PET FOOD BY UTILIZING MEAT INDUSTRY WASTE, TRIPE

PRE-DISSERTATION REPORT

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CERTIFICATE

This is to certify that VINOD SURENDRAN (Registration No. 11706176) has personally completed M.Sc. Dissertation-I entitled, **"Pet food by utilizing meat industry waste; tripe"** under my guidance and supervision. To the best of my knowledge, the present work is the result of her original investigation and study. No part of pre-dissertation has ever been submitted for any other purpose at any University.

The project report is appropriate for the submission and the partial fulfillment of the conditions for the evaluation leading to the award of Master of Food Technology.

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ACKNOWLEDGEMENT

Every work accomplished is a pleasure a sense of satisfaction. I would like to express the deepest appreciation to my esteemed supervisor **Dr. Anil Panghal**, Associate Professor Lovely Professional University, who has the attitude and the substance of genius. Without his guidance, valuable time and persistent help this dissertation would not have been possible. I would also like thank Lovely Professional University for providing the required instruments, material which is needed for carrying out the project. Last but not the least, I would like to thank my family.

DECLARATION

I hereby declare that the work presented in the dissertation 1 entitled **"Pet food by utilizing meat industry waste; tripe"** is my own and original. Work will be carried out by me at School of Agriculture, Lovely Professional University, Phagwara, Punjab, India under the guidance of Dr. Anil Panghal, (20785), Research & Consultancy Coordinator & Associate Professor, School of Agriculture, Lovely Professional University, Phagwara, Punjab, India, for the award of the degree of Master of Science in Food Technology.

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I certified that the above statement made by the student is correct to the best of my knowledge and belief.

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CHAPTER 1: INTRODUCTION

Pet food is a product prepared by combining plant and animal products which is used for feeding pets, and it is available in almost all pet shops and supermarkets. Contents in the pet food varies depending up on the type of animals, like cat food or dog food and it is not regarded as human grade. Pet food industries are considered as a continuous part of slaughter houses, human food and other crop processing industries. The importance of pet feed is that, it creates a market for the products like slaughter house offals, grains etc.… that are not suitable for human consumption and related waste products are exploiting and converting it in to income. The waste portions are gut, viscera, udders, esophagi, meat leftovers like blood, fat, bone, poultry by-products (like unfertile eggs, dead embryos etc..), tail, liver, ears, feet, kidney, lungs, spleen, animal plasma, rumen, tripe, and fish wastes such as head, bones, scales, skin, viscera etc. inedible by-products of rendering industries like grease, tallow, and meat cum bone meal is also utilizing (Newman et al 1994). Everything except dressed meat produced by or from the animals are considered as by- product in united states. 2 major classifications of animal by-products in America, are edible and inedible. Almost all slaughter houses, classify offal (waste) into 2 classes mainly red and white. Red includes head portion, liver, tail, lungs, tongue etc. and white includes fat, guts and bladder, tripe(rumen), legs and other types of trimmings.

Majority of meat by-products are not used in human diet due to the unwanted chemical and physical properties, so with suitable modifications and formulations these by-products can give a better yield and profit to meat technology units. Pet food is one among the major application of utilizing waste meat by- products. The waste management system of majority of meat technology units and slaughter houses in India is very poor, so the effectual management of waste generated from slaughter houses is very important. Blood which is considered as a waste product from slaughter houses can be maximum utilized for its pharmaceutical properties and other waste materials can be rendered for edible and inedible products (k. jayathilakan et al 2011)

Incorporation of functional foods in the feed of pets like cats and dogs generally have an improved health benefits. Modifications in the gastrointestinal physiology, improvement in the functioning

of brain, enhanced changes in the biochemical parameters, and a reduction in the development of certain kinds of pathogens etc.… are the major functions of functional foods (Hasler,et al 1998).

Due to high amount of amino acids, hormones, minerals, vitamins, fatty acids etc... some meat by-products can also be applicable in medicinal field. Compared to meat, certain meat by-products such as lungs, kidney, brain, spleen, etc… have a high moisture content. (k.jayathilakan et al 2011)

The present review has been set up to describe the existing data on waste products from slaughter houses that can be incorporate in pet food and its pharmaceutical and nutraceutical properties.

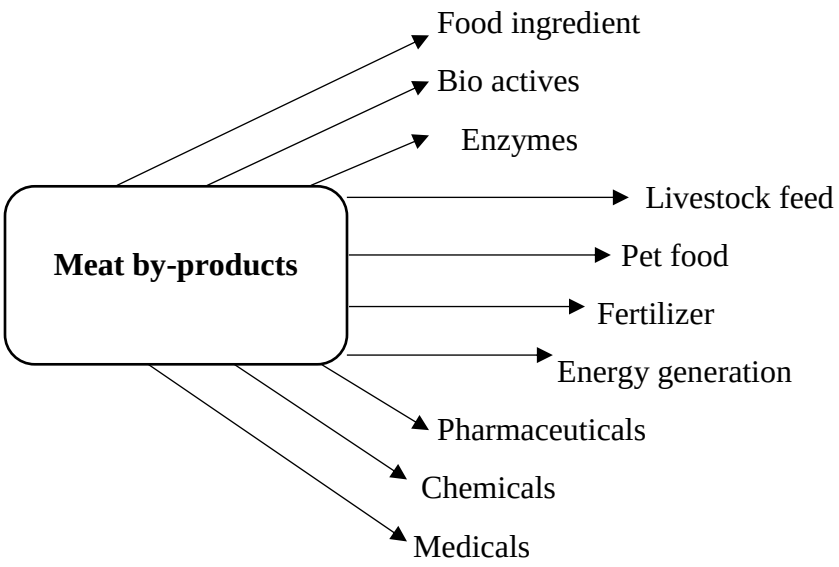


Figure 1 Main routes of applications for meat by-products (Fidel Toldrá et al 2016)

The present review has been set up to describe the existing data on waste products from slaughter houses that can be incorporate in pet food and its pharmaceutical and nutraceutical properties.

Typical bone shaped pet food image (Source: https://www.google.com/)

CHAPTER 2: PROBLEM BACKGROUND

In our modern society there is a great demand for products having a high nutritional standard. The gradual increase in the price and lack of raw materials and more seriousness of environmental pollution, which shows the importance of recovery and recycling of wastes. Meat and meat processing industries have a huge amount of waste products having a high nutritional quality and this can be incorporated in animal feeds particularly to dogs and cats to minimize the cost of production and to maximizing the profit and to produce high value-added products. The waste materials and other unwanted substances are collected and modified to useful product of better quality by using suitable processing methods and efficient in plant treatment. The aim of this study is to prepare a good quality pet food, by utilizing waste materials from the slaughter houses or from meat industries. The pet food should provide high palatability, acceptability and having good pharmaceutical properties to the animals.

CHAPTER 3: REVIEW OF LITERATURE

Statistics and production of by products

In india there are about 2,702 slaughter houses which are registered and they are basically doing only the slaughtering & dressing process. Cattles, goats, buffalos, pigs, poultry & sheeps are the major livestock used for slaughtering in india. According to the current data provided by the Ministry of Agriculture, livestock population range is around 204.5 million cattles, 115.3 million goats, 84.2 million buffaloes, 12.8 million pigs, 307.1 million poultry & 50.8 million sheep. About 1.5 million tons of meat was produced in the year of 1992-93 and it was then raised to 2 million tones in 1997-98. In the case of live stock holding India is top in the world and the total amount of meat by products obtained from large slaughter houses in the country is found to be higher than 21 lakh tones/annum.

TABLE: 1 Quantity of solid waste generated from the bovine, goat, sheep and pig slaughter houses and the specific waste index for slaughter houses with respect to the type of animal.

Animal	Quantity of solid waste		% of animal weight	Animal	Specific waste index [a]
	Kg/head	Kg/TLWK			
Bovine	83	275	27.5	Cow	0.56
Goat/sheep	2.5	170	17	Calf	0.87
Pig	2.3	40	4	Pig	0.2
				Sheep	0.2

K. Jayathilakan et al 2012, Russ and Pittroff 2004, USDA 2001

Nutritional composition of slaughter house wastes utilized in pet food

Meat by-products are rich source of essential nutrients such as amino acids, fatty acids, minerals, vitamins, and certain hormones which are having some medicinal properties. As compared to meat, meat by-products like Lung, kidney, brains, spleen, tripe and other related parts are having a greater amount of moisture. Carbohydrate content of organs like kidney and liver is very high as compared to other meat portions (Devatkal et al 2004b). Tail of pork is considered as a meat by-

product having a higher amount of fat and a lower amount of moisture. Cattles feet and ears are good source of collagen, and the protein content of parts like liver, tail, ears, and feet are similar to the tissues of lean meat. (Unsal and Aktas 2003). The protein content of brain, fatty tissue and chitterlings is very low. The amino acid profile of meat by-products has a higher variation from the tissues of lean meat as a result of more amount of connective tissues. Because of this, amino acids such as proline, hydroxy proline and glycine are present in a larger amount and tryptophan & tyrosine content is smaller in meat by-products like feet, tripe, ears, lungs and stomach. Meat by-products are having a high vitamin composition as compared to lean meat tissue. As compared to lean meat, the parts like liver and kidney are having a higher riboflavin content (1.697-3.630mg/100g) that is about 5-10 times more. Liver contains a good amount of vitamin B12, B6, vitamin A, niacin, Ascorbic acid, and Folacin. Kidney is a good source of vitamin B12, B6 & folacin.

A 100-g serving of liver from pig or meat provides vitamin A which is about 450% – 1,100% of the RDA, vitamin B12 which is about 3,700% of the RDA, vitamin B6 which is of 65% of the RDA and ascorbic acid which is about 37% of the RDA. Iron content is very high in Sheep kidneys, lungs, spleen & liver. Livers of sheep, meat, and veal are good source of copper which adds about 90 – 350% of the RDA (2 mg/day). Liver is also a good source of minerals like manganese (0.128 – 0.344 mg/100 g), potassium (360 – 433 mg/100 g) and phosphorus (393 – 558 mg/100 g) (Devatkal et al. 2004b). Most of the meat by products contains a very low range of sodium except kidney, brain, spleen, lungs, and ears. Meat which is mechanically deboned contains a higher amount of calcium (315 – 485 mg/100 g). As compared to lean tissue, the polyunsaturated fatty acid content is higher in organ meats. The monounsaturated fatty acid content is low in chitterlings, Brain, liver, lungs and kidney as compared to polyunsaturated fatty acids. (Liu 2002). In organ meat the cholesterol content is about (260 – 410 mg/100 g) which is 3-5 times more than in the lean meat and also contains a higher amount of phospholipids. Cholesterol content is very high in animal heart (1,352 – 2,195 mg/100g).

TABLE 2 Proximate Composition in Major Constituents per 100 g of Beef, Pork, and Lamb Edible By-products

Organ	Species	Energy (kcal)	Protein (g)	Fat (g)	Carbohydrates (g)
Liver	Beef	130	21	3	5
	Pork	140	22	45	3
	Lamb	150	21	5	5
Heart	Beef	115	17	5	0.5
	Pork	115	17	5	0.5
	Lamb	120	17	5.5	0.5
Kidney	Beef	100	16	4	1
	Pork	90	16	3	1
	Lamb	95	17	3	1
Brain	Beef	120	10.5	8.5	<1
	Pork	125	10.5	9	<1
	Lamb	120	10.5	8	<1
Tongue	Beef	185	16.5	13	0.5
	Pork	180	16	13	0.5
Spleen	Beef	110	18	35	1
	Pork	105	18	25	-
	Lamb	95	17	3	-
Blood	Beef	70	16.5	0.4	0.1
	Pork	70	17	0.4	0.1

Herbert W. Ockerman et al 2014, Honikel, K.O., 2011

Utilization of blood and blood plasma

The first by product which is mainly obtained from slaughter house animals are blood. Blood collected from animals that are having good health conditions will be sterile, and it should be recovered under stabilized and hygienic manner. Bovine blood contains about 17.3% protein, 80.9% water 0.07% carbohydrate, 0.62% minerals and 0.23% lipid. (Duarte et al., 1999). Animal blood contains a high amount of proteins in which the most essential compound is hemoglobin, also called as iron-containing protein (Ofori and Hsieh, 2012). Blood can be incorporated into

different kinds of meat products and it is then filled into artificial or natural casings followed by water cooking, chilling and cold smoking prior to rechilling.

An increased amount of blood level in certain sausage products may badly effect to its color and flavor, so blood level should be maintained near 0.5% in such kinds of products. Certain products like black sausage and black pudding contains a higher level of blood level. (Nollet and Toldra´, 2011). With the help of a process called as centrifugation, blood can be split into cellular portion and plasma portion (Ofori and Hsieh, 2011). WBCs, platelets and RBCs are present in the cellular portion which helps to enhance the color of sausage products. Due to the negative sensory results and dark colored nature of hemoglobin, its usage is very limited to certain products only. (Ofori and Hsieh, 2011). RBC portions present in the blood of animals like pork, sheep, red deer and cattle are having a good antioxidant property (Bah et al., 2016). In the food sector plasma proteins are having a wide range of applications. Serum albumin, fibrinogen, and immunoglobulins are certain plasma proteins that have a high emulsification and gelation properties, while high water binding capacity, foaming, protein cross-linking or proteins enrichment are the characteristics of some other kinds of plasma proteins. (Del et al., 2008). Plasma proteins are used in ground meat products for meat emulsion stabilization process due to its high tendency to form gels and good water binding capacity on heating. (Ofori and Hsieh, 2012).

Medicinal and pharmaceutical uses of blood

The components present in animal blood contains so many medicinal properties. The highest portion in the animal blood is fluid plasma (63.0%). The albumin content is about (3.5%), fibrinogen & globulin content is (4.0%). Certain blood products are having a wide range of applications in the microbiology lab such as major component in blood agar, nutrient source for the tissue culture media, and as peptone source for microorganisms (Kurbanoglu et al 2004). Components like sphingomyelins, globulins, catalase, glycerophosphates and albumins are having a beneficial application in biological process. Serotonin, fibrinolysin, kalikreninsa, fibrinogen, plasminogen & immunoglobulins are some other blood constituents that has medicinal properties (Young and Lawrie 2007). The blood loss or fluid loss in animals can be overcome with the help of purified bovine albumin. In humans it is needed to test the Rh factor, applied in antibiotic sensitivity tests and also used as vaccine stabilizers.

Gelatin from hides and skins

Gelatin is an edible food ingredient which is obtained from collagen (water insoluble) formed from protein by a method of hydrolysis under controlled circumstances. The raw materials used to produce gelatin are hide, skin and bones. Bones & hides are good source of collagen. The important steps involved in the production of gelatin from skin and hides are rejection of portions which are not collagenous, from the raw materials. Next step is the production of gelatin from collagen by a process of controlled hydrolysis followed by the drying and collection of end product.

Utilization of bone

The percentage of bone present in animals like pig, beef and sheep based on their whole carcass is 11%, 15% and 16% respectively. The bone marrow is also consumed as food. Animal carcass contains about 4.0 – 6.0% of bone marrow. (West and Shaw 1975). Bones of aniaml can be used for the preparation of gelatin and soups. Mechanically deboned meat which is produced from animals like pig, cattle and sheep is now widely used as meat products in several countries (Field 1981). Meatcum bone meal (MCBM) is a major product obtained from the rendering plant in which bone is used as a major ingredient. Meatcum bone meal (MCBM) contains a huge amount of proteins, vitamins, minerals and some essential amino acids so that it is incorporated in pet food and other kinds of animal food as a good nutrient source. Meatcum bone meal can also be used as phosphorus fertilizer and as a fuel source.

Utilization of glands and organs

Meat by-products such as animal organs & glands are having a higher nutritional content and also responsible for a variety of textures and flavors. After processing it can be utilized for the preparation of pet foods. Heart, kidney, spleen, lungs, brain, tounge, pancreas of cattle, udder, stomach, thymus & testes of lamb & pork, pork uterus, tripe, reticulum, abomasum & omasum of lamb & cattle etc... are the widely used organs and glands as meat by products. (Liu 2002).

Fish waste/by-products utilization

Fat, proteins and minerals are the major nutrients obtained from the fish wastes. A different variety of functional foods can be prepared from keratin or collagen which is present in fish waste & slaughter house wastes with the help of hydrolysis using enzymes. Bones from yellow tailed fish & wastes from pork skin are good source of Collagens & are used as the major ingredients for the preparation of peptides and protein hydrolysates & the hydrolysates thus obtained is used as ingredients in food. (Morimura et al. 2002). Fish waste contains peptides which are biologically active and certain enzymes that are used for the preparation of fish sauce, fish feeds and fish silage (Gildberg 2004). The viscera of fish which is considered as a waste material is used to produce peptone hydrolysates by a method of auto-hydrolysis and it is applied in the culture media to promote the growth & enhance bacteriocin production by lactic acid bacteria (Vanquez et al 2004). The processed fish waste such as fish mince, fish gelatin etc.… are used in fish protein concentrates, nutraceutical food products and fish based meals. Fish waste is also used as ingredients in pet food and poultry food (Hammoumi et al 1998). Waste from fish processing units are collected and it is then fermented biologically with the help of starter cultures like lactobacillus & saccharomyces species and result in the production of protein rich food products for pets. Studies shows that different type of anticarcinogenic & antimicrobial compounds like gelatin, proteins & enzymes can be extracted from sea food wastes. Crab shell & shrimp are good source of Chitosan, which has a higher medicinal value and also use in cosmetic industries (Arvanitoyannis and Kassaveti 2008). Due to the antimicrobial characteristics of Chitosan it applicable as food grade preservatives during the packaging of food products in which it forms a film. Chitosan also helps to inhibit the growth of yeast, fungi & bacteria (Rabea et al. 2003; Shahidi et al. 1999). The fish oil contains a higher amount of unsaturated fatty acids. The oil extracted from fish waste can be used as a source of fuel in diesel engines after treating with ozone.

Rendered products in pet food

Rendering is a heat processing method that involves the conversion of waste animal parts or tissues in to stable, value added materials. The materials used for rendering is collected from slaughter houses, also includes restaurant grease, trimmings from butcher shops and expired meat from grocery stores, which includes fatty tissues, bones and offals and the carcasses of animal condemned at slaughter houses, and those animals that are died on farms etc. can be utilized.

Edible rendering may result in the production of a thick, lumpy "stew" which is sold to pet food industry and it is widely used in tinned cat and dog foods. Lard and edible tallow is also produced using edible rendering process. Meat cum bone meal which is a major ingredient of pet food industry is produced by using inedible rendering process (Ellin et al 2012). Meat parts, meatcum bone meal, poultry wastes, poultry by-products and fish wastes etc. are the major rendered protein meals that are widely used in pet foods (Murray et al 1997)

Meat cum bone meal (MCBM)

Meat cum bone meal is a major product of the rendering plant. It contains up to 48-52% of protein, ash 33-35%, 8-12% fat and moisture 4-7%. It is mainly used as an ingredient in the feed of animals to enhance the amino acid content of the feed. In united states MCBM is widely used as cheap-cost ingredient in cat & dog food. MCBM is mixed with corn or wheat flour to produce a better-quality pet food. Meat cum bone meal is prepared from Slaughter house wastes and dead animals. Slaughter waste includes parts of animals that are not consumed by humans, such as trimmings, carcasses, contaminated carcasses, contaminated livers, bones and inedible offal(lungs). Normally blood, and hairs are excluded. The carcasses of dead animals are rendered to destroy the pathogens and made in to meatcum bone meal. MCBM is the richest source of supplemented protein, phosphorus, calcium, and vitamin-B & it is also a good energy source. MCBM can be classified in to two based on the protein level in the final product.

1.Meat meal (protein content >55%)

2.MCBM (Protein content around 40-55%)

If higher the ash content, then higher will be the amount of bones and it is meat cum bone meal. If the ash content is lower, then it is meat meal. Similarly, if the phosphorus content is greater than 4.5%, then it is considered as meatcum bone meal and if it is lower than that level it is called as meat meal. Meat cum bone meal is very cheap and it is available in almost all meat technology units. (Manli Liu ,2000 may). MBM is mainly used as a major ingredient in animal feed to increase the amino acid content of the feed and it is used as a low-cost meal in united states for dogs and cats. Meat cum bone meal is mixed with corn or wheat midds to produce a good quality pet food (Hendriks et al 2002).

Table 3 Proximate composition of meat cum bone meal

Composition of meat and bone meal		Mineral content in MCBM		Amino acid profile of meat and bone meal	
Components	Meat and bone meal (%)	Items	MBM (%)	Amino acid (%)	MBM
Crude protein (cp)	50.4(43.8-56.7)	Ash	25.6	Protein	50.4(43.8-56.7)
		Ca	10.30	Arginine	3.46(3.04-4.57)
Gross Energy(GE)	4001(3392-4359)	P	5.10	Cystine	0.64(0.32-1.16)
		Non phytate P	5.10	Histidine	0.91(0.51-1.52)
Ash	29.2(22.4-34.5)	K	1.45	Isoleucine	1.38(0.82-1.63)
		Na	0.7	Leucine	3.07(2.08-3.90)
Non-phytate P	5.1(3.8-6.6)	Cl	0.69	Lysine	2.59(1.57-3.23)
		Mg	1.12	Methionine	0.63(0.48-1.02)
Dry Matter(DM)	93	S	0.5	Phenyl alanine	1.65(1.12-2.12)
		Fe(mg/kg)	490	Tyrosine	0.98(0.53-1.25)
Fat	10(7.8-13.9)	Mn (mg/kg)	14	Threonine	1.59(0.99-1.84)
		Cu(mg/kg)	2	Tryptophan	0.31(0.23-0.35)
Calcium	10.30(6.7-12.9)	Se(mg/kg)	0.25	Valine	1.97(1.37-2.42)
		Zn(mg/kg)	93		

Manli Liu 2000, Wang & Parsons 1998, Waldroup and Adam (1994), Batterham et al. (1980)

Spent hen meal based pet food

"Spent hens" are commercial type of egg laying hen breeds in which the production level is almost completed. With the addition of 10-20% of spent hen meal, a pet food with better nutritional quality and palatability can be developed and it will have a shelf life of approximately 45 days at room temperature. The first stage is the crushing of the whole carcasses of spent hen, after that by the method of dry rendering it is processed in the laboratory by cooking it for a time period of 20-minute at a temperature range of 100°C, then sterilize it at 140°C for about 20minute and finally dry the product at 100°C for 60minute (Rojas et al 2013). Fat can be removed from the cooked materials by centrifuging it in the basket centrifuge at 1000rpm for about 20minute. Then the SHM is cooled, dried and grounded in a hammer mill after that it is packed in LDPE bags and stored in

a place which is cool and dry at room temperature (35±2°C). To check the yield and quality of final product the moisture content, crude protein content, ether extract, total ash, crude fiber content, nitrogen free extract, Ca, P, and metabolize energy by the method of NIR spectroscopy and the amino acid content of SHM is need to be determined. The approximate rate of pet food which constitutes about 10 to 20% SHM would be 18-22.75 rupees (Karthik P, et al 2009)

Chemical composition of spent hen meal		Amino acid as % protein	
Components	Spent hen meal (%)	Amino acid	SHM (%)
Moisture	5.5 ± 0.44	Methionine	1.61
		Cystine	1.75
Ether extract	9.5 ± 0.48	Lysine	5.14
		Threonine	3.87
Total ash	12.3 ± 0.25	Tryptophan	0.85
		Arginine	6.35
Phosphorus	2.2 ± 0.04	Isoleucine	2.31
Crude protein	72.1 ± 1.13	Leucine	4.27
Crude fiber	0.2 ± 0.02	Valine	3.01
		Histidine	1.30
Ca	4.4 ± 0.06	Phenyl alanine	2.43

Table 4 Proximate composition of spent hen meal (Karthik P et al 2010)

CHAPTER 4: PROPOSED RESEARCH OBJECTIVES

The mandate of the present study is as under –

1) Physico-chemical analysis, deodorization & standardization of raw tripe.
2) Recipe standardization; Preparation and standardization of the pet food by utilizing meat industry waste.
3) To optimize the conditions for the preparation of pet food.
4) To study storage quality of the pet food through physicochemical, microbial and organoleptic evaluation for a time period.

CHAPTER- 5 PROPOSED RESEARCH METHODOLOGIES

Pet food is prepared by utilizing tripe as major ingredient & Maida, corn flour, meatcum bone meal, & egg as minor ingredients through a continuous process of chopping, mincing, mixing, shaping & cooking.

Equipments required for preparing pet food

1. Meat mixer
2. Meat mincer
3. Hot air oven
4. Tray
5. Mould

The flesh products used in pet foods must first be rendered, or processed, to separate the water, fat, and protein components, including soft offals (viscera) and hard offals (e.g. bones and hoofs). Frozen tripe is used for dry foods.

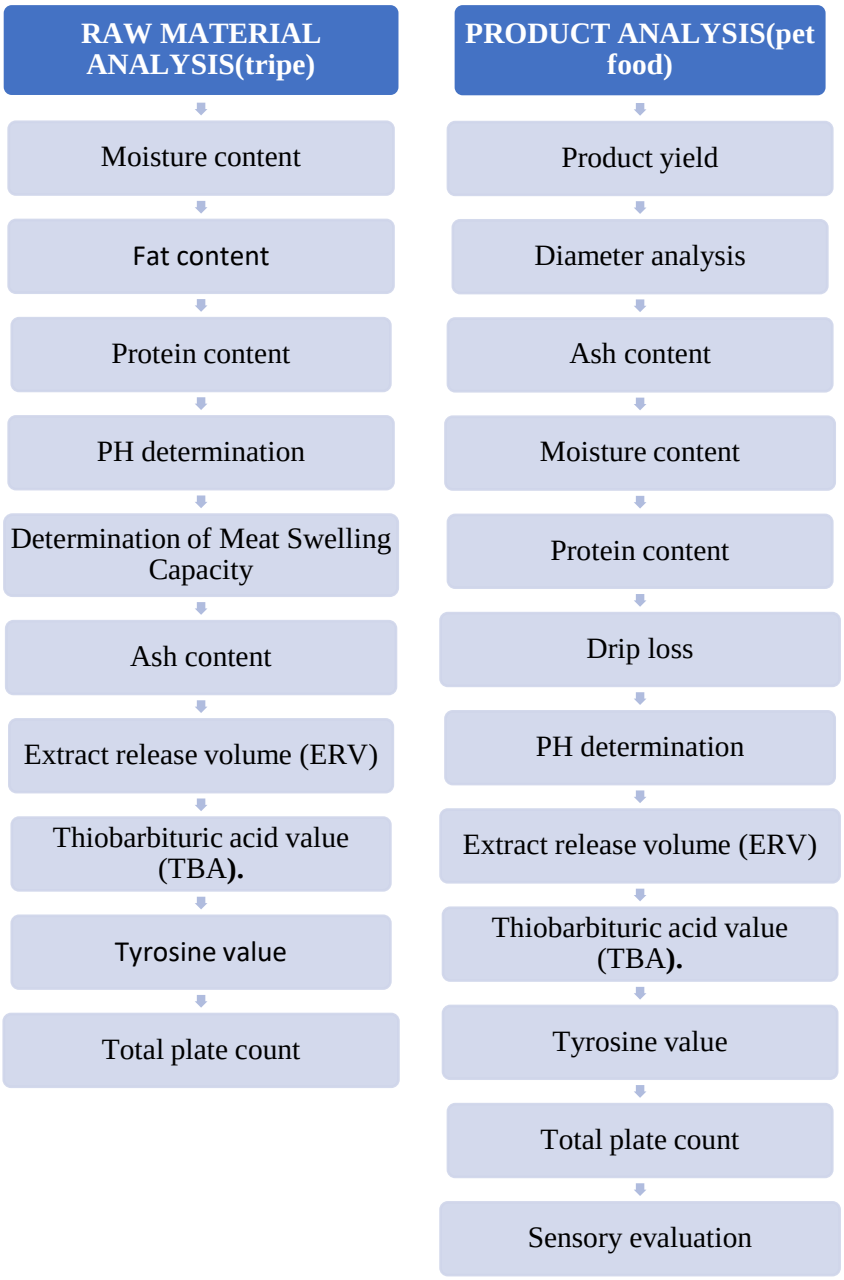
Grinding and pre-cooking the tripe

- The tripe is coarsely ground to the desired texture.
- To facilitate further processing, the ground tripe is cooked in a continuous cooker with live steam at the appropriate temperature.
- The flesh products are reground after initial cooking to produce a more uniform consistency.

Blending and shaping

- Make the tripe in an emulsion form
- The tripe mixture is blended with other ingredients.
- Dry and semi-moist foods are usually heated so the mixture will partially dextrinize, or thicken, the starch. Make the mix in to suitable shapes using mold
- Grease the tray & arrange the product.
- Cook it in an oven at 80°C.

PHYSICO-CHEMICAL ANALYSIS



Raw material analysis(tripe)

1) Moisture content

Moisture of Pet food sample will be determined by using hot air oven method at 105°C for 90-100 minutes. Drying process will continue until the constant weight will reach, then percent moisture will be determined (AOAC 1990).

Calculation:

% moisture content= Initial weight-final wt/ initial wt x 100

2) Fat content

Take sample of pet food in a dry thimble. Petroleum ether will be used to extract with the boiling range of 600-800C for 3 hours. The fat extract will be collect in a weighed dry flat bottom flask, separated from the solvent by evaporating over a hot water bath. The flask will be dry in an oven at 800-1000C and cooled till constant weight is obtained (S. Butool and M. Butool., 2013).

Calculation:

%fat= final wt- empty wt of beaker/sample wt

3) Protein content

Ground the powder place sample (accurately weighed) in a Kjeldahl flask, Add acid and catalyst, digest until clear to get complete breakdown of all organic matter. Protein= Sulfuric acid/Heat, catalyst= (NH4)2SO2

Dilute digest with water add sodium thio-sulfate to neutralize the sulfuric acid. The ammonia formed is distilled into a boric acid solution containing the indicators methylene blue and methyl red (AOAC Method 991.20).

Calculations- Moles of HCl = moles of NH3

= moles of N in the sample

A reagent blank should be run to subtract reagent nitrogen from the sample nitrogen.

%N=NHCl× Corrected acid volume/ g of sample × 14gN/ mol ×100

where: NHCl = normality of HCl, in mol/1000ml

Corrected acid vol. = (ml std. acid for sample) – (ml std. acid for blank)

14 = atomic weight of nitrogen A factor is used to convert percent N to percent crude protein. Most proteins contain 16% N, so the conversion factor is 6.25 (100/16 = 6.25). %N/0.16 = %protein or %N×6.25 = %protein. (Kjeldahl Method).

4) Determination of total ash

Weigh accurately about the 5 gm of sample in a silica crucible. Flame the dried material in the dish left after the determination of moisture with the flame of a burner till charred this process is charring. Transfer to a muffle furnace maintained at 550 – 600°C and continue ignition till grey ash is obtained. Cool the crucible with ash in a desiccator and weigh. Repeat the process of heating, cooling and weighing at half hour intervals till the difference in weight in two consecutive weightings are obtain is less than 1 mg. Note the lowest weight. If ash still contain black particles add 2-3 drops of pre-heated water at 60°C. Break the ash and evaporate to dryness at 100-110°C. Re-Ash at 550°C. Until and until ash is white or slightly grayish in color.

Weight of empty crucible = W (g)

Weight of crucible + sample before ashing = W1 (g)

Weight of crucible + sample after ashing = W2 (g)

$$\text{Ash content (\%)} = \frac{\text{Weight after ashing (g)}}{\text{Weight of sample(g)}} \times 100$$

(OR)

$$\text{Ash content}(\%) = \frac{(W2 - W)}{(W1 - W)} \times 100$$

5) pH determination

Digital pH meter

1. Blend 15 gm meat with 30 mL distilled water at 27-300C.

2. Note the pH with a glass electrode pH meter.

Reference method: Chicken broth flavor and pH by Pippen et al. (1965), Poultry Sci. 44: 816-823

6) Determination of meat swelling capacity

This test determines the freshness of meat. Swelling capacity of meat increases during spoilage due to protein degradation and penetration of more amounts of water in protein matrix. A method of measuring the water binding capacity of muscle proteins with low water holding forces known as meat swelling (SW).

a) Take 25 gm of meat in 100 mL of distilled water

b) Blend it for 2 min

c) Centrifuge 35 mL of homogenate at 2000 rpm for 15 min

d) Measure the volume of supernatant (S)

e) Record the volume and denote it as "S".

Percent meat swelling can be determined as

% Meat Swelling = (35-S-7)/7 X 100

Reference method:

Determination of meat swelling capacity as a method for investigating the water binding capacity of muscle proteins with low water holding forces. II Application of the swelling methodology by Wierbicky et al (1963) Fleischwirtschaft 15: 404.

7) Determination of extract release volume (erv):

a) Take 25 gm meat sample in 100 mL distilled water

b) Bend it with in pestle and mortar

c) Filter through celluose based qualitative filter paper, folded thrice so as to make eight sections.

d) Allow the homogenate to seep between the folds

e) Collect the extract in 100 mL graduated cylinder for 15 min.

f) Record extract release volume and interpret results

(15 g of minced stored sample was blended with 60 ml of distilled water in a homogeniser and homogenate was transferred as quickly as possible into a funnel, equipped with a Whatman filter paper No.1. The volume of filtrate collected in the first 15 min was recorded as ERV of the respective sample.)

Reference method:

Jay (1964), Release of aqueous extract by beef homogenates and factors. Food Technol.18:129-132. Jay (1964), Beef microbial quality determined by extract release volume (ERV) (1964), Food Technol. 18: 132-137

8) Thiobarbituric acid value (tba).

Trichloroacetic acid extracts of each sample were used for measuring the absorbance at 532 nm. TBA value was calculated as mg malonaldehyde per kg of meat sample by referring to a standard graph prepared using known concentration of malonaldehyde.

TCA extract. Twenty grams of meat were blended with 50 ml of cold 20% trichloroacetic acid (TCA) for 2 min. The blender contents were rinsed with 50 ml of water, mixed together, and filtered through a Whatman #1 filter. This filtrate is termed the TCA extract and is used

in the TBA and tyrosine tests.

TBA number. A 5-ml aliquot of the TCA extract was mixed with 5 ml of 0.01 M 2 thiobarbituric acid. Either of two procedures was used for TBA color development. One procedure involved storage for 14 h at room temperature (ca. 20 C) and the other for 30 min at 100 C. Color

development, measured as Absorbance (A) at 532 nm, was identical when either color development procedure was used with standard solutions of tetraethoxypropane or with TCA extracts of meat. Absorbance at 532 nm is reported as the TBA number.

9) Tyrosine value.

Two and one-half ml of the TCA extract were diluted with 2.5 ml of water. To this 10 ml of 0.5N NaOH were added followed by 3 ml of Folio's Reagent (diluted 1 Folin's:2 water). After mixing, the color was developed for 15 min at room temperature before reading at 660 nm. The "tyrosine" value is reported as mg of tyrosine/ g of meat.

10) Enumeration total viable count (TPC) / aerobic plate count (APC)

For evaluating total viable counts of the microorganisms, standard pour plate technique is used. Take 0.1ml of 10-4, 10-5 and 10-6 dilutions (in duplicate) of inoculum in the Petri plates to which pour molten agar having temperature around 45-500C and mix thoroughly by rotating plate clockwise and anticlockwise for five times. Allow the plates to solidify and then keep the plates for incubation at 370C for 24-48 hrs.

Product analysis (pet food)

1) Product yield

The weight of each roll was recorded before and after cooking, the product yield was calculated and expressed as percentage.

$$Product\ yield = \frac{Weight\ of\ cooked\ rolls}{Weight\ of\ raw\ rolls} \times 100$$

2) Diameter analysis

The diameter of rolls was measured before and after cooking with a digital vernier caliper at three random locations. The reduction in diameter shrinkage (DS) was expressed as percentage.

Ash content, Moisture content, protein content, determination of extract release volume, PH determination, Thiobarbituric acid value, Tyrosine value, Total plate count etc… are determined by the same procedure as used in raw material analysis.

3) Drip loss

It is determined by reweighing blotted slices of roll after one week of storage at 4 ± 1 _C

$$Drip\ loss = \frac{Weight\ loss}{Initial\ weight} \times 100$$

4) Sensory evaluation

The pet food prepared from tripe is evaluated on the basis of texture, colour, appearance, and overall acceptability by a panel of 9-10 judges by using 9-point hedonic scale assigning scores from like 9- extremely to 1- extremely (Appendix- 1) dislike and score of 5.5 and above was considered as acceptable (Amerine et al 1965).

The product is also tested on selected dogs and the preference is determined.

APPENDIX- 1 (Hedonic Rating Scale)

Name: **Date:**

Product:

Evaluate the given samples and check how much you like or dislike each one. Use the appropriate rating from the scale to show your attitude by checking at the point that best describe your feeling about the sample. Please give a reason for your attitude.

Score Preference	Code
Like extremely	9
Like very much	8
Like moderately	73
Like slightly	6
Neither like nor dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

Sample code: **Remarks:**

Score:

Signature:

CHAPTER- 6 EXPECTED RESEARCH OUTCOMES

In this research main aim is to prepare nutrient rich as well attractive pet food by using tripe, which is considered as a meat industry waste. Meat and meat processing industries have a huge amount of waste products having a high nutritional quality and this can be incorporated in pet food particularly to dogs and cats to minimize the cost of production and to maximizing the profit and to produce high value-added products. The waste materials and other unwanted substances are collected and modified to useful product of better quality by using suitable processing methods and efficient in plant treatment.

CHAPTER- 7 PROPOSED WORK WITH PLAN TIMELINE

Work plan	Jan	Feb	March	April	May	June	July	Aug	Sept	Oct	Nov
Review of Literature	√	√	√								
Report Submission				√							
Product standardization						√					
Product development						√					
Product Analysis							√				
Result compilation								√	√		

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