Exclusivité Atavik

La preuve par le frais

Analyse nutritionnelle comparée du frais et du déshydraté dans les croquettes pour chiens et chats.

L’étude scientifique qui démontre les bénéfices du frais dans les croquettes

Une étude de l’Université de Nottingham en Grande-Bretagne l’atteste : les croquettes à base d’ingrédients frais sont plus digestes que les croquettes à base de farines animales !

On l’avait constaté sur nos chiens et nos chats. Mais maintenant, c’est prouvé par la science !

Cette recherche, menée par les biochimistes et docteurs en nutrition Aboubakry Diallo, Sandra Hill et Angelina Swali, a analysé la digestibilité de tous les nutriments de croquettes à base d’ingrédients frais face à celle des nutriments de croquettes à base de sous-produits déshydratés. Les résultats sont massivement en faveur du frais !

Plus de protéines

Avec une recette ne contenant que des farines animales, seules 61% des protéines sont digestes. Donc, près d'une protéine sur deux dans les produits à base de viande déshydratée ne sert à rien.

En revanche, 95% des protéines animales des croquettes à base d'ingrédients frais sont digestes !

	Croquettes à base de sous-produits animaux déshydratés	Croquettes à base d'ingrédients frais
Digestibilité protéique (%)	61	95
Lysine disponible (g/kg protéine)	41	65

Plus de protéines

Avec une recette ne contenant que des farines animales, seules 61% des protéines sont digestes. Donc, près d'une protéine sur deux dans les produits à base de viande déshydratée ne sert nutritionnellement à rien.

En revanche, 95% des protéines des croquettes à base d'ingrédients frais sont digestes. La quasi-totalité ! La déperdition est minimale, et les protéines de ces aliments sont donc 55% plus digestes que celles des produits sans trace de viande fraîche.

Parmi ces protéines, la lysine est l'une des plus importantes pour les carnivores. Elle développe les muscles, contribue donc à la santé du squelette et des articulations, renforce le système immunitaire, etc

Pour 1 kg de protéines dans les croquettes, il y a 58% plus de lysine dans des croquettes à base de frais que dans les croquettes aux farines animales. Soit près de 25g de protéines pures et digestes en plus, ce qui est énorme !

De meilleurs lipides

Dans 100g de croquettes avec viande fraîche, vous trouverez 50% d'acides gras de plus que dans les croquettes à base de farines !

	Croquettes à base de sous-produits animaux déshydratés	Croquettes à base d'ingrédients frais
Oxydation lipidique (meqO2/kg huile)	1,15	1,24
Acides gras non-estérifiés	6,72	10,11

Plus de lipides

Les Omega-3 et Omega-6 sont aussi primordiaux à la santé des chiens et chats. Santé de la peau et du poil, santé neurologique, vertus anti-inflammatoires : leurs bénéfices sont essentiels pour les carnivores.

Les lipides s'oxydent très facilement. Plus ils sont oxydés, moins ils sont utiles à l'organisme. Les acides gras essentiels sont moins oxydés lorsque les croquettes sont à base d'ingrédients frais que lorsque elles sont à base de farines animales déshydratées.

Plus de vitamines

+15% de vitamine A dans un aliment à base d'ingrédients frais !

2 à 3 fois plus de vitamine D dans un aliment à base d'ingrédients frais !

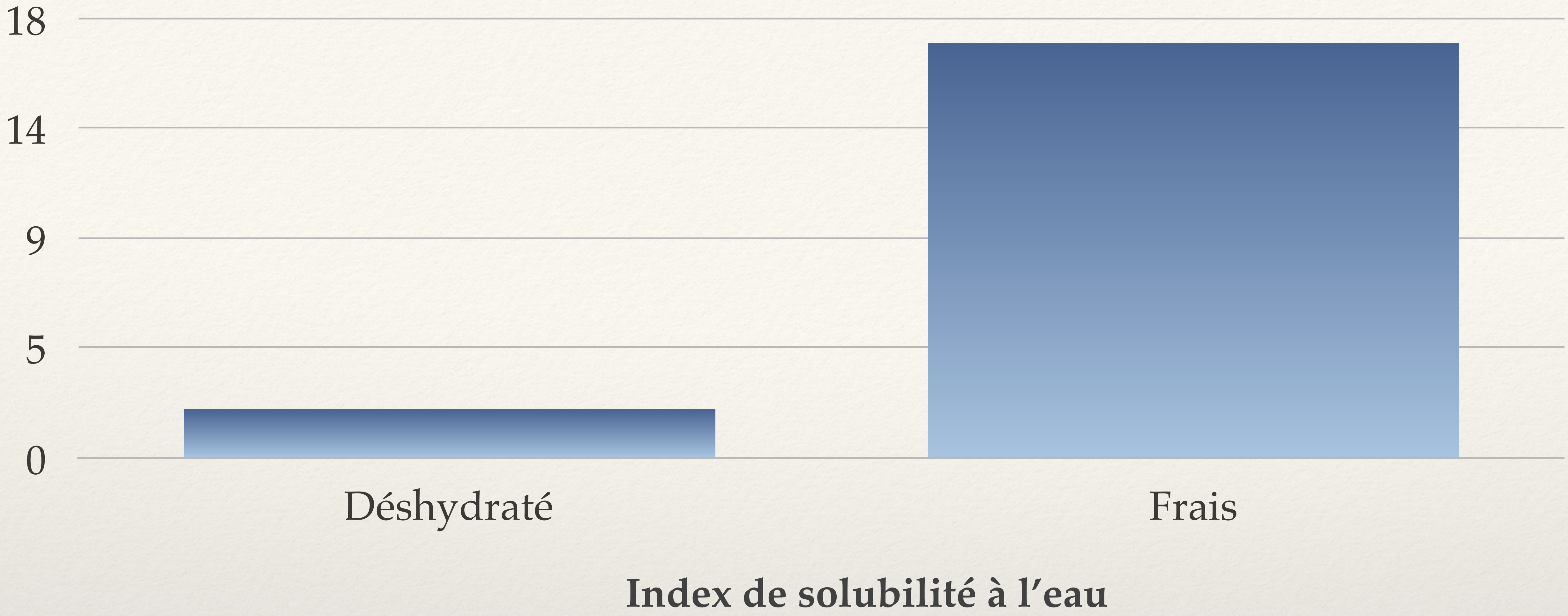
	Croquettes à base de sous-produits animaux déshydratés	Croquettes à base d'ingrédients frais
Vitamine A (iu/kg)	5890	6760
Vitamine D (iu/kg)	<500	1240
Vitamine E (iu/kg)	81	60

Plus de vitamines

+15% de vitamine A en plus dans un aliment aux ingrédients frais !

+150% de vitamine D en plus dans un aliment à base d'ingrédients frais !

Les vitamines A et D servent aux os, aux globules rouges et blancs, etc



Des nutriments plus solubles !

Si les croquettes à base d'ingrédients frais sont plus digestes que celles à base de sous-produits animaux déshydratés, c'est simplement grâce à l'eau que les ingrédients frais contiennent. Cette hydratation des ingrédients les rend plus solubles dans l'estomac.

Il est donc très important pour la bonne nutrition de vos chiens et vos chats de regarder la composition de leurs aliments et de connaître la cuisson de chaque ingrédient. Les protéines commencent à se dégrader à partir de 200ºC, et l'on trouve des croquettes à base de farines animales ou de « viandes déshydratées » cuites à plus de 600ºC.

Solubilité comparée de croquettes à base d'ingrédients frais et de croquettes à base de sous-produits déshydratés
Simulation d'acidité et d'activité stomacale canine, 40°C

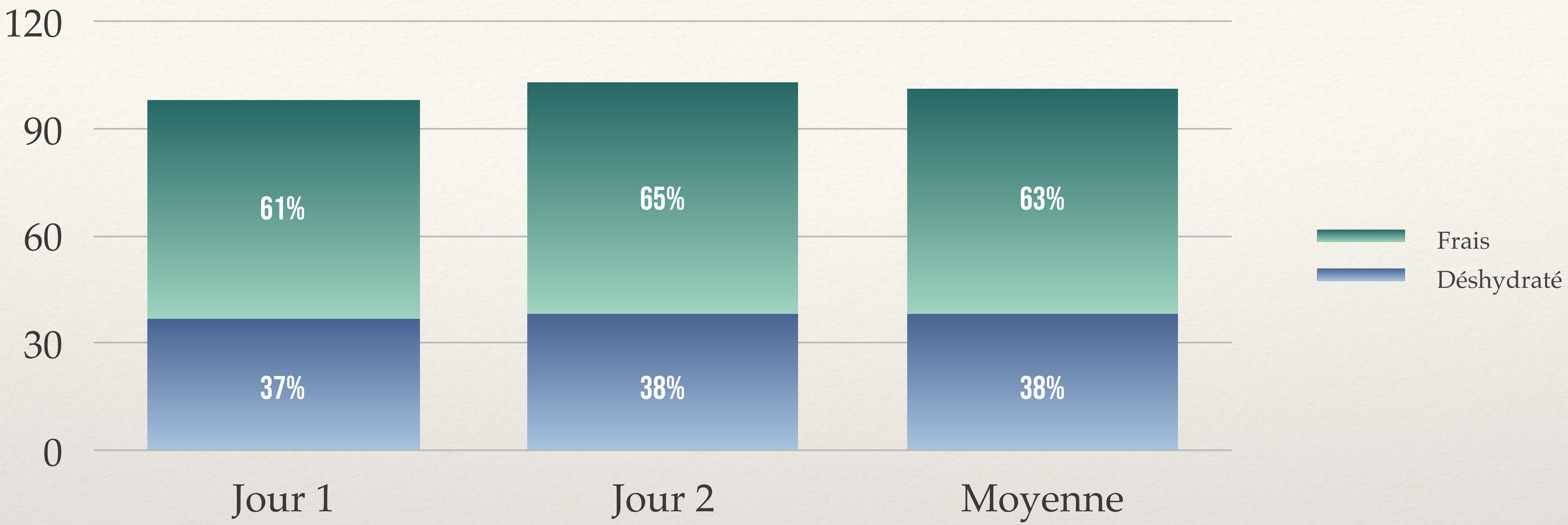
Illustration de la solubilité à l'eau

Des glucides sans conséquence pour la santé !

Les glucides sont présents dans le régime alimentaire naturel du carnivore sous forme de glycogène, dans le foie de ses proies. Dans les croquettes, l'amidon utilisé nécessaire à l'extrusion multiplie la quantité de glucides complexes par 5 par rapport à une proie naturelle. On pensait jusqu'à présent que cette surreprésentation des glucides dans le bol alimentaire entraînait des problèmes hépatiques et pancréatiques. Cette étude nous offre une réponse : cela dépend de la nature des ingrédients qui fournissent l'amidon et de ceux associés à l'amidon.

C'est la révélation majeure de cette étude : l'amidon se décompose 8 à 9 fois mieux en présence d'ingrédients frais qu'avec du déshydraté ! La production d'amylase pancréatique est donc 8 à 9 fois moindre. L'utilisation de croquettes à base d'ingrédients frais **diminue conséquemment les risques d'hépatite et de pancréatite** par rapport à une croquette à base de farines animales.

Test d’appétence sur un panel de 18 chiens de toutes tailles et toutes races



Plus d’appétence !

Il a été donné 2 gamelles à un panel de 18 chiens, de toutes tailles et toutes races, contenant dans l’une des croquettes riches en farines animales, et dans l’autre des croquettes riches en viandes fraîches.
Le test a été effectué sur 2 jours, et les chiens avaient donc accès librement à ces 2 gamelles.
Les résultats sont sans appel : les chiens ont dévoré en moyenne environ 66% de croquettes à base de viandes fraîches de plus que de croquettes à base de sous-produits animaux.

D’après ce test, nous pouvons donc conclure que ces 18 chiens ont naturellement été plus attirés par les croquettes à base de viandes fraîches, plutôt que celles à base de farines animales.

Abstract

GC-MS and Xray Micro-CT are two examples of advanced analytical tools which can be used as predictors of eating quality

GC-MS: MEASUREMENT, IDENTIFICATION AND COMPARISON OF VOLATILES

General Principle

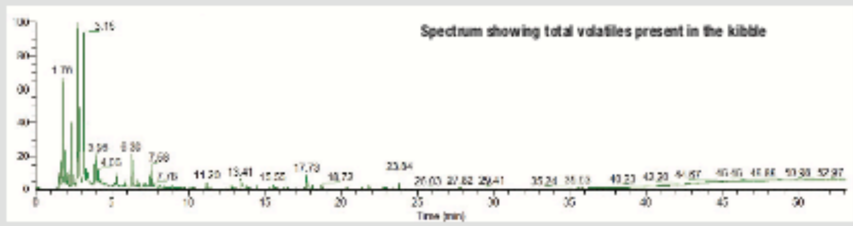
GC-MS allows the separation of chemical mixtures and identifies the components at a molecular level. Individual substances that make the flavour are identified by mass spectrometry based on the mass of the molecule.

The instrument used was an ISQ-SPME Headspace (Thermo scientific).

Powdered samples are placed in the GC-MS and the volatiles are extracted by solid phase micro extraction.

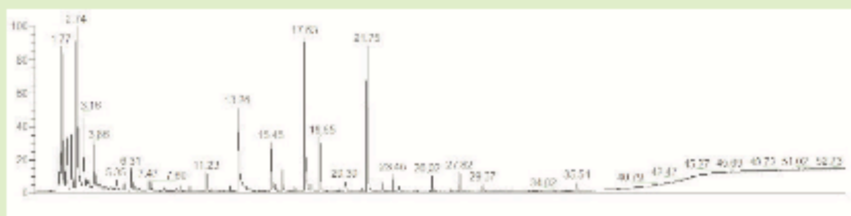
Conventional Process

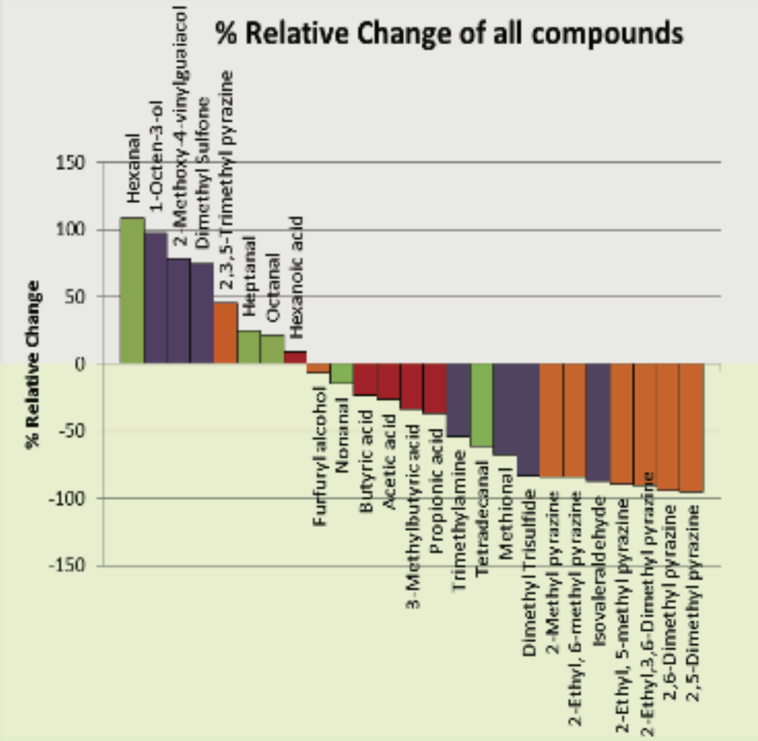
*Meat meal and rice



Novel Thermal Process

*Freshly prepared meat and rice





MICRO-CT: MEASUREMENT AND IDENTIFICATION OF STRUCTURE

General Principle

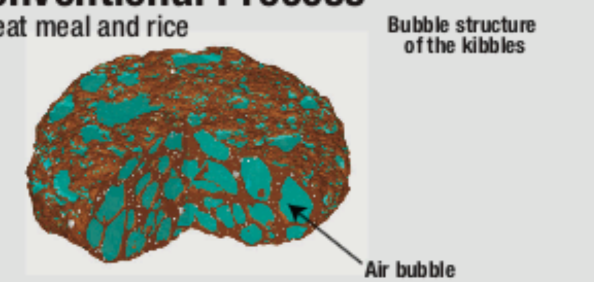
Micro CT scans produce 2D representations of a slice of the kibble based on material density measured by X-ray transmissions.

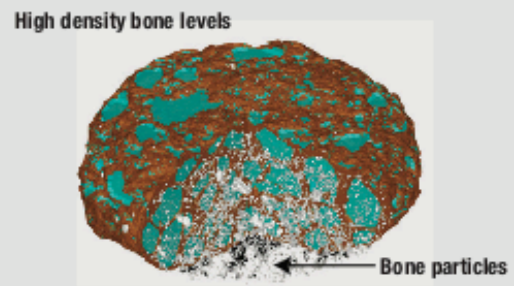
The instrument used was a Phoenix Nanotom (GE).

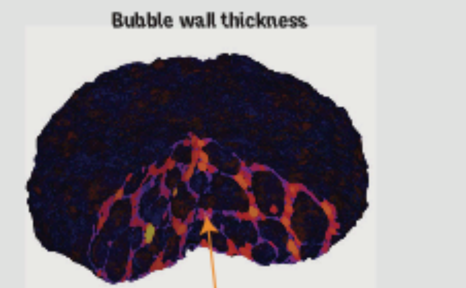
Since a CT scan is a mathematical representation of an object rather than a true image, it is suited to quantitative analysis of structure and geometry.

Conventional Process

*Meat meal and rice

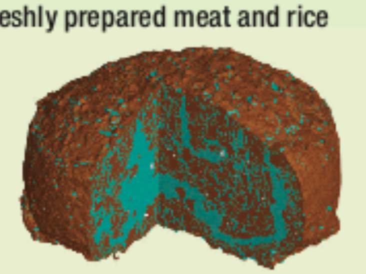


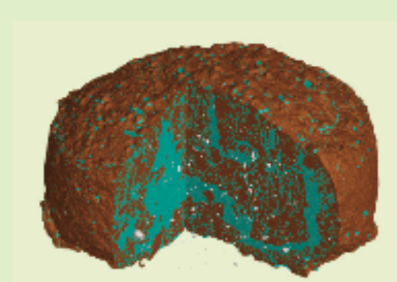


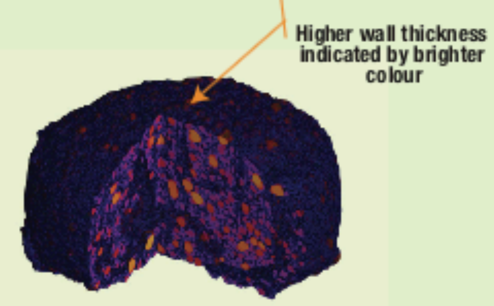


Novel Thermal Process

*Freshly prepared meat and rice







Conclusions

Two dry pet foods manufactured using meat meal and freshly prepared meat, respectively were compared for palatability indicators.

Volatile assessment using GC-MS showed significant variations in profile and intensity that may be due to material and processing.

Texture analysis using X-ray micro-CT also reveals major differences in the internal structures of the kibble such as number and distribution of bubbles, wall thickness and density.

Major differences were found using the methodologies.

These findings, if adequately modelled with in vivo palatability trials would represent significant tools for the early prediction of food acceptance.

Acknowledgements: *The authors wish to acknowledge the University of Nottingham's Flavour group (volatile analyses) and the Hounsfield Facility (X-ray Micro Computed Tomography) for their assistance in obtaining the data used in this poster.*

À suivre, les résultats de l'étude originale complète.

Benefits of using freshly prepared meat in dry pet food by utilizing novel meat processing and extrusion technologies

Aboubakry Diallo, PhD
Research Associate, University of Nottingham

Petfood Forum USA

Where the **Global Petfood Industry** does business

Content

1. Introductions and definitions
2. Protein sources for kibbles
3. Demonstration of protein quality retention
4. Limitations and challenges for commercial production
5. Eating quality

Benefits of using **freshly prepared meat** in dry pet food by utilizing novel meat processing and extrusion technologies

- Terminology for meat by-products

Definition of meat

- What is meant by freshly prepared meat and dried rendered meat
  - The EU regulation N°68/2013 catalogue of feed materials- Definition of meat
    - Definition of meat as skeletal muscle

	Moisture	Protein	Lipid	Ash
*Meat (typical composition) *g per 100g*	75	23	1	1

* *FAO 2007-* **Nutritional composition of meats and other food sources per 100g- Value from chicken meat**

Dried rendered meat (DRM)

- What is meant by dried rendered meat
  - DRM: Variability in the type of material, collection storage condition and production method
  - Typical composition

	Moisture	Protein	Lipid	Ash
Meat	75	23	1	1
Dried rendered meat	5	61	12	16

Freshly prepared meat (FPM)

- What is meant by freshly prepared meat
  - FPM: Starting material- Chilled carcass processed through a deboning machine- may be subsequently frozen
  - Pasteurized- Separated- Concentrated

	Moisture	Protein	Lipid	Ash
Meat	75	23	1	1
Dried rendered meat	5	61	12	16
Freshly prepared meat (FPM)	50	32	13	2

Facts and trends

- Commercial advantages/benefits from using freshly prepared meats
  - Search for new ingredients
  - Offer of high end products and differentiation
  - Growing demand for "fresh" nutritional petfood
  - Trend spreading to the dry petfood market

Protein sources for kibbles

- Dried animal protein
- Dried vegetable protein
- High moisture protein

– *Is it necessary to consider using FPM when rendered meat can offer the necessary nutrients in a convenient dry ingredient?*

– *What are the benefits from utilizing FPM?*

What are the differences when using FPM or DRM

- Variations in proximate composition

	FPM	DRM
Moisture	50	5
Protein	32	61
Lipids	13	12
Ash	2	16

What are the differences when using FPM or DRM

◆ Quality indicators of freeze-dried FPM and DRM, respectively mixed with rice

	Freeze-dried FPM recipe			DRM recipe		
Protein digestibility % (AA equivalent)	95			61*		
Available lysine (g/kg protein)	65			41*		
Vitamin A, D, E (iu/kg)	6760	1240	60	5890	<500	81
Lipid oxidation-Peroxides (meqO_2/Kg oil)	1.24			1.15		
Free fatty acids	10.11			6.72*		
Anisidine value	< 0.5			< 0.5		

**Value significantly different from the FPM, p<0.05*

Are the benefits of FPM sustained throughout the extrusion process?

- Experimental design
  - Pilot scale extrusion- Twin screw Prism 24 MC Thermo Fisher extruder
  - **Freeze-dried** FPM + white rice

Are the benefits of FPM sustained throughout extrusion?

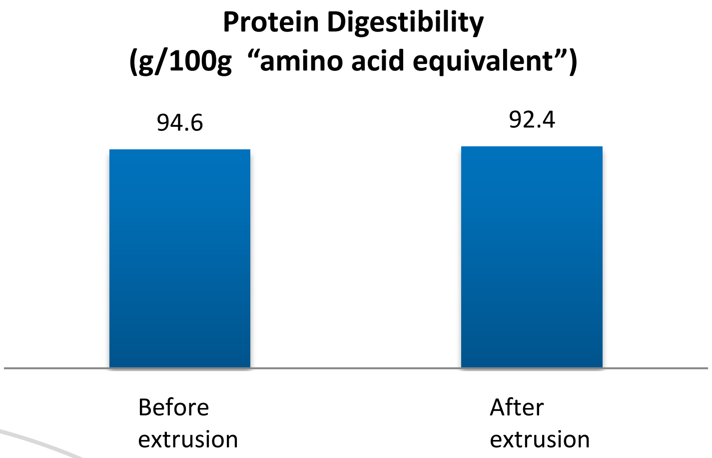
- Experimental design
  - 4 tests: Range of SME applied (25-60 kwh/t)
  - Temperature range inside the barrel 60°C-140°C

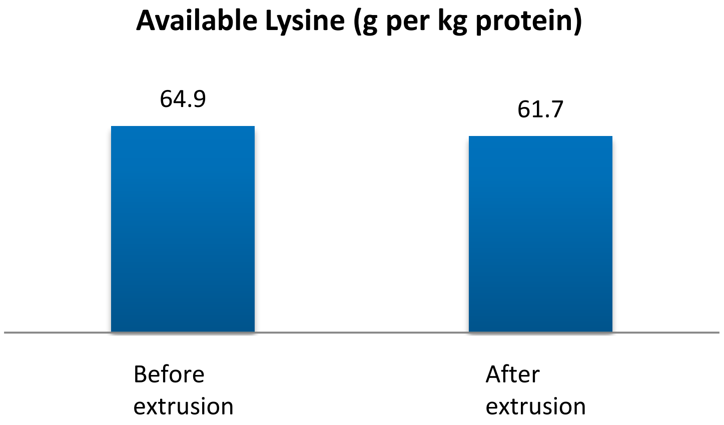
Expérimental design

Screw speed (rpm)	Barrel Temperature (°C)									SME (Kwh/t)	Water content %
300	61	59	85	100	119	119	119	119	138	35.7	64
400	60	58	87	100	119	119	119	120	140	47.62	64
200	60	59	89	100	120	120	120	120	140	23.83	64
400	60	59	88	101	119	119	119	119	140	59.16	32

Are the benefits of FPM sustained throughout extrusion?

- Nutritional and quality indicators of the extrudates





Are the benefits of FPM sustained throughout extrusion?

◆ Nutritional and quality indicators of extrudates

	Freeze-dried FPM recipe	
	Before extrusion	After extrusion
	iu/kg	
Vitamin A	6760	6000 ± 2305
Vitamin E	73	60 ± 3.5
Vitamin D	1240	< 500

	Freeze-dried FPM recipe	
	Before extrusion	After extrusion
Peroxides ($meqO_2$/kg)	1.15	2.46 ± 0.3
Free Fatty Acid (g/100g oil)	6.7	3.9 ± 0.5
Anisidine value*	< 0.5	0.7 ± 0.1

- measures the secondary oxidation products like aldehydes, carbonyls, trienes, ketons
- The AV is empirically defined as 100 times the absorbance of a solution resulting from 1 g of fat or oil mixed with 100 ml of isooctane/acetic acid/*p*-anisidine reagent, measured at 350 nm

- *Is it necessary to consider using FPM when rendered meat can offer the necessary nutrients in a convenient dry ingredient?*
- *What are the benefits from utilizing FPM?*

- *There are nutritional benefits, particularly protein quality*
- *And these benefits seem to be retained during extrusion*

- But the FPM used was freeze-dried

Limitations and challenges of using FPM

	Moisture	Protein	Lipid	Ash
Mechanically separated meat	75	16	7	1.1
Freeze dried FPM	5	61	25	4

- Dry extrudate manufacturing
  - Moisture
    - Inherent high moisture content of meat will not allow significant inclusion level
  - Lipids
    - Along with moisture, fat will reduce mechanical energy

Limitations and challenges of using FPM

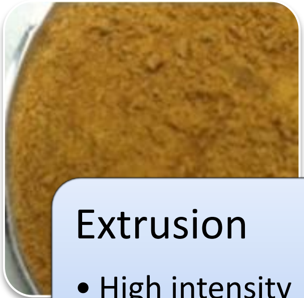
- Logistics
  - – Sourcing- Level of supply chain
  - – Storing conditions- microbiological control

The novel thermal process

Mechanically separated meat

FPM Preparation

- Logistics-conditioning
- Particle size reduction
- Pasteurization
- Concentration



Extrusion

- High intensity preconditioning
- Thermal twin extrusion

Drying

- Pre-drying
- Final drying

The novel thermal process (FPM preparation)

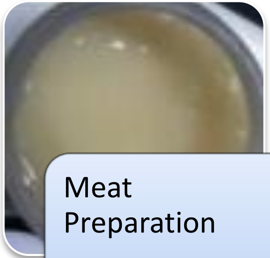
- Reception and storage of the meat
  - Maintain cold chain
  - Pathogen control
    - Microbial load
    - Lipid Oxidation
    - Biogenic amines

FPM Preparation

- Particle size reduction
- Pasteurization
- Concentration

The novel thermal process (meat preparation)

- FPM Preparation
  - Particle size reduction
  - Pasteurization- Pathogen control

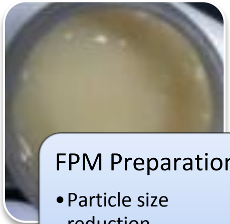


Meat Preparation

- Particle size reduction
- Pasteurization
- Concentration

The novel thermal process (FPM preparation)

- FPM Preparation
  - Separation- Water and/or Fat separation by centrifugation
  - Concentration- Low temperature evaporation



FPM Preparation
- Particle size reduction
- Pasteurization
- Concentration

	Moisture	
	Before	After
Preparation	70-80%	50%

← FPM

Fat content can also be manipulated to suit application

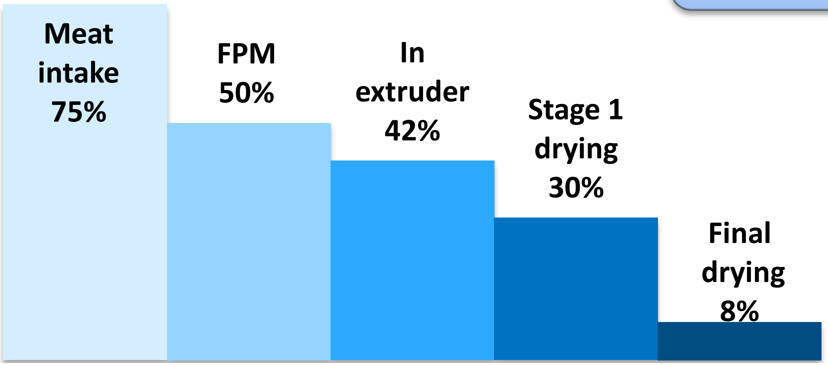
The novel thermal process (Extrusion)

Extrusion
- High intensity preconditioning
- Thermal twin extrusion

- Dry Extrusion
  - High intensity Pre-conditioner *(Model 1500 Wenger)*
    - Blend wet FPM with dry ingredients
    - Uniform hydration- *Moisture content 42%*
  - Thermal twin extrusion
    - Wenger Thermal Twin extruder TT3630
      - Thermal “cook” vs mechanical

The novel thermal process (Drying)

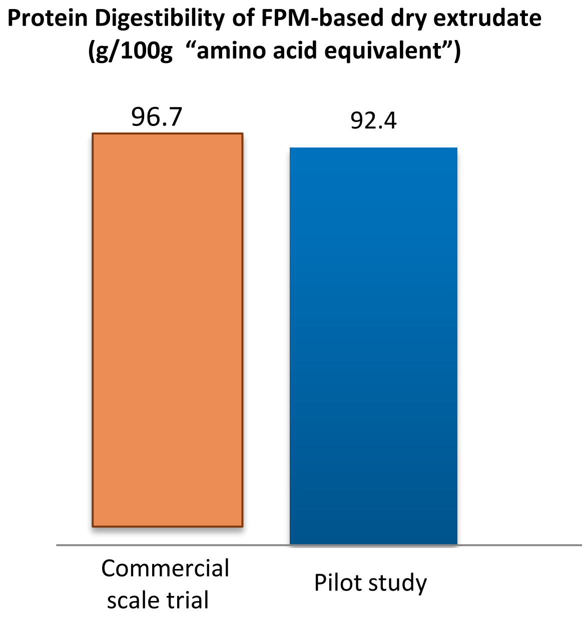
- Dry Extrusion
  - Pre-drier-Stage 1 drying
  - Drier-Stage 2 drying



Moisture changes throuhout the process

Extrusion trials of FPM in comparison with freeze dried FPM

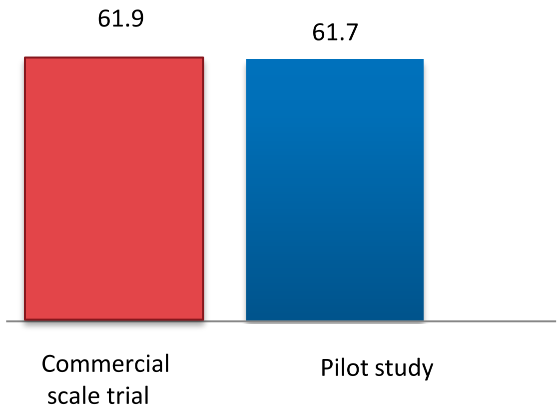
- Commercial scale study
  - Freshly prepared meat and rice
- Pilot scale
  - Freeze dried FPM and rice



Extrusion trials and comparison

- Commercial scale study
  - Freshly prepared meat and rice
- Pilot scale
  - Freeze dried FPM and rice

Available Lysine of FPM-based dry extrudate (g per kg protein)



Extrusion trials and comparison

- Commercial scale study
- Pilot scale

	Commercial scale	Pilot extrusion	Codex Standard for Fats and Oils from Animal Sources
Peroxides (meqO_2/kg)	5.9	2.5	Up to 10
Free Fatty Acid (g/100g oil)	4.5	3.9	Up to 1.25
Anisidine value	1.8	0.7	-

The novel thermal process

- Summary
  - FPM presents high nutritional qualities
    - High protein quality
      - High digestibility
      - Relatively high levels of available lysine after extrusion
    - Low levels of lipid oxidation products
  - Those qualities are retained throughout the thermal process

How about the eating quality?

FPM+Rice	Thermal twin extruder	Dual drying	SME= 16kwh/t
DRM+RICE	Thermal twin extruder	Dual drying	SME= 44kwh/t

Physical structures

FPM+rice

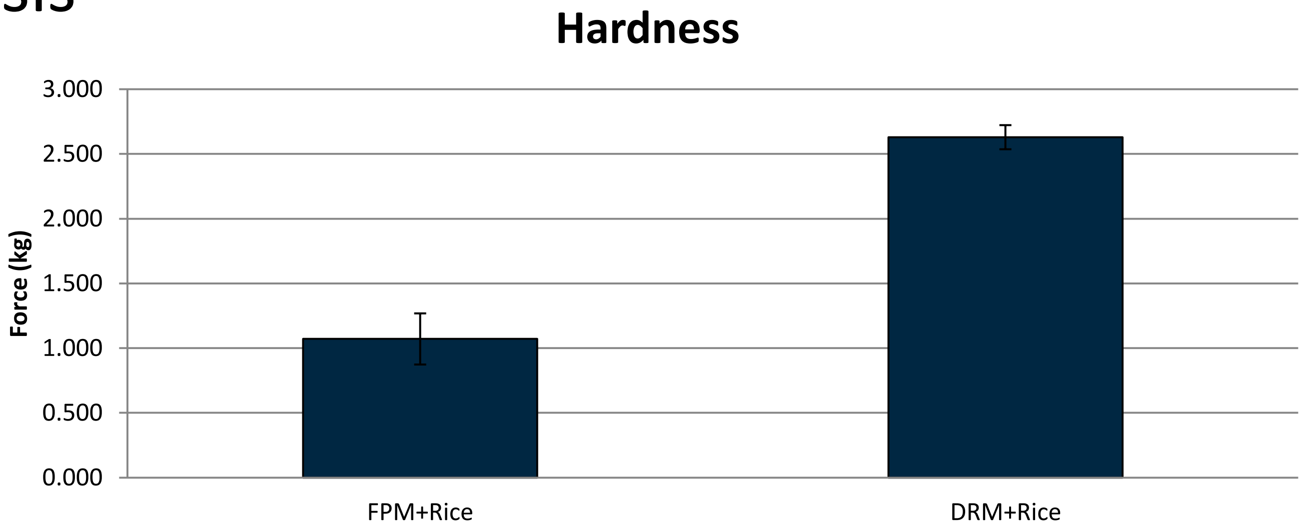
DRM+rice

Physical structures

– Texture analysis

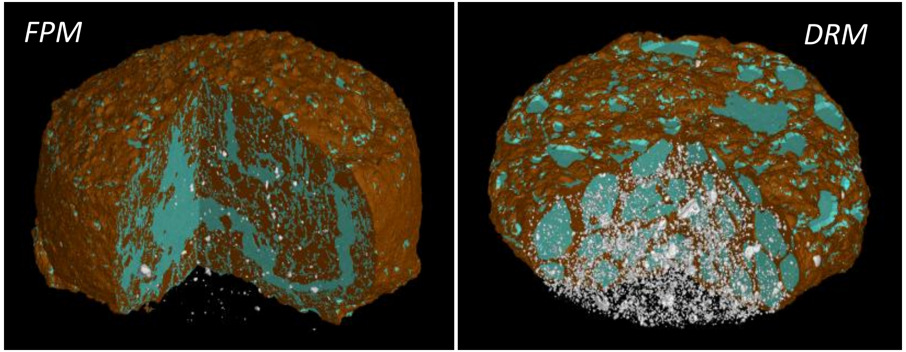
Moisture of tested kibbles 6-7%

Test speed: 1mm/s

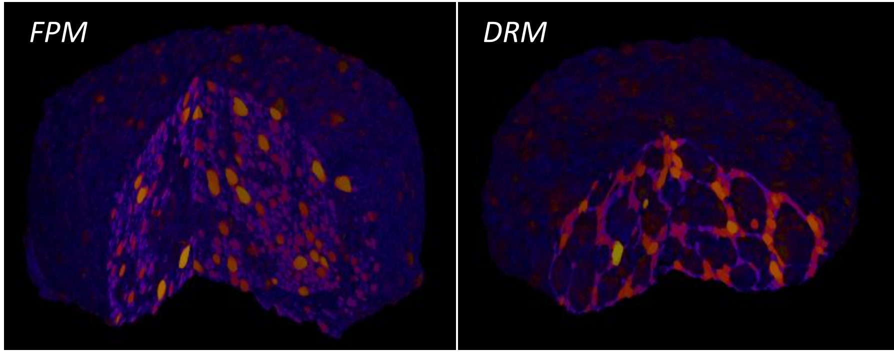


Physical internal structures

- Internal structures
  - X-ray Micro CT scans



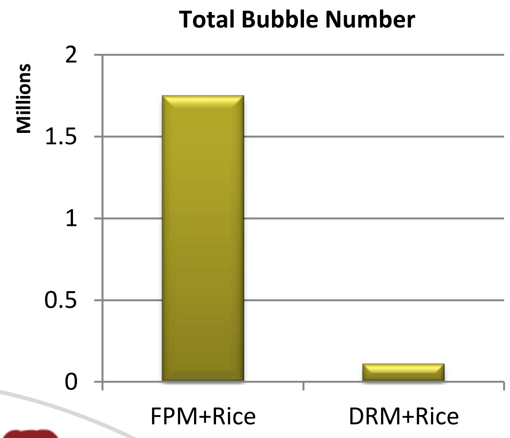
Bubble structure

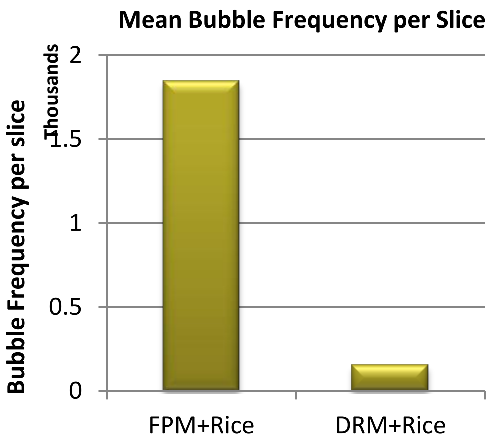


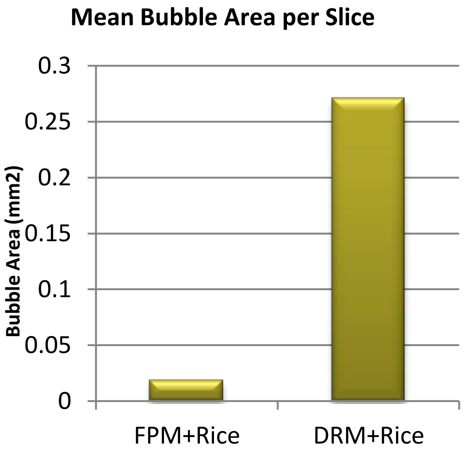
Density map

Physical structures

- Internal structures
  - X-ray Micro CT scans

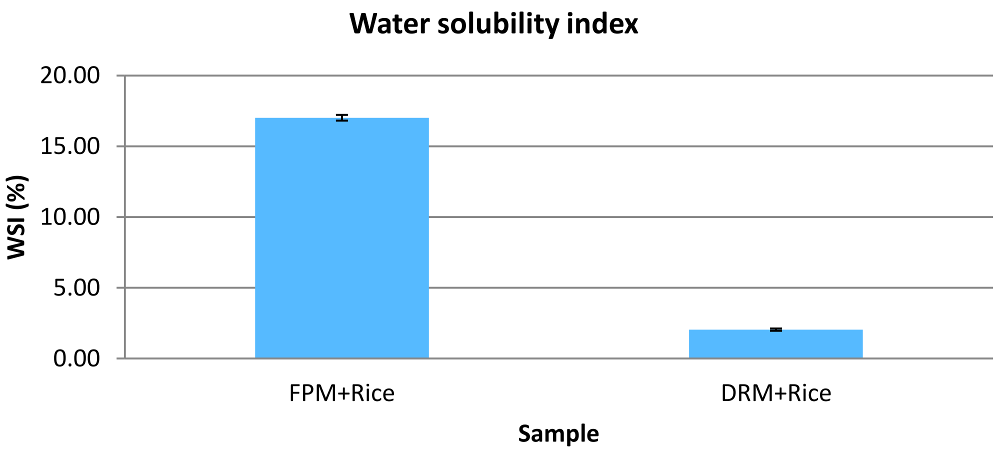






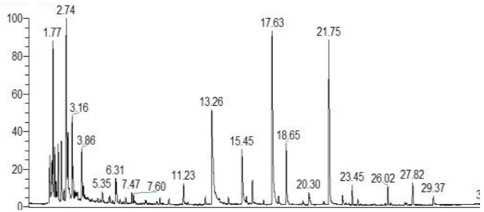
Water Solubility Index

WSI is related to the amount of soluble solids, and often used as an indication of degradation of starch

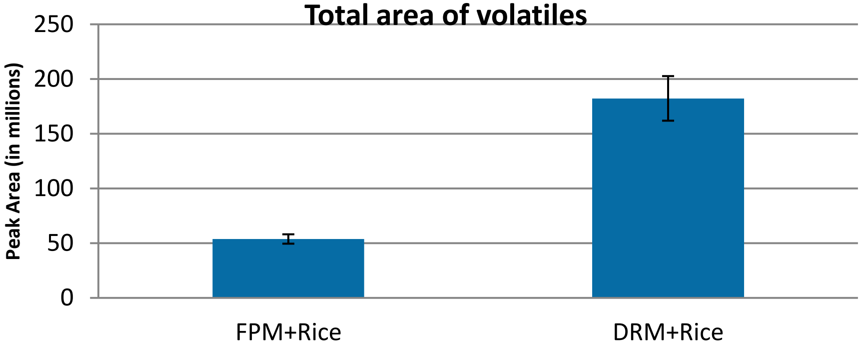


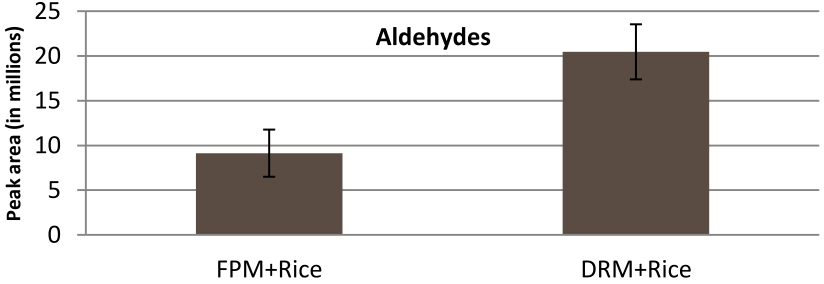
Aroma

– Volatile profiling using GC-MS



GC-MS spectra





Palatability-Dog feeding trial

Panel Composition				
Breed Size	Normal RDA	Number in Panel	% of Panel	% UK Population
Small and Toy	150 - 300g	5	27.80%	26.50%
Medium	300 - 450g	5	27.80%	26.0%
Large	400 - 600g	8	44.4%	43.80%
Giant	600g	0	0.00%	3.80%

Summary Results

Product code	**Percentage eaten of amount fed**		
	Day 1	**Day 2**	**Mean**
FPM+Rice	**61%**	**65%**	**63%**
DRM+Rice	**37%**	**38%**	**38%**


FPM+Rice


DRM+Rice

99.9% probability that the diets are different

Summary and Conclusions

– Commercial scale systems exist that allow dry extruded pet foods to be manufactured using freshly prepared meat

– Certain quality indicators such as protein digestibility, available lysine are benefits found in FPM that are carried through into dry extruded products

Summary and Conclusions

– Market trends and proven benefits associated with FPM makes the use of FPM in dry petfood an area worthwhile investigating further

– Further work to associate the major differences in texture and volatile with food preferences for pets.

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Article

Low temperature processed meat in extruded dry petfood: 2-Effect of commercial scale processing on nutritional and eating qualities.

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Simple Summary:
Although using low temperature processed meats should be advantageous as part of an animal feed, the commercial use of this material is difficult due to its high water content. It has been shown that a dried dog diet can be manufactured by adapting the pre-processing stages and using a specialised thermal twin extruder and drying stages. Products produced from the fresh meat and rice were compared with a meat meal control sample. The new products had different textural properties and outperformed the control sample in terms of in vitro protein digestibility and in dog feeding trials based on preference.

Abstract: Although significant evidence of highly nutritious kibbles has been demonstrated when a low temperature processed meat is used in extruded petfoods, the high inherent moisture content limits the use of such material in commercial production. Using novel thermal processes that utilized adaptation of the pre-processing steps of blending/particle size reduction, pasteurization, separation and concentration and the introduction of a specialized thermal twin extruder, a recipe containing freshly prepared meat (at 60% moisture) and rice was extruded and compared to a dry rendered meat (DRM) based recipe. The nutritional quality of the kibbles, produced from three separate trial runs for each sample type, was evaluated using protein digestibility and available lysine *in vitro* assays, as well as lipid oxidation indicators. The *in vitro* protein digestibility measured was significantly higher in the fresh meat extrudate compared with the DRM kibbles (91% vs 68% ($p<0.05$)). Available lysine was also higher in the fresh meat based kibbles (58% vs 43%). Peroxide value (PV), free fatty acids (FFA) and anisidine value (AnV) were used as indicators of lipid quality. The difference in AnV and PV measured was not significant, however the DRM extrudate had a significantly higher FFA content (9.2% vs 4.1 %; $P<0.05$). The eating qualities of the extrudates were also assessed. The kibbles' structural characteristics and aroma profiles were different between the two samples. X ray micro CT images showed a significant change in the internal structure compared to the conventional meat meal recipe. Although the rendered meat kibbles were significantly harder, the fresh meat products were much denser (0.6 vs 0.4g/cm^3). The x-ray analyses also revealed the presence of a higher number of bubbles formed in the fresh meat kibble (1842 vs 150 bubbles/2D slice), but were of significantly smaller size (0.016 vs 0.27mm^2). These structural and aroma differences would be expected to have a significant impact in the eating quality. A dog feeding trial was completed to measure the acceptance of the products. There was a significant preference for the fresh meat product, however, that preference could not be attributed to a single characteristic of the kibble. The simple recipe used in this study indicates that there are significant positive changes in the eating and nutritional qualities when freshly prepared meat is

used as the only animal protein source in an extruded kibble and this kibble can be made on a commercial scale.

Keywords: Freshly prepared meat; commercial process; nutrition; palatability

1. Introduction

Pet owners are becoming more demanding for quality foods of known provenance. In response to these rising expectations, petfood manufacturers have been looking at the diversity of ingredients available and gaining an understanding of the necessary processing conditions required for the utilization of new ingredients. Much of the animal protein used in petfoods comes from sources that require rendering and drying before use. Although the quality of the protein could be superior before the extremes of pH and drying required in the creation of meat meals, the high moisture contents of the recovered meat reduces the ease of its utilization in commercial manufacturing without additional processing. Thus to use these mechanically separated animal proteins, without the deleterious effects of extreme processing, several critical steps would seem to be necessary: supply lines need to be secure, the protein concentrations need to be increased without loss of quality, adaptation of thermo-mechanical extruders is required to handle higher moisture contents, care is needed in the final handling and then drying of the samples.

Previous work [1], showed that blended, pasteurised, separated and evaporated material (known as freshly prepared meat (FPM)) could be freeze dried and used as an ingredient to make thermally extruded kibbles in a pilot scale thermo-mechanical extruder. This type of extruder could not create kibbles if the moisture content exceeded 30% and thus the FPM required drying. The protein digestibility of the extruded kibbles made from the freeze dried FPM was substantially greater than equivalent products made from meat meal. This demonstrated that the superior quality of the FPM could be retained through the subsequent process. However, to make an economically viable product, use of high moisture ingredients that then negates the need for additional drying steps for the FPM is necessary. The development of a thermal twin extruder, drying and handling technologies which can handle moisture contents of up to 50% and allows fragile materials to be dried, have made the vision of utilizing low temperature concentrated meat materials (FPM) directly into an extrusion process for the production of petfood kibbles possible.

The main aim of the work described in this paper was to establish if a non-complete dog food diet could be created using FPM (moisture content 60%) using a thermal twin extruder at a commercial scale. The diets used a concentrated poultry meat slurry (achieved in the same way as described in the previous paper [1]) without any additional initial drying as the protein source, along with rice, to create non-complete dog diet. The resultant samples were compared with those created using a poultry meat meal of typical moisture content (<10%) using the same extruder. The kibble samples manufactured from the meat meal and the freshly prepared meat were compared in terms of their nutritional qualities, physical structuring properties, aroma profiles and preference feeding.

2. Experimental Section

1. Kibble manufacture

The objective was to create two products that would represent the basis of dog diet using the same extruder (Thermal Twin Extruder TT 3630 (Wenger, Sabetha, Kansas USA)). The carbohydrate source (Table 1) used was white rice (supplied by GA Petfood Partners (Leyland, Lancashire, UK). The major protein sources were both based on poultry, one being supplied as dry rendered meat meal (DRM) and the other as a frozen meat slurry. The proximate analyses of these materials is given in Table 1. The meat slurry was processed at GA Petfood Partners by blending/particle size reduction, pasteurization, separation and concentration by low temperature vacuum evaporation. The resultant material is named freshly prepared meat (FPM) and its composition is shown in Table 1.

Table 1: Proximate composition of raw materials

	Moisture	Oil	Protein	Ash
Poultry dry rendered meat (DRM) ‡	5.0	12.0	61.0	16.0
Frozen meat slurry †	67.6	19.3	12.8	<0.1
Freshly Prepared Meat †	60.0	10.0	25.3	1.7
(FPM) ‡	5.0	24.0	60.0	4.0
White rice †	11.0	1.5	7.5	1.0
Poultry Fat †	0.5	99.0	-	0.5

† wet weight basis

‡ at 5% wet weight

As indicated in Table 1 the lipid content on an equivalent moisture basis was higher for the FPM compared to the rendered meat meal. To balance the oil content in the final kibble additional poultry fat was used in the diets for the DRM product. The diet formations are given in Table 2.

Table 2: Inclusion levels of ingredients used to create the non-complete dog diets and proximate analyses of the extrudates produced.

	FPM+Rice	DRM+Rice
	% Inclusion Dry basis	
Poultry meat meal	-	28.5
Fresh meat	30	-
White rice	70	70
Poultry fat	-	1.5

Proximate composition (measured using AACC approved methods). As a percentage of the total wet weight

	Moisture*	Oil*	Protein*
FPM+Rice Extrudate	$6.3^{a}\pm0.7$	$7.3^{a}\pm4.5$	$23.3^{a}\pm1.0$
DRM+Rice Extrudate	$8.0^{b}\pm0.8$	$5.6^{a}\pm1.8$	$23.2^{a}\pm2.1$

n=3; Different letters denote statistical differences at the 0.05 level
FPM: Freshly Prepared Meat
DRM: Dry Rendered Meat

To create the DRM + rice extrudates, the rice, DRM and additional oil were blended in a pre-conditioner and 12-14% water was added. The extruded kibbles were dried to a target moisture of 8%. Three replicate extrusions were performed and the specific mechanical energy calculated as between 40-45 kWhr/ton for the three trials.

For the FPM + rice extrudates, the FPM was kept hot (~70°C) after preparation and pumped directly into the pre-conditioner. The same temperature profile was used as for the DRM samples. On exiting the extruder the kibbles were sent to a pre-drier before the main dryer to achieve moisture contents of <10%. Three replicate extrusions were carried out and the SME calculated were between 12 and 20 kWhr/ton.

3. Nutritional indicators

3.1. In vitro protein digestibility and available lysine

Protein digestibility was assessed using an *in vitro* method. The *in vitro* protein digestibility assay used in this study was a two stage method developed and based on the method reviewed by Boisen and Eggum [2]. In the first stage of digestion, 1.6% (v/v) pepsin (≥ 250 units/mg solid), (Sigma Aldrich, Dorset, UK), (20% w/v in 0.1M HCl), was added to ground test sample (8% w/v in citrate buffer 30mM, pH 2.5, 0.1% (w/v) sodium azide) and incubated for 6 hours at 40°C. After the first incubation stage, the pH of the digestion system was increased to 6.8 by the addition of 2 M sodium hydroxide. Pancreatin (1.6% v/v) (Sigma Aldrich), (20% in 50mM sodium phosphate) was then added to the system and incubated at 40°C for 14 hours. At the end of the digestion, the sample was centrifuged and an aliquot clarified through a syringe filter with a 0.45 µm pore size cellulose membrane. The latter filtrate was diluted 1/500 to measure amino acid content using ninhydrin reagent. The amount of amino acid in the filtrate was measured using a calibration standard of amino acid mixture for protein hydrolysis (A9781) (Sigma Aldrich). Available lysine was also measured as an indicator of protein quality. This was assessed by a contract laboratory using an FDNB method.

3.2. Lipid quality

The quality of the lipid in the extruded kibbles was also assessed. Free fatty acid content, peroxides and anisidine values were measured as indicators of lipid quality using a FoodLab Fat Analyzer (CDR, Florence, Italy).

4. Physical Characteristics and eating quality

4.1. Water Absorption Index (WAI) and Water Solubility Index (WSI)

Physico-chemical properties and functionality of extruded starchy/proteinaceous food products are highly influenced by processing variables and this will essentially impact water absorption and solubility properties [3, 4]. Raw material properties can also have an effect on water solubility and absorption behaviors. WSI and WAI of the extruded FPM and DRM products were measured based on the method described by Anderson [5]. A 2.5g sample of ground product was suspended in 8mL distilled water at 30°C and left to hydrate for 30 minutes in a 50mL tarred centrifuge tube with intermittent mixing. The tube was subsequently centrifuged at 3000xg for 10 minutes. The WAI was expressed as the weight of the gel obtained after separation of the supernatant per gram of dry sample. The supernatant was dried and the amount of dried solids recovered to determine the WSI as a percentage of initial dry sample.

4.2. Texture analysis

The hardness of the all six kibbles (expressed as the amount of force required to break the kibble under the test conditions) was measured using a texture analyzer TA XT plus (Stable Micro Systems, Godalming, UK). Twelve kibbles from each product were evaluated by penetration using a probe P/2 with the following settings: Pre-test speed: 1.00 mm/sec, Test-speed: 1.00 mm/sec, Post-test speed: 10.00 mm/sec, Target Mode: Distance - 2.00 mm and Trigger type: auto (Force).

4.3. Internal structures

X-ray Micro Computed Tomography was also used to evaluate the structural properties of the kibbles. X ray microCT was performed in the Hounsfield Facility (University of Nottingham) using a Phoenix Nanotom (GE Measurement and Control, Boston, USA) with an X-ray source of 70 kV, 180 mA and a 7.5um spatial resolution. Detailed image visualization as well as quantitative information such as number of air bubbles, bubble size and distribution were calculated.

4.4. Aroma profiling using Gas Chromatography

The aroma profiles of the extruded kibbles were determined using gas chromatography to identify and quantify the aroma compounds in the samples. The gas chromatography was coupled to a mass spectrometer as the detection technique. The instrument used was an ISQ-SPME Headspace (Thermo Fisher, Loughborough, UK).

Two grams of powdered sample were poured into an auto sample vial (3 replicates of each sample were analyzed). Volatiles were extracted by solid phase micro-extraction (extraction time: 10 min, agitator temperature: 60°C, desorption time: 5min).

4.5. Palatability Trials

A dog palatability feeding trial has been contracted in order to assess acceptability of the extruded FPM and DRM products using dog as the model animal. The trial was carried out on 18 dogs of various breeds and sizes from small through to giant, the mix being representative of the UK dog population. Two introductory days of feeding was performed where the dogs' usual food was mixed in with the trial foods. This was then followed by the two trial days. The two foods are presented simultaneously to the breed with appropriate weighed amounts. Both bowls are removed the moment one food was completely consumed, or in situations where neither bowl has been completely consumed at the end of 5 minutes the trial was concluded. The trial was repeated the next day with the food that was on the right being presented on the left to remove left or right hand bias.

5. Results and Discussion

5.1. Comparison of nutritional indicators

A major driver for these studies was to establish if the use of freshly prepared meat could have nutritional benefits and whether the necessary changes in the processes may induce any negative quality indicators. The diets were formulated to have the same proximate compositions. Table 2 shows that there is no major difference in the proximate composition of the kibbles formed. The total protein levels in the kibbles formulated from the FPM and DRM are the same. However, the digestibility of these proteins as assessed by an *in vitro* test were very different (Table 3). Protein digestibility (90.8% vs 68.1%) and available lysine (58.3 vs 42.7 g/kg protein) were both significantly higher in the fresh meat sample compared to the meat meal extrudate ($p<0.01$). Similarly high protein quality was recorded when the low temperature processed meat was freeze-dried and extruded with rice in the pilot study [Diallo et al. Submitted]. This shows that, when used in a wet form, those attributes of the meat remain when extruded on a commercial system.

Table 3: Physical properties, nutritional and quality attributes of extruded kibbles

	FPM+Rice extrudate	DRM + Rice extrudate
PD[1]	90.9[a]±6.0	68.1[b]±5.8
AvL[2]	58.3[a]±4.8	42.7[b]±3.3
PV[3]	8.7[a]±4.2	7.2[a]±1.5
FFA[4]	4.1[a]±0.9	9.2[b]±2.8
AnV[5]	1.8[a]±0.4	2.8[a]±1.5
	3.4±0.0	3.9±0.3
	2.8±0.2	4.0±0.2
WAI[6]	3.7±0.0	3.8±0.1
	3.3±0.4	3.9±0.1
	13.7±0.2	2.1±0.3
	17.0±0.1	7.7±0.1
WSI[7]	5.1±1.2	5.4±0.1
	12.0[a]±6.2	5.1[a]±2.8
Hardness[8]	10.5 [a] ±6.7	25.8 [b] ±13.0
Bulk Density[9]	0.6 [a] ±0.0	0.4[b]±0.0
Total Volatiles[10]	$5.4*10^7$ [a] $\pm 3.2*10^6$	$1.8*10^8$ [b] $\pm 7.4*10^6$
Aldehydes[10]	$9.1*10^6$ [a] $\pm 1.2*10^6$	$2.0*10^7$ [b] $\pm 4.6*10^6$
Fatty Acids[10]	$2.1*10^7$ [a] $\pm 1.8*10^6$	$1.3*10^8$ [b] $\pm 4.2*10^6$
Mean bubble area per slice[11]	0.016[a]±0.03	0.27[b]±0.15
Mean bubble frequency per slice[12]	1842.8[a]±858.9	149.6[b]±52.0

[1] Protein Digestibility (g/100g protein as amino acid equivalent); [2] Available Lysine (g/kg protein); [3] Peroxide Value (meq O_2/kg oil); [4] Free Fatty Acids (%); [5] Anisidine Value; [6] Water Absorption Index; [7] Water Solubility Index (n=12); [8]Hardness (as force required to break kibble (n=12)) (*N*); [9]Bulk density (g/cm^3); [10]Total volatile, Aldehydes and Fatty acids (peak area-arbitrary unit); [11]Mean bubble area (mm^2); [12]Bubble Frequency (bubble per 2D slice)

-Different letters denote statistical differences at the 0.05 level between FPM and DRM products

The level of lipid oxidation (PV, FFA, AnV) was also assayed and shown in Table 3. Due to the high lipid levels and the multiple processes that some of the fats have undergone the level of degradation of these materials and the potential negative nutritive factors are of concern in human foods [6, 7] and are therefore of relevance to petfoods. Interestingly the peroxide and anisidine values were higher in this study than in the previous pilot study by [Diallo et al. Submitted]. However, in both studies, there were no significant differences between the samples created from the DRM and FPM. In the pilot study the FPM samples had lower free fatty acid values than measured for the meat meal extrudates. This was again true for the kibbles manufactured in this study using the thermal twin extruder (see Table 3).

5.2. *Eating qualities-Physical structures*.

Food texture and appearance has a significant impact on eating quality and preferences for both the pet owner and the pet [8-11]. Texture related attributes are used in food product development to understand eating preferences and measurement of hardness is an effective test that has been used to find eating differences between products.

The kibbles extruded using fresh meat were different in appearance (see Figure 1) to the meat meal based kibbles. The fresh meat extrudate was lighter in colour, probably reflecting the less aggressive thermal treatment of the fresh meat (processing temperatures not higher than 90°C) compared to rendering where processing temperatures can reach up to 150°C [12, 13].

Image 1: Kibbles after processing and drying. a) FPM extrudate - b) DRM extrudate

The amount of carbohydrate inclusion in these extrudates was 70% on a dry weight basis. The degradation of the starch during extrusion is often considered to dominate the structural characteristics of the expanded products. The major differences in the extrusion would be in the moisture content and the concomitant lower SME for the

FPM samples and this could be expected to reduce starch degradation in comparison with the DRM samples. However, both extrudates show low water absorption and the FPM, even with the low SME, shows a tendency for greater water solubility index (See Table 3). This increase in solubility may indicate that it is the protein that is soluble rather than the starch and this solubility would relate to the high digestibility observed.

Hardness tests were performed on both sets of kibbles using the method described in section 4.2. The results showed that the rendered meat meal based product was significantly harder than the fresh meat extrudate (Table 3). Bulk density measurements also showed that the FPM sample was denser than the DRM product (0.6 vs 0.4 g/cm^3, respectively) despite showing a less hard profile. This bulk density parameter is often used as a key indicator during process and this difference in density can reflect the nature of the proteins in FPM and DRM as well as the level of starch conversion. However, in the current study, the additional drying required for the FPM extrudates may well have caused collapse and densification of the surface of the kibble.

To establish why the denser FPM product also had a lower break strength than the DRM, the samples were analyzed using X-ray micro CT techniques to reveal the internal structures of the kibbles. Visualization of x-ray images provided evidence of significant structural differences between the two extruded products (Image 2). Large air voids or bubbles were present in the meat meal product, which could be resulting from the product expansion at the die. In contrast, very large voids could be seen within the FPM kibble plus a large number of tiny bubbles. Despite the large voids seen in the FPM samples the small bubbles dominated and gave rise to the average bubble area for the fresh meat product being 0.018 mm^2 compared with 0.27 mm^2 for the meat meal extrudates (see Figure 3). The number of bubbles was evaluated and the results showed mean bubble frequency 10 times higher for the fresh meat sample compared to the meat meal extrudate (1842 bubbles per slice vs 149). The fresh meat product also appeared denser (Image 2), probably indicating the quality of the bubble walls between the tiny bubbles. This may indicate an inability to expand at the extruder die. This could be due to the difference in the nature of the proteins found in fresh meat with high binding properties, as was speculated by Kadri et al [14] but also the levels of starch conversion and high moisture levels would have major impacts on water vapor flash off and expansion of the matrix [15, 16]. The hardness values for the FPM kibble denoted a more easily fracturing material. The texture measures look at large deformation failure and it is possible that this behavior was dominated by the very large voids observed in the samples created from the FPM +Rice. The materials connecting these voids would seem to have dense wall structure with intact bubbles within the walls. For the DRM extrudates the bubble pattern shows thin walls, with few air inclusions. There are clear cracks across many of the air cell walls and these may also reduce the perceived hardness of the kibble. The internal structure of the kibbles is very different for the dry material and also could be expected to be different when hydrated ether during feeding or during digestion.

Image 2: Comparison of internal structures. X-ray micro CT of products FPM (a) and DRM (b) and quantitative analysis of internal structure showing average bubble sizes (c) and bubble frequencies per 2D slice (d)

5.3. Eating quality- Aroma profile

Composition of food, as well as the manufacturing parameters, greatly affects the texture but also the flavour developments [14, 17, 18]. For an aroma comparison, the volatile profiles of the respective extruded kibbles were determined using gas chromatography and a quantitative measure of the amount of each component was provided. Significant differences were noticed in the total amount of volatile detected (see Figure 3a) but also in specific groups of volatiles such as aldehydes and fatty acids (see Figure 3b and 3c, respectively). Higher levels of aldehydes and fatty acids were detected in the meat meal product. These compounds are indicators of lipid quality with aldehydes being secondary oxidation products which might result from the oxidation of fatty acids. The high presence of these volatiles in the meat meal extrudate might be originating from the severe thermal application in the manufacture of dry rendered meat.

5.4. Feeding trial

Flavour description of volatiles is common practice in human food research [19, 20] and has been used in new product development to understand attributes of food that drive acceptance and palatability, and now this application is finding its way into research in petfoods [21, 22]. Although individual volatile compounds have been determined using gas chromatography, this paper did not attempt to give a flavour description of the extruded kibbles. Instead, the two products have been used in a preference dog feeding trial. As well as aroma, other factors such as textural properties will also have an influence on the preference trial. Following the method described in section 4.5, the feeding trial showed that there was a significant preference for the FPM product on both trial days. Although we have seen some significant structural and aroma differences between the two products, this level of acceptance may not be attributed to a single factor. However, it is very likely that the difference came from the different processing regimes of the animal protein source used in the kibbles.

4. Conclusions

It has been previously demonstrated that using a low temperature processed meat as an animal protein source in extruded petfood, could provide high nutritional benefits. In a previous pilot study, mechanically separated meat has been pasteurized, separated and evaporated to subsequently create a dry product using low temperature drying techniques. Utilizing this material in extruded petfood has shown that high protein digestibility and lysine availability could be achieved. The level of lipid deterioration was reduced when compared to an equivalent product made with rendered meat. The freeze drying technique used to achieve this low temperature processed meat would not be economically viable. In the current study, high levels of the wet meat slurry produced have been incorporated, without any additional drying, to make an extruded dry petfood by using a high intensity preconditioner, a thermal twin extruder and specialist drying techniques. Higher nutritional attributes have been recorded for the kibbles, formed using this process, when compared to an equivalent rendered meat. The protein quality was significantly higher in the freshly prepared meat product and further deterioration of lipids was not noticed. Furthermore, a preference feeding trial has shown that there was a significant preference for the FPM product when fed to dogs against the DRM kibbles. This may be partly explained by the significant differences found in the texture of the two products as well as the difference in their volatile profiles. At this point it is not clear what attributes will drive palatability and whether dogs will have a preference for relatively softer kibbles or if there is a combination of specific texture and aroma and flavour release. The main challenge of the work carried out and reported in this paper was to verify whether the process put in place to incorporate a wet meat ingredient could produce a petfood product that would have beneficial key quality and nutritional indicators. It has been shown that a product can be produced, that is preferred by dogs and retains the quality of the starting meat based materials.

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Author Contributions

Aboubakry Diallo, Sandra Hill, James Lawson and Angelina Swali planned the study and formulated the recipes and prepared the manuscript. Aboubakry Diallo, Brian Streit and James Lawson manufactured the samples. James Lawson and Brian Streit reviewed and provided guidance for preparation of the paper.

Conflicts of Interest

"The authors declare no conflict of interest".

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