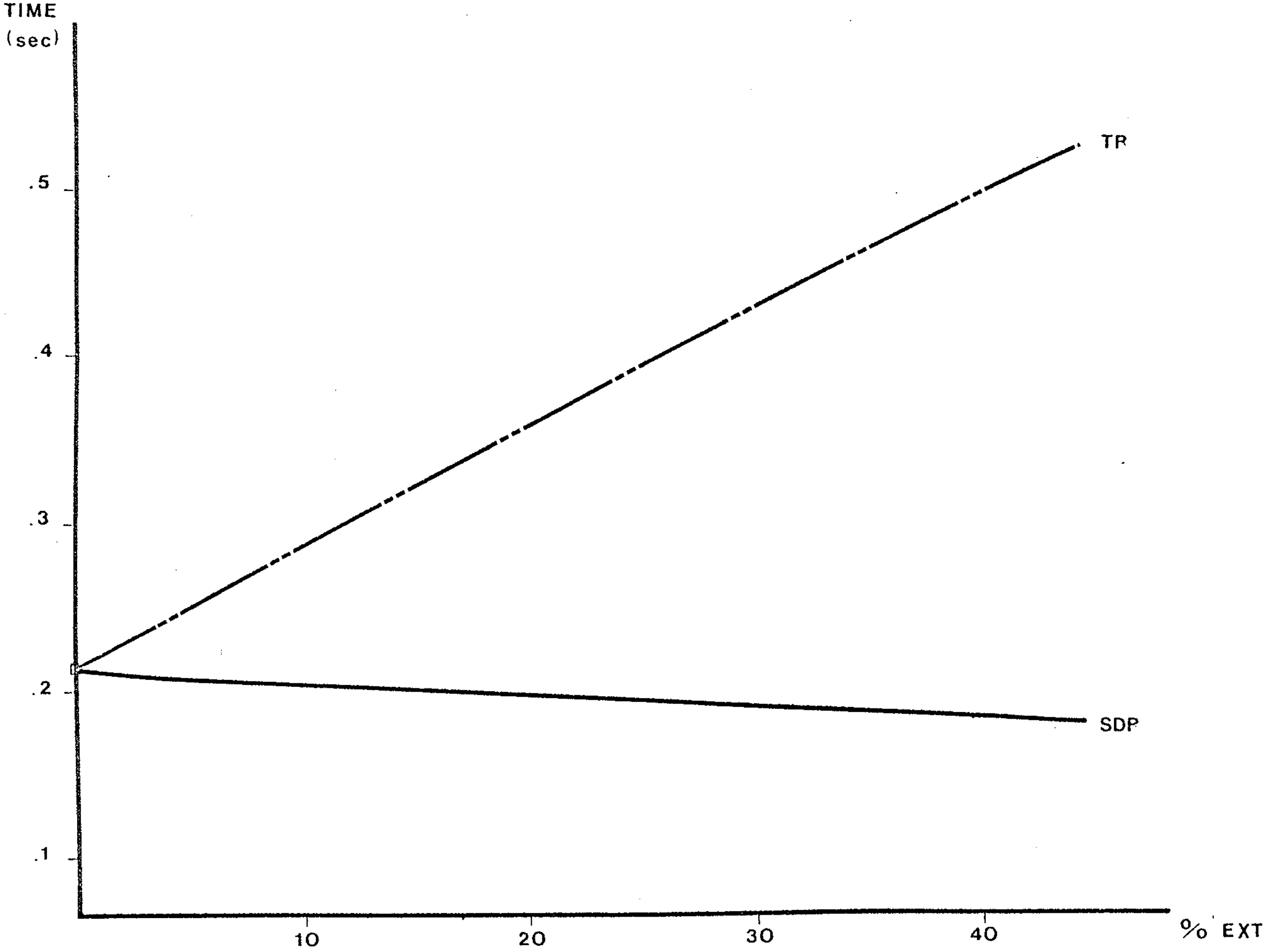


Fig 24 Effect of protein extender on relaxation time.

Experiment II

Figure 25 represents a typical response obtained with a sample in the extrusion cell. The following profile was obtained from each curve (Voisey and Larmond, 1977):

1) compactability - the distance required to compress the sample to a mass behaving like a viscoelastic solid;

2) the force at the point of true rupture;

3) firmness - as calculated from the slope of the linear portion following compaction;

4) extrusion toughness - the slope of the line after onset of rupture;

5) maximum force;

6) total energy used during the test as determined from the area under the curve.

Figure 26 shows an example of the force deformation curves obtained over six cycles on the same sample. Each sample was put through the extrusion cell several times in order to simulate the human biting and chewing actions (Friedman _et_ _al_., 1963). The number of cycles was limited to six as further recycling proved to yield no additional change in the sample. This may be equated with the state ready for swallowing in sensory terms.

The average value over six cycles for all parameters was plotted against level of extension in Figures 27 to 32. Lines were drawn from the solutions of regression equations.

Figure 27 shows the effect of extender on MTPA firmness. Both protein extenders led to an increase in force, the increase being much greater for TP. Figure 13 reveals that the sensory response for firmness yielded results quite different from those in Figure 27. Hence, the objective and sensory measurements of firmness do not show agreement. In Figure 28 MTPA compression forces obtained in sausage extended with both proteins are given. Products extended with SDP showed a greater increase in compression forces. In Figure 23 the results of compression to rupture data from Experiment I reveal that compressing samples between two plates led to a much different relationship, TP leading to a greater increase in compression force in this case. Again, there appears to be no relationship between sensory data and the measurements obtained instrumentally.

The effects of extension on maximum force are shown in Figure 29. Samples possessing less cohesiveness were expected to have low maximum forces of extrusion. Products extended with both proteins showed an increase in this parameter, greater for TP than for SDP. No similarities in responses to either sensory chewiness or firmness were observed. According to Figure 30 TP and SDP had a similar effect on toughness, both causing a slight increase in the characteristic over level of extension. Sensory firmness data indicated an increase in toughness as greater levels of protein were added to the sausages. Figure 31 shows that rupture forces increased substantially as more TP was used in the formulation and also, but to a lesser extent, with SDP. Again, this was not reflected in sensory data.

The total energy used over all cycles should correlate with chewiness scores as the effort required in mastication is a measure of the muscle "energy" required to reduce a sample to the state ready for swallowing. Figure 32 reveals that total energy increased over level of extension, also indicating that more energy was required to chew samples extended with SDP than TP. This is quite contrary to observed sensory chewiness data as presented in Figure 12, hence another contradiction between objective and sensory measurements.

The MTPA revealed that in all parameters except toughness there was a difference between products extended with TP and those with SDP. The observations recorded for the MTPA, however, cannot be verified with data from sensory evaluation. Indeed, the responses observed instrumentally directly contradicted those from sensory analysis in some cases. The best verification of observed sensory response which can be made instrumentally was obtained with the Warner-Bratzler shear cell despite all the criticism this procedure has received (Szczesniak, 1968; Voisey and deMan, 1976; Korth, 1982).

The results obtained in these attempts to evaluate texture differences between the sausages serve to illustrate the difficulties inherent in describing and quantifying the mechanical properties of food. This is especially true in the case of meat products where so many factors contribute to overall impressions as perceived by the human being (Stanley, 1976). The observations drawn from the MTPA, therefore, should not be dismissed as insignificant simply because they contradicted the sensory data. Each of the parameters observed may not have been perceivable by the panelist as a separate sensory characteristic. In this sense the instrument may be more sensitive than the mouth. Alternatively, it is possible that it is the mouth that is more sensitive and which can detect differences which our instruments cannot. If the factors contributing to the texture of a product such as pork sausage were defined in exact engineering terms an instrumental measurement of differences induced by incorporation of a protein extender may be more easily attainable.

The observations made over the course of this study indicate that extension of pure pork sausage with TP and SDP pea protein has the effect of altering instrumental measurements of texture. These changes become more significant with increasing levels of substitution with either protein. Similar patterns of response were obtained upon sensory evaluation of texture although a direct comparison between sensory and instrumental measurements could not be achieved.

Fig 25. Instrumental Texture Profile: Sources of data

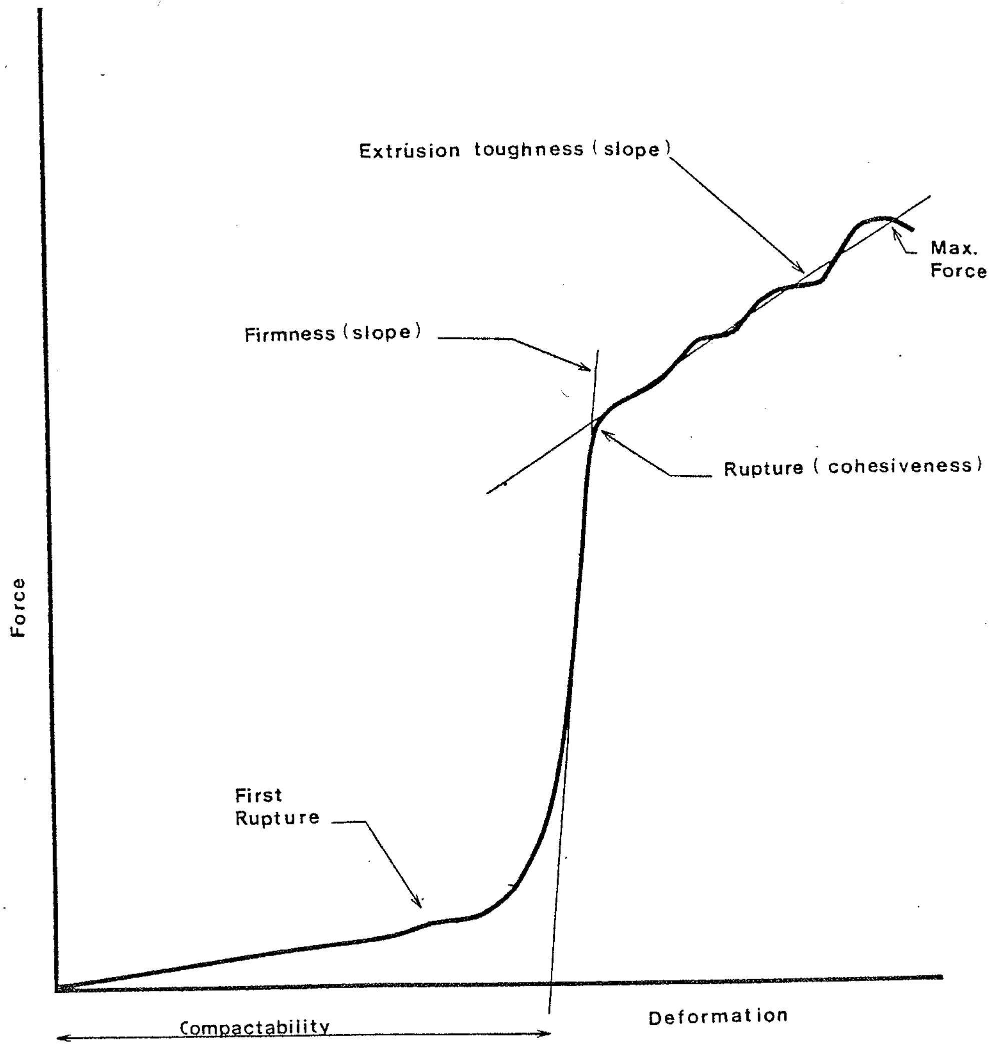


Fig: 26. Example of force deformation curves over six cycles on the same sample

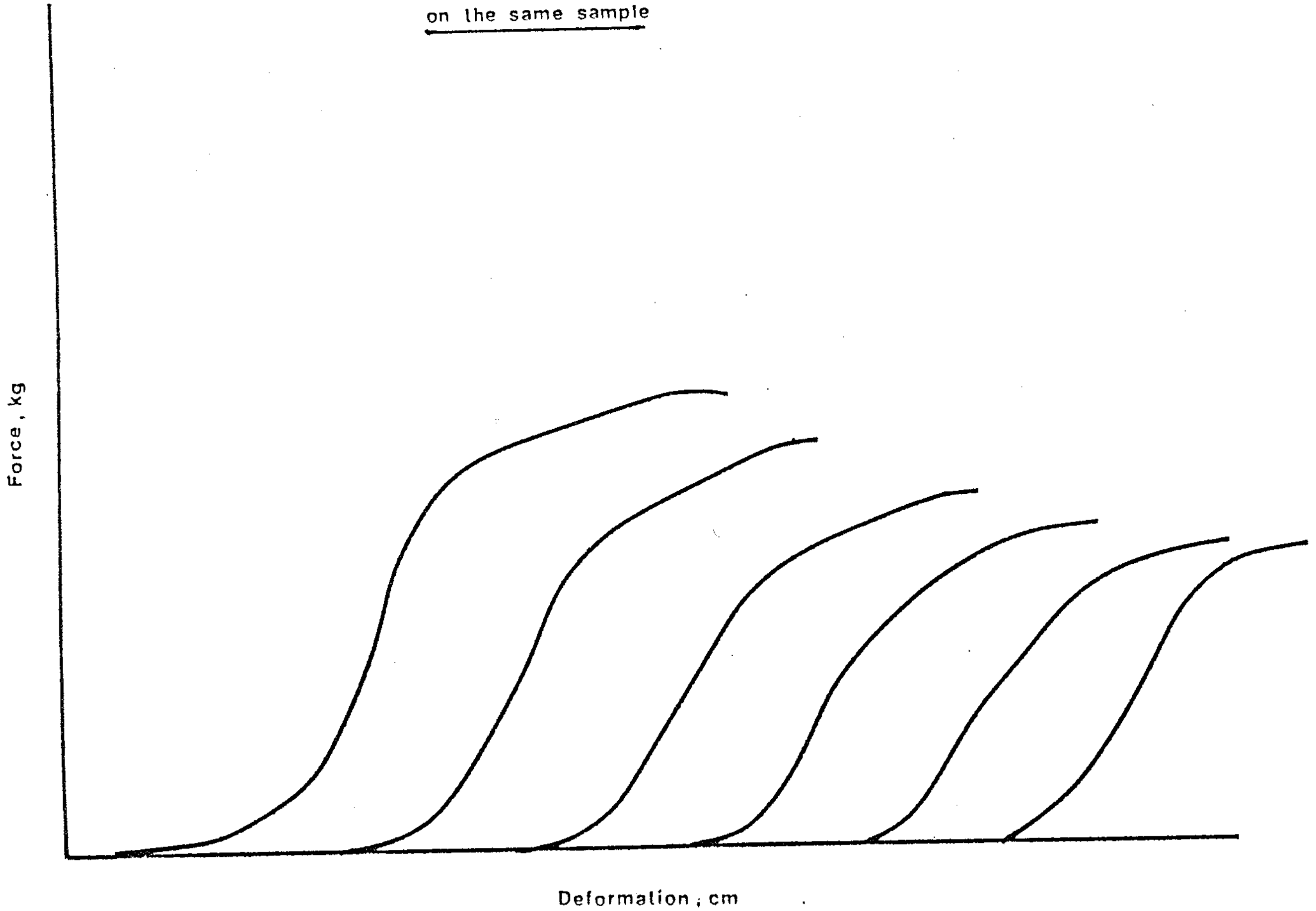


FIG 27 . MTPA : EFFECT OF EXTENDERS ON FIRMNESS

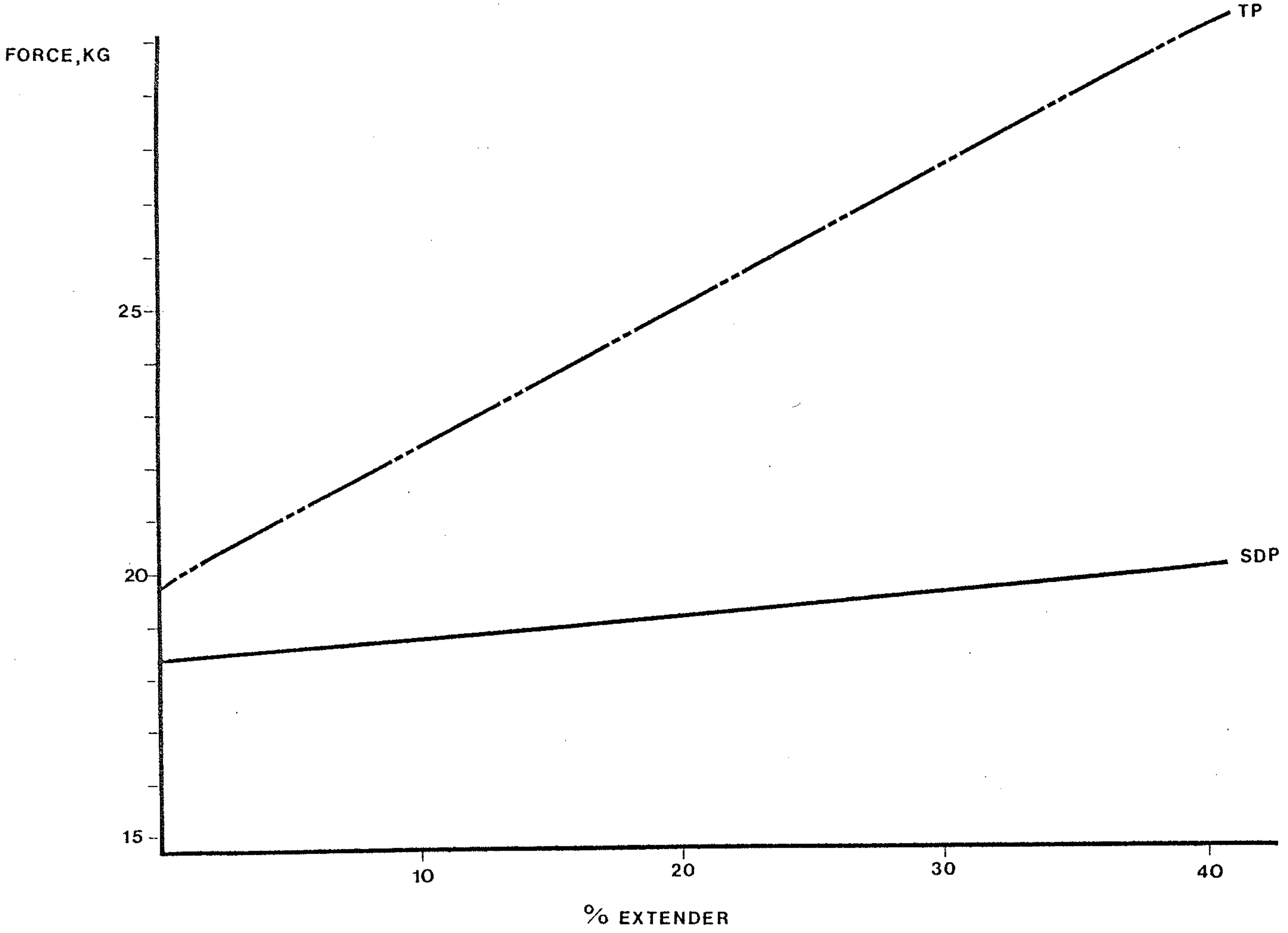


FIG 28. MTPA : EFFECT OF EXTENDERS ON COMPRESSION

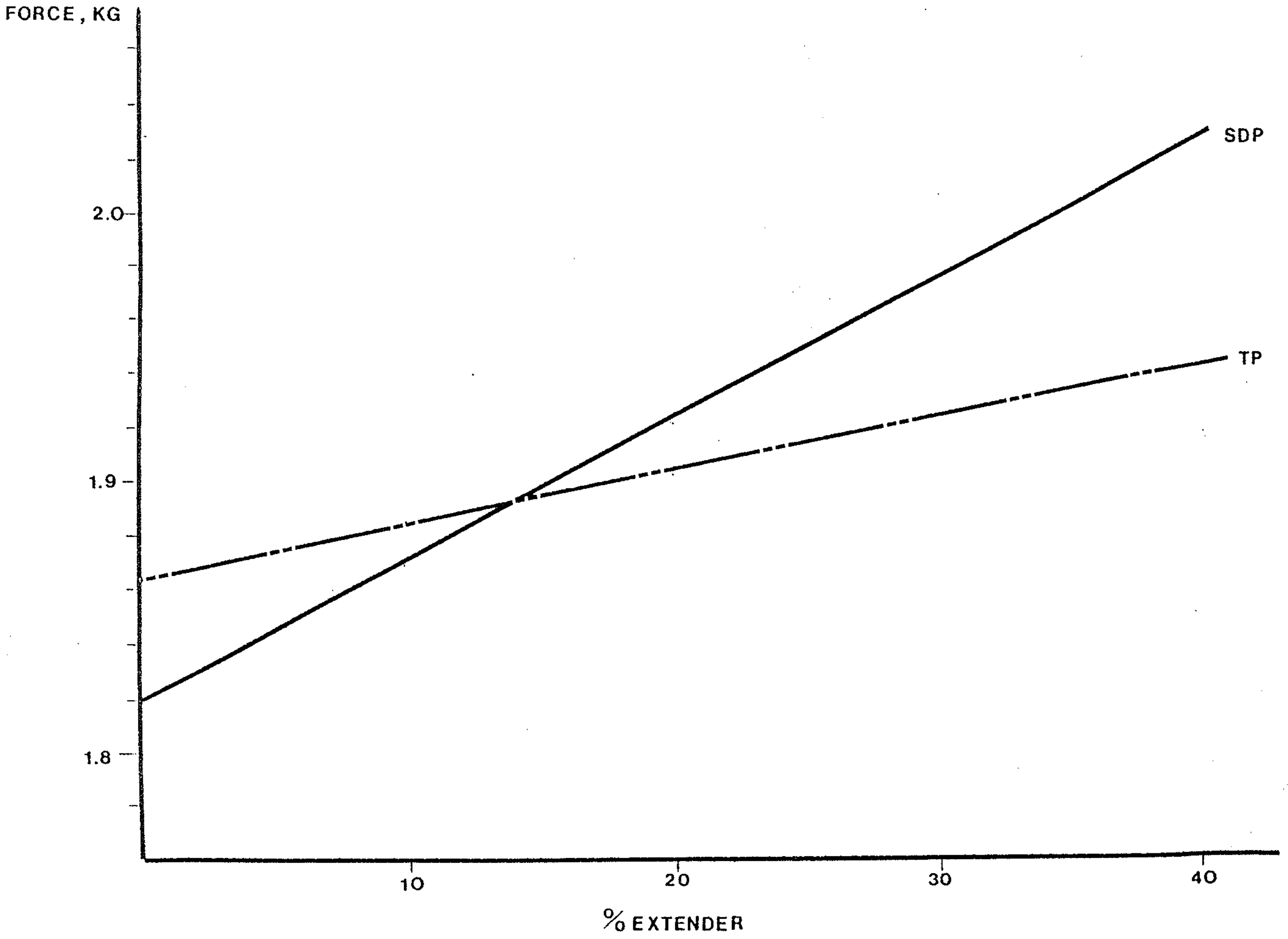


FIG 29 MTPA: EFFECT OF EXTENDERS ON MAXIMUM FORCE

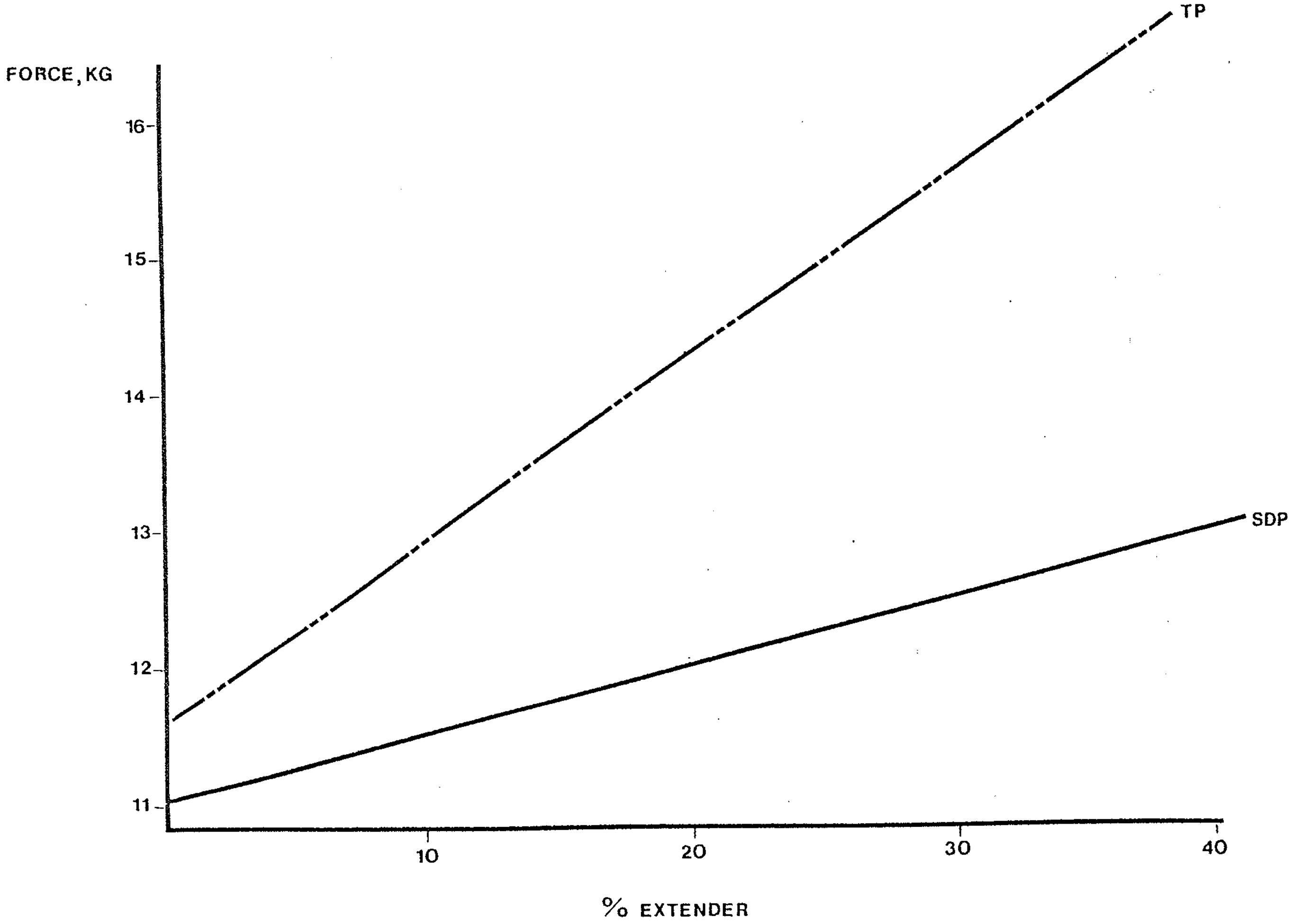


FIG 30. MTPA : EFFECT OF EXTENDERS ON TOUGHNESS

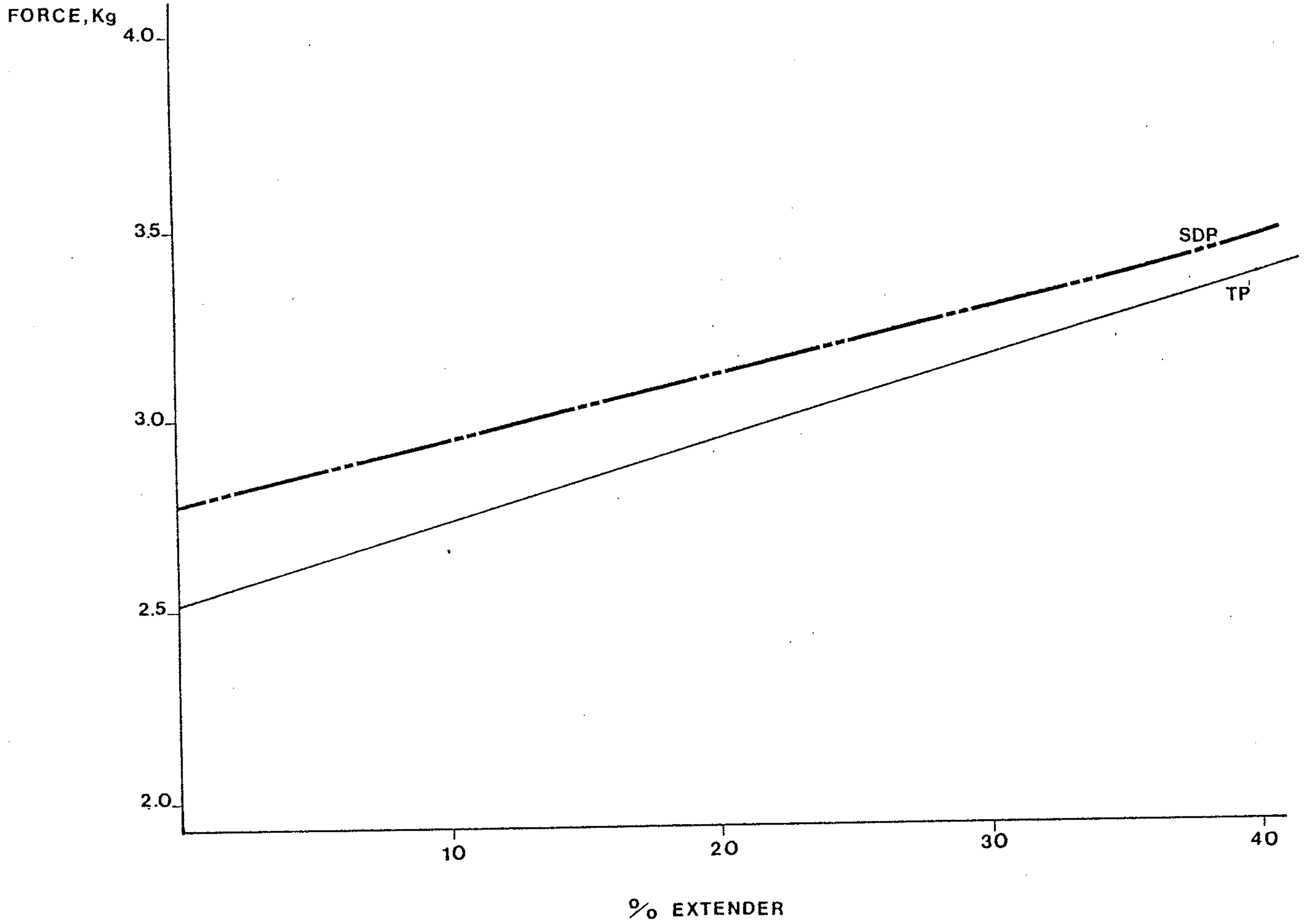


FIG 31. MTPA: EFFECT OF EXTENDERS ON RUPTURE

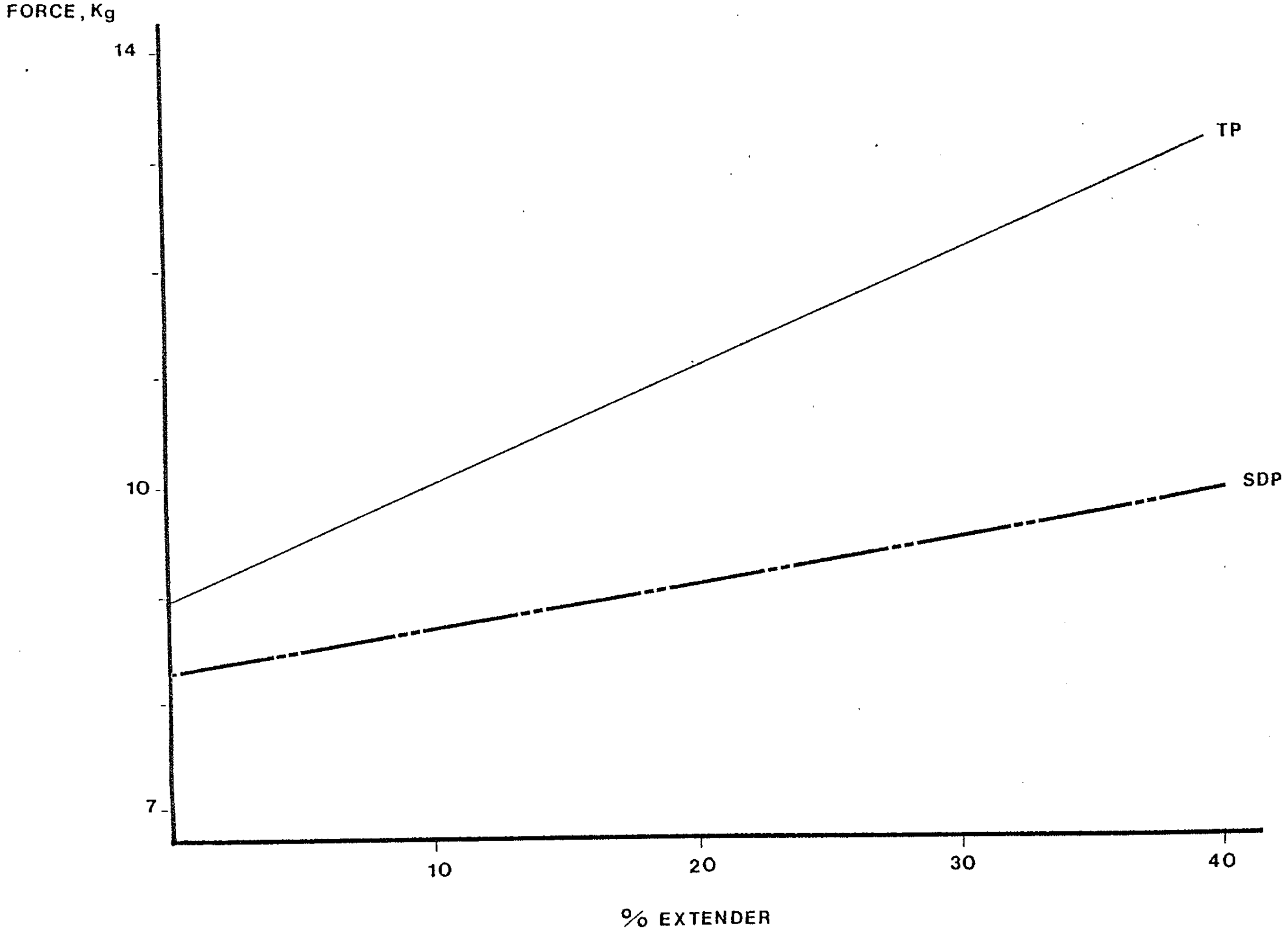
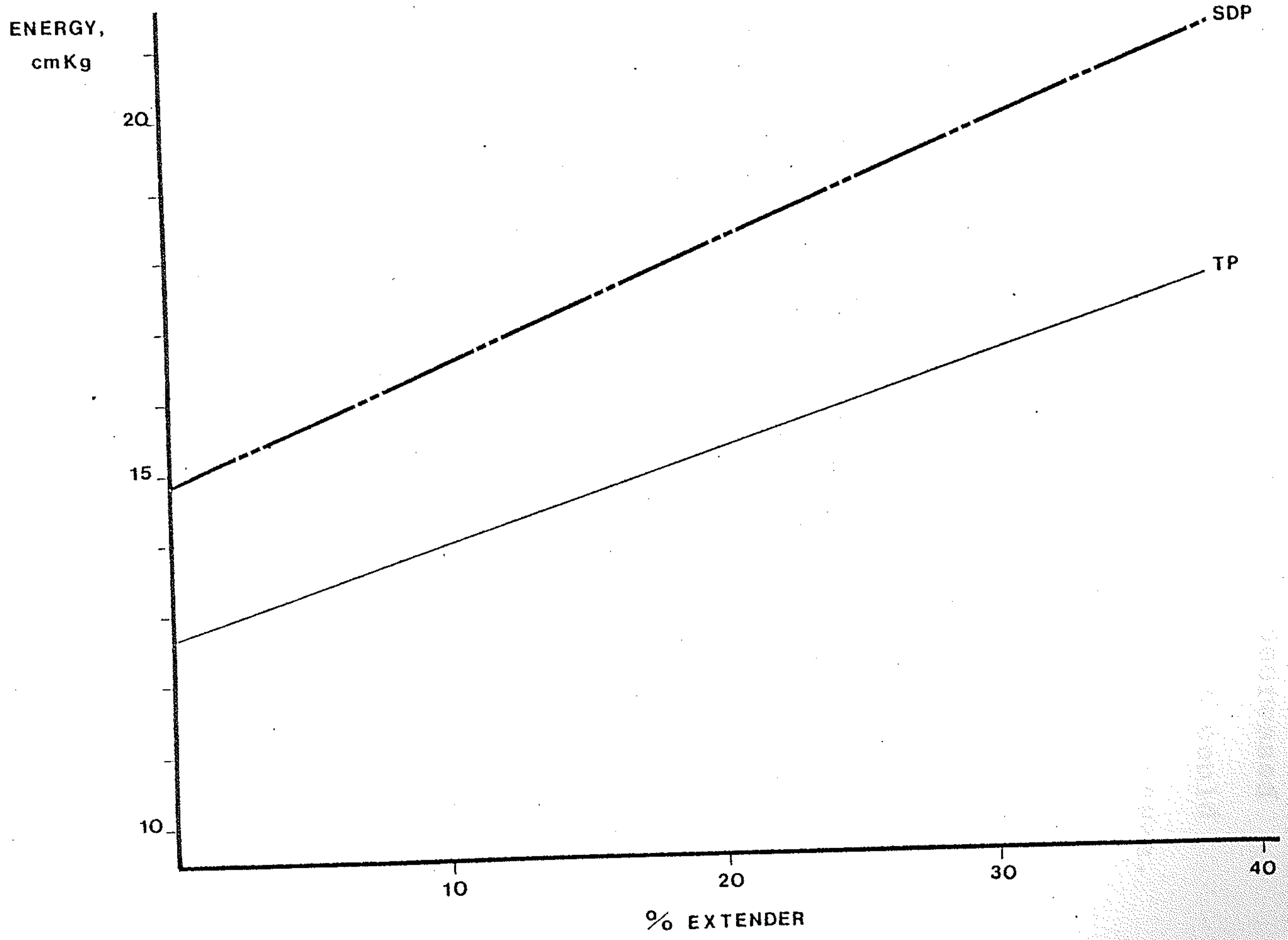


FIG 32. MTPA : EFFECT OF EXTENDERS ON TOTAL ENERGY



E. SUMMARY AND CONCLUSIONS

The pea PMM protein used to substitute meat in pure pork sausage at levels of 10, 20, 30 and 40% by whole weight did not influence the microbiological shelf life of the products, either in spray-dried form (SDP) or after texturization (TP). The use of both extenders decreased cooking losses by holding more water and fat. This could be of advantage to a producer in reducing processing losses. Sensory evaluation demonstrated that the textured extender had less effect on firmness, chewiness, flavour and overall acceptability than the untextured extender. No differences in juiciness were observed but TP decreased greasiness scores while SDP did not. Some flavour problems were observed at higher levels of extension with both proteins due to the appearance of a beany off-flavour. A decrease in chewiness and firmness scores was also observed.

Attempts to determine the effect on texture with the OTMS using techniques reportedly successful with such products showed the difficulties in obtaining instrumental measurements of texture that will reflect sensory observations. Warner-Bratzler shear offered the best agreement with sensory data. In this study, therefore, a single point measurement proved most useful in establishing a comparison between the two approaches to the evaluation of texture. Sensory evaluation indicated that the textured protein performed well as a meat substitute and substitution at levels up to 20% in pork sausage would likely be acceptable at the consumer level. The purpose of meat substitution or extension with non-animal sources of protein is to replace or add to the normal complement of animal protein without displacing the meat system itself. The textured pea protein extender satisfied this requirement to a greater degree than the spray-dried, untextured pea protein extender.

F. RECOMMENDATIONS FOR FUTURE STUDY

Because of questions raised as to the safety of extended meat products, innoculation studies with pathogens such as Staphylococcus aureus should be conducted to determine whether these products can support their growth to a greater extent than pure meat formulations. PMM appears to have excellent water and fat binding properties. These could be better exploited in finely comminuted products such as wieners. This aspect should be investigated.

PMM fibers could be further altered by addition of flavours, fats and colour to produce a true meat analogue. This would require finding methods of extrusion suitable for production and means of causing individual fibers to bind together to simulate the appearance of meat. Finally, much more work is required in the field of meat texture. Means must be found to define mechanical characteristics of meat and meat products more accurately. This would ease interpretation of data from currently available instrumentation.

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APPENDIX 1. Friedman rank sums test (Hollander and Wolfe, 1973).

Data is blocked to obtain n blocks given k treatments. A rank is assigned to each observation from least to greatest within each block.

The S statistic is calculated using rank sums according to:

$$S = \left[\frac{12\ n}{k\ (k+1)} \sum_{j=1}^{k} Rj^{2} \right] - 3n\ (k+1)$$

where n = number of blocks
k = number of treatments
Rj = rank sum of jth treatment

The hypothesis tested is that all treatment effects are equal against the alternative that they are not.

H_0 is rejected if $S \geq s\ (\alpha, k, n)$
accepted if $S < s\ (\alpha, k, n)$

where the constant $s\ (\alpha, k, n)$ satisfying the equation $P_0 \quad S \geq s\ (\alpha, k, n) \quad =$ is found in tables for Friedman's S statistic.

(Continued)

APPENDIX 1 . (continued)

Sausage Extended with SDP

1. SPC 30

Ranked data:

Days of Storage	Levels of Extension				
	0	10	20	30	40
1	5	1	3	4	2
3	5	4	2	3	1
5	1	3	2	5	4
Rank Sum	11	8	7	12	7

$$S = \frac{12 \times 3}{5(5 + 1)} (11^2 + 8^2 + 7^2 + 12^2 + 7^2) - 3 \times 3 (5 + 1)$$

$$= 2.93$$

Tabulated s (.05, 5, 3) = 8.4

$S < s(.05, 5, 3)$

Therefore, H_o is accepted and it is concluded that all treatments are the same.

2. SPC 4

Days of Storage	Levels of Extension				
	0	10	20	30	40
1	4	3	1.5	2	1.5
3	5	3	4	1	2.0
5	2.0	2.0	4.5	2	4.5
Rank Sum	11	8	10	5	8

(Continued)

APPENDIX 1 . (continued)

$S^1 = 5.52$

∴ there are no differences.

3. VRB

Days of Storage	Levels of Extension				
	0	10	20	30	40
1	4	5	3	1.5	1.5
3	2	3	5	1	4
5	5	4	3	2	1
Rank Sum	11	12	11	4.5	6.5

$S^1 = 3.12$

∴ there are no differences.

4. PDA

Days of Storage	Levels of Extension				
	0	10	20	30	40
1	5	2	4	1	3
3	5	4	3	1	2
5	3	1	4	2	5
Rank Sum	13	7	11	4	10

(Continued)

APPENDIX 1 . (continued)

S = 6.7

∴ there are no differences.

Sausage Extended with Tp

1. SPC 30

Days of Storage	Levels of Extension				
	0	10	20	30	40
1	5	3	4	1	2
3	5	4	1	3	2
5	1	2	3	5	4
Rank Sum	11	9	8	9	8

S = .8

∴ there are no differences.

2. SPC 4

Days of Storage	Levels of Extension				
	0	10	20	30	40
1	3.5	3.5	2	5	1
3	3	1	4.5	2	4.5
5	1.5	1.5	3	4	5
Rank Sum	8	6	9.5	11	10.5

(continued)

APPENDIX 1 . (continued)

$S^1 = 2.28$

∴ there are no differences.

3. VRB

Days of Storage	Levels of Extension				
	0	10	20	30	40
1	3	2	4.5	4.5	1
3	1	4.5	2	3	4.5
5	1	3.5	5	3.5	2
Rank Sum	5	10	11.5	11	7.5

$S^1 = 3.67$

∴ there are no differences.

4. PDA

Days of Storage	Levels of Extension				
	0	10	20	30	40
1	5	2	3	4	1
3	3.5	1	5	3.5	2
5	2.5	1	5	4	2.5
Rank Sum	11	4	13	11.5	5.5

$S^1 = 8.16$

∴ there are no differences.

APPENDIX 2

ANOVA's for Sensory Evaluation.

Abbreviations:
FIRM = firmness
CHEW = chewiness
JUIC = juiciness
GRES = greasiness
FLAV = flavour
OVER = overall impression

A. Effect of extension with SDP : Mean squares from analysis of variance

Source	Df	Firm	Chew	Juic	Gres	Flav	Over
Panelists	6	458.9	195.7	130.4	1218.4^{**}	1867.4^{**}	1272.6^{**}
Level	3	620.5^{*}	90.0	763.2^{*}	163.6	1632.9^{**}	3215.4^{**}
Replicate	1	175.0	37.8	4.0	1.1	0.0	0.2

$*$: Significant at $\alpha = .05$

$**$: Significant at $\alpha = .01$

Duncan's multiple range test for means of the scores observed over levels of substitution : means in the same column followed by the same letter are not significantly different at $\alpha = .05$

Level	Firm	Chew	Juic	Gres	Flav	Over
10	25.1^{a}	19.1	19.6^{a}	23.4	31.8^{a}	31.6^{a}
20	19.6^{ab}	17.1	21.5^{a}	21.4	28.6^{a}	29.7^{a}
30	20.3^{ab}	17.2	13.1^{b}	18.7	21.5^{b}	19.5^{b}
40	15.8^{b}	15.6	13.1^{b}	22.0	18.2^{b}	13.1^{b}

B. Effect of extension with TP : Mean squares from analysis of variance

Source	Df	Firm	Chew	Juic	Gres	Flav	Over
Panelists	6	358.4	172.1	471.4^{**}	1341.4^{**}	1382.2^{**}	1554.2^{**}
levels	3	175.2	72.6	1197.4^{**}	1387.5^{**}	2190.1^{**}	2427.9^{**}
Replicates	1	18.3	0.2	34.6	23.1	2.6	6.5

Duncan's multiple range test for means of the scores observed over levels of substitution : means in the same column followed by the same letter are not significantly different at $\alpha = .05$.

Level	Firm	Chew	Juic	Gres	Flav	Over
10	27.6	21.6	19.4^{a}	22.9^{a}	33.7^{a}	33.7^{a}
20	25.8	19.5	18.9^{a}	22.7^{a}	33.2^{a}	32.0^{a}
30	23.6	19.4	14.6^{b}	19.3^{b}	26.5^{b}	26.2^{b}
40	23.1	18.6	7.9^{b}	10.6^{b}	18.3^{b}	16.8^{b}

C. Effect of type of protein extender : Mean squares from analysis of variance

Source	Df	Firm	Chew	Juic	Gres	Flav	Over
Protein	1	655.8**	177.5**	84.0	175.0*	231.4*	386.3*
Panelists	6	707.6*	300.3	457.1	2335.7**	3120.5**	2740.4**
Levels	3	703.5**	138.9	1757.5**	807.6**	3685.2**	5500.9**
Replicates	1	40.1	21.4	31.1	17.3	1.5	4.3

Duncan's multiple range test for means of the scores observed with the two different proteins : means in the same column followed by the same letter are not significantly different at $\alpha = .05$.

Protein	Firm	Chew	Juic	Gres	Flav	Over
TP	25.0^{a}	19.8^{a}	15.2	18.9^{b}	27.9	27.2^{b}
SDP	20.2^{b}	17.3^{b}	16.9	21.4^{a}	25.0	23.5^{a}

APPENDIX 3. Cooking losses.

	Weight loss %		liquids lost/g meat	
Fiber 0	32.9	40.0	.112	.115
10	32.1	--	.079	--
20	25.2	24.2	.061	.065
30	26.9	25.7	.054	.055
40	17.0	22.6	.047	.033
Spray 0	32.9	40.0	.112	.115
10	32.6	28.7	.070	.115
20	30.2	27.4	.042	.047
30	26.6	26.6	.046	.024
40	20.4	21.5	.038	.033

1. Fiber level x weight loss, $r = -.89$, $z = 1.422$, $N = 10$

eq'n = $y = -.389\ x + 35.610$

$x = 10$, $y = 31.7$

$x = 20$, $y = 27.8$

$x = 30$, $y = 23.9$

$x = 40$, $y = 20.0$

Spray x weight loss, $r = -.91$, $z = 1.528$, $N = 10$

$y = -.351\ x + 35.700$

(Continued)

APPENDIX 3. (continued)

$x = 10$, $y = 32.20$

$x = 20$, $y = 28.7$

$x = 30$, $y = 25.2$

$x = 40$, $y = 21.7$

---

2. Fiber X liquid lost / g meat. $r = -.96$

$y = -.002x + .106$

$x = 10$, $y = .089$

$x = 20$, $y = .071$

$x = 30$, $y = .053$

$x = 40$, $y = .036$

Spray X liquid lost / g meat. $r = -.88$

$y = -.002x + .107$

$x = 10$, $y = .085$

$x = 20$, $y = .064$

$x = 30$, $y = .043$

$x = 40$, $y = .022$

Differences in r's tested by using the Fischer Z statistic (Huntsberger and Billingsley, 1975).

1. $H_o : r_{TP} = r_{SDA}$

$r_{TP} = .89$ $\qquad$ $r_{SDA} = .91$
$Z = 1.4222$ $\qquad$ $Z = 1.528$ $\qquad$ (Fisher's Z statistic)

(Continued)

APPENDIX 3 . (continued)

Standard error of the difference between two Z:

$$S_{DZ} = S_{Z1}{}^2 = S_{Z2}{}^2$$

$$= \left(\left(\frac{1}{N_1 - 3}\right)^2 + \left(\frac{1}{N_2 - 3}\right)^2\right)^{1/2}$$

$$= .556$$

$$Z^1 = \frac{Z_{SDP} - Z_{TP}}{SDZ}$$

$$= \frac{.106}{.556}$$

$$= .191 \qquad .191 < 1.96$$

$\therefore H_o$ is accepted.

There is no difference between r's.

2. $H_o = r_{TP} = r_{SDP}$

$r_{TP} = .96$ $\qquad$ $r_{SDP} = .88$

$Z = 1.946$ $\qquad$ $Z = 1.376$

$$S_{DZ} = .556$$

$$Z = \frac{1.946 - 1.376}{.556}$$

$$= \frac{.570}{.556} = 1.025 \qquad 1.025 < 1.96$$

$\therefore H_o$ is accepted.

APPENDIX 4

ANOVA's for Experiment I in texture.

Abbreviations: WB = Warner Bratzler
CTORUP = Compression to rupture
RELAX = Relaxation time

A. Effect of extension on W.B. : Mean squares from analysis of variance

Source	Df	Mean square
Protein	1	86.5^{**}
Level	4	22.0
Rep.	2	4.8

* : Significant at α =.05
** : Significant at α =.01

Duncan's multiple range test for means observed with both proteins: means followed by the same letter are not significantly different at =.05

Protein	Mean
TP	7.42^{a}
SDP	6.49^{b}

B. Effect of extension on RELAX:

Source	Df	Mean square
Protein	1	55.82 **
Level	4	14.8 *
Rep.	2	.1

Duncan's multiple range test for means.

Protein	Mean
TP	.37^{a}
SDP	.19^{b}

C. Effect of extension on CTORUP:

Source	Df	Mean Square
Protein	1	28.76 **
Level	4	22.0 **
Rep.	2	1.6

Duncan's multiple range test for means,

Protein	Level
TP	10.08^a
SDP	8.03^b