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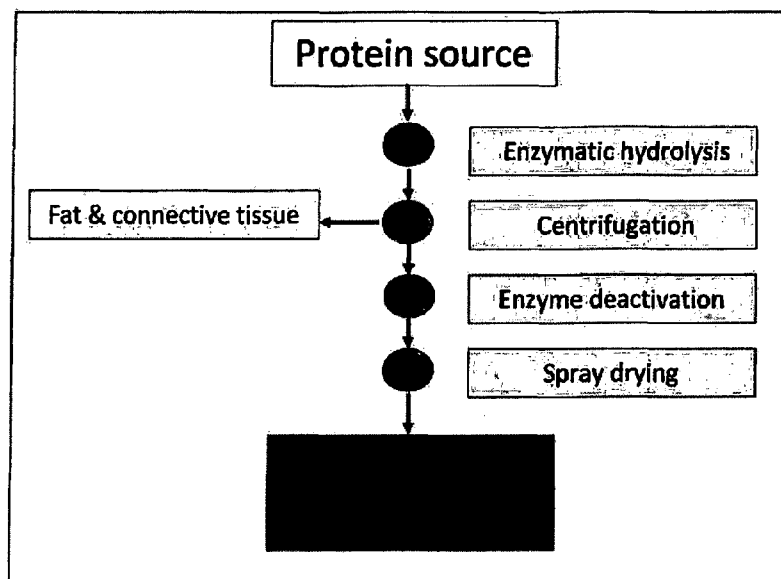
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- (54) Title: EDIBLE COMPOSITION AND METHOD OF MANUFACTURE

FIGURE 1



- (57) Abstract: This invention relates to a hydrolysate composition isolated from a meat source characterised in that, the hydrolysate contains 60 to 90% w/w protein which has a moisture level of below 20%.



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EDIBLE COMPOSITION AND METHOD OF MANUFACTURE

TECHNICAL FIELD

This invention relates to an edible composition and its method of manufacture.

BACKGROUND ART

- 5 The present invention relates particularly to hydrolysates which are hydrolysed bio-matter typically prepared into compositions for human consumption.

Hydrolysates can be used for nutrient supplements for bodybuilders and endurance athletes, nutritional support of the malnourished including cancer patients, elderly and post-operative patients, pregnancy nutritional support, or treating malnutrition
10 in Aids patients, and so forth. The usefulness of hydrolysates stems from the compositions containing nutrients that can aid the recovery, maintenance and improvement of human health.

In the past, hydrolysates have been prepared from whey, milk or soy; partially due to their characteristic cost-effectiveness and availability. However, increasing
15 demands in these industries have resulted in an increased price of these commodities. This leads to an increase in the cost of the product for the end-consumer.

Furthermore, the nutrients available from these sources often do not effectively match the nutrient requirements of the human body. This is particularly so in the
20 case of elderly people who have high requirements for amino acids. Often, in order to provide suitable nutrient levels through currently available hydrolysates, a large volume of hydrolysate must be consumed This is often undesirable, as many elderly people (for example) may not be able to consume large amounts of the

composition due to reduced diet intake. Similarly, if large doses are required to be taken in order to get the required nutrients, other desirable food components such as fat, salt, sugars, flavourings or preservatives can also be consumed, potentially lead to their side effects.

- 5 Some nutrients (e.g. essential amino acids) that are particularly advantageous to humans are not well provided by currently available hydrolysates (e.g. those from vegetable sources). Amino acids play an important role in human body by acting as building blocks for different tissues.

10 In addition, the techniques used to prepare the hydrolysate can be detrimental to the resulting nutrient levels in the composition, where the nutrients can be lost during the processing steps, leading to loss of their effectiveness. Plus, many hydrolysates can have a short shelf life, and many of the nutrients can be lost during storage. This can lead to a reduction in their effectiveness when consumed by the user.

- 15 Furthermore many hydrolysates can have undesirable flavour profiles. Often the flavour level can be overbearingly salty or bitter. In order to overcome unpalatable flavours of the hydrolysates, synthetic flavoring has often been used, however with limited success. This can be a further disadvantage as people prefer natural products lacking synthetic additives.

- 20 Some materials sourced for preparing hydrolysates can also represent a hazard for disease transmission. In many countries, the regulations can be ineffective to prevent disease transmission through human consumption of animal products. Diseases that are transmitted through an animal population (e.g. Bovine Spongiform Encephalopathy, or "Mad Cow Disease" in cattle) can significantly
25 hinder use of the animal products. Radical treatment means can often be required

to ensure the disease is not transmitted to humans. Regardless, the consumer's trust in the meat quality can make commercialization non-viable. Therefore, it is critical to identify a source, be it animal or non-animal, that not only has stringent regulatory approval, but also has consumer confidence.

- 5 Often, nutrients within the hydrolysates can be poorly absorbed by the body, or are absorbed at the inappropriate positions within the digestive tract. This is often a result of poor pharmacokinetic properties of the composition (e.g. solubility levels, pH suitability, and absorption across the intestinal tract). Absorption is also highly dependent on the source and method of preparation. This can be a major concern
- 10 as the consumer may falsely believe they are receiving adequate levels of the nutrient (as labeled on the product) when in fact they are not.

There can also be many associated problems that arise during the preparation of hydrolysates.

- For example, it can be difficult to identify an appropriate enzyme mixture if the hydrolysis is prepared enzymatically. As different enzymes will cleave proteins at
- 15 different amino acid positions, the choice of enzymes utilised depends on the level of hydrolysis and amino acid profile required.

- Also, the timeframe of the hydrolysis must be closely monitored. Alterations in the source and type of meat source can dramatically affect the timeframe required for
- 20 adequate hydrolysis. Furthermore, the purpose of the product must be considered when assessing the timeframe of the hydrolysis. The level of hydrolysis can also affect flavour e.g. can lead to generation of bitter peptides. As such many variables must be considered when determining the appropriate length of hydrolysis.

Also an additional hydrolysis step may be required to remove un-wanted components. This can take up valuable time, equipment and/or can lead to a reduction of nutrients lost during the additional hydrolysis step.

It is therefore important to improve the efficiency of preparing the hydrolysate,
5 particularly for source(s) which have unwanted components.

A further problem with many hydrolysates is that they are not well adapted for numerous purposes. Often, the hydrolysates can be well suited to one given purpose (due to high levels of a particular nutrient), but can be rather ineffective for other purposes. It has been a goal to develop a well-rounded hydrolysate that may
10 be used effectively either as a supplement or to treat multiple ailments.

It is an object of the present invention to address the foregoing problems or at least to provide the public with a useful choice.

All references, including any patents or patent applications cited in this specification are hereby incorporated by reference. No admission is made that any
15 reference constitutes prior art. The discussion of the references states what their authors assert, and the applicants reserve the right to challenge the accuracy and pertinency of the cited documents. It will be clearly understood that, although a number of prior art publications are referred to herein, this reference does not constitute an admission that any of these documents form part of the common
20 general knowledge in the art, in New Zealand or in any other country.

Throughout this specification, the word "comprise", or variations thereof such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements integers or steps, but not the

exclusion of any other element, integer or step, or group of elements, integers or steps.

Further aspects and advantages of the present invention will become apparent from the ensuing description which is given by way of example only.

5

DISCLOSURE OF THE INVENTION

According to one aspect of the present invention there is provided a hydrolysate composition isolated from a meat source

characterized in that

10 the hydrolysate contains 60-90% w/w protein.

Measures of dietary protein quality are of fundamental importance in nutrition. The nutritional value of dietary proteins depends on the composition of amino acids and their bioavailability for metabolic utilization. The latter is determined by digestion and absorption processes in the small intestine. Lean red meats are
15 acknowledged as being sources of superior quality protein and important micronutrients that are essential for good health throughout life. As we age, the ability to eat and digest proteins such as those contained in red meat is reduced in some people. This is due to many reasons including changes in taste and smell, poor oral health resulting in difficulties with chewing and/or swallowing, decreased
20 appetite and reduced food intake. This reduced food intake is a special problem because protein-energy malnutrition is correlated with a higher rate of mortality and morbidity.

Protein-energy malnutrition accentuates the physiological loss of skeletal muscle

mass that occurs with advancing age, known as sarcopenia, resulting in a higher requirement for superior quality dietary protein. Ingestion of high quality proteins is of particular importance in order to provide sufficient amounts of the essential amino acids for the stimulation of skeletal muscle protein synthesis. Essential amino acids are the amino acids which must be obtained from the diet. If an individual's diet is deficient in one or more of these amino acids, the usefulness of all other amino acids is affected even if they are present in otherwise sufficient quantities. Although targeted amino acid supplementation may indeed be beneficial in cases involving accelerated protein catabolism (e.g. advanced sarcopenia, cachexia and trauma) for the majority of older adults the most practical means of increasing skeletal muscle protein anabolism is to include a serving of protein of high biological value during each meal (Paddon-Jones *et al.* 2008).

Throughout the specification the term hydrolysate should be taken as meaning any biological material that has been hydrolysed or otherwise *broken down, digested or cleaved*, either enzymatically or non-enzymatically, into smaller components than normally present in the un-modified state of the starting biological material.

Preferably the hydrolysate is provided in a dry soup mix or powder. However this should not be considered limiting – the applicant acknowledges that other means of providing the hydrolysate may include a slurry, tablet, injectable liquid or drink, bolus, etc or any other form of food or nutraceutical. Providing the hydrolysate in a dry powder or soup mix may help to increase the shelf life, reduce space requirements during storage, and the need to refrigerate the composition. Indeed, studies performed by the inventors suggested the hydrolysate stored at room temperature was very stable over a temperature range between -20°C and 37°C over a 24 week trial period.

Throughout the specification the term meat source should be taken as meaning any resource that contains proteins.

Preferably the meat source is a red meat. The meat source may include ligament, cartilage, fat, muscle, skin or other tissue.

- 5 Preferably, the meat source is lamb. However, this should not be taken as limiting. Someone skilled in the art would appreciate that other sources of protein, including for example cow, goat, deer, rabbit, etc may be suitable. The applicant notes that there are minor variations in the amino acid profiles or other characteristics (e.g. protein level or fat level) between each meat source and each may have a
- 10 beneficial effect.

The inventors have found that lamb meat may provide particularly advantageous levels of nutrients (e.g. amino acid profile) which closely match what may be required by the human body during growth and development, recovery and/or treatment. In particular, the amino acid profile of hydrolysed lamb meat very

15 closely matches the human muscle amino acid profile. There is convincing support that amino acids play an important role in enhancing human muscle repair and growth.

Other meat sources such as cattle, sheep, poultry and pigs can also be used.

More preferably, the meat source is from New Zealand.

- 20 Sourcing the meat from New Zealand holds numerous advantages. New Zealand represents a country with a clean green image, both locally and overseas. There is considerable trust and reliance on products sourced from New Zealand. As such, hydrolysates prepared using New Zealand meats represents considerable advantages to consumer satisfaction and product image.

Additionally, stringent regulations and safety requirements implemented in New Zealand help to ensure that the meat used for preparing the hydrolysate will be disease free. This may include ensuring the animal meat that is sourced is disease free, and/or avoidance of introducing disease/infection during the processing stage
5 of the hydrolysate.

Secondly, New Zealand is renowned for raising healthy well-nourished animals in the food sector. This may partially be due to the living conditions in which the animals are raised. One outcome of a healthy upbringing may be an improved amino acid profile of the meat, which in turn may benefit the end user of the
10 resulting Hydrolysate prepared from said meat.

Preferably, the meat source is derived from mechanically separated (MS) meat. Preferably, the meat source is meat off cuts or offal. However the source is not restricted to these and can also include any form of meat.

Use of MS meat and/or off cuts avoids the need to use premium quality meat.
15 However, the mechanically separated meat may still has high nutrient levels, but not typically considered appropriate for eating (e.g. due to appearance and texture).

A further advantage of using MS meat or off cuts is that the hydrolysate may utilise by-products that would otherwise be discarded. This may help to reduce costs in
20 sourcing the meat used for the hydrolysate, or potentially even free. Additionally, providing a use for off-cuts may help to boost the profitability of the meat industry in New Zealand and other countries.

The term protein should be taken as meaning any polypeptide or peptide that consists of one or more amino acid, which may be naturally occurring or a

derivative thereof.

The hydrolysate contains between 60-90% w/w protein. Typically, currently available meat hydrolysates include relatively low levels of protein, often in the range of 10-30% w/w.

- 5 Preferably, the hydrolysate contains 83-88% w/w protein. Most preferably, the hydrolysate contains 83.4% protein. This amount may include approximately 1.5% collagen.

Furthermore, the inventors have found that a protein range of 60%-90% w/w is what the preferred source (mechanically separated lamb meat) will most often
10 provide using the preferred method outlined in the Best Modes section of this patent specification. The novel range of protein in the present invention has many advantages over the prior art. For example, a high protein hydrolysate may be particularly beneficial to those who:

- are malnourished and/or have underlying deficiency in protein/amino acid
15 levels (e.g. in third world regions);
- are susceptible to infection (due to reduced immunity);
- have disorders which may otherwise lead to loss of protein/muscle (e.g. muscle wastage);
- do not wish to, or cannot eat large amounts of solid protein yet require a
20 protein boost (e.g. the elderly or those in rest homes, or those recovering or convalescing from illness);
- have a high quality verified source of protein (e.g. body builders or infant formulators);

- seek natural and/or organic supplements;
- want to differentiate the product from competitors (e.g. by providing a “functional soup”).

The inventors have found that the preferred hydrolysates of the present invention
5 include an appropriate balance of amino acids, including the essential amino acids.

The most important amino acids are those that cannot be biosynthesised but have to be obtained from the diet. Nine amino acids are generally regarded as essential for humans: Isoleucine, leucine, lysine, threonine, tryptophan, methionine, histidine, valine and phenylalanine. If any particular essential amino acid is lower than
10 required for protein synthesis, overall protein synthesis can be limited.

Preferably, the protein is rich in sulphur-containing amino acids, namely cysteine and methionine.

These are both important amino acids for the human body's proper functioning. Methionine represents one of the essential amino acids which the body cannot
15 make. Therefore, the body must utilise amino acids present in the diet to account for methionine.

The inventors have found the amino acids in the hydrolysate of the present invention have high ileal absorption demonstrated in rat and pig models.

The inventors have found that a particularly preferred composition of the
20 hydrolysate has the following true ileal amino acid digestibility characteristics. True ileal amino acid digestibility ranged from 87% for glycine to 101% for threonine with complete digestibility (>99%) observed for threonine, serine, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine and arginine. The mean

true digestibility across all amino acids was 98% and the mean digestibility for all amino acids excluding glycine was greater than 99%. The true digestibility values indicate that the hydrolysate was almost completely digested and absorbed from the small intestine.

- 5 The amino acid profile indicates that the hydrolysate is an amino acid source high in valuable lysine. This hydrolysate may have particular application to humans consuming diets high in cereals, for which lysine is often the first limiting amino acid. It may also be important for humans with muscle atrophy, such as the elderly or those undergoing rehabilitation after surgery or for athletes aiming to increase
10 muscle mass.

The absorption of individual amino acids is highly dependent on the protein source. For example, the digestibility of many amino acids in humans differs between soy and milk proteins and between individual milk proteins, for example beta-lactoglobulin and casein. For milk proteins, only 50% of the ingested protein is
15 absorbed between the stomach and the jejunum and 90% is absorbed by the time the food reaches the ileum. Thus it is important to assess the bioavailability of any meat source used in supplements.

The inventors consider it may be particularly advantageous to have highly hydrolysed protein in the hydrolysate in which a large percentage of the peptide
20 links are severed by the hydrolysis to result in a mixture of small peptides or individual amino acids leading to their better absorption.

Most preferably, the level of hydrolysis is approximately 40% or greater (meaning more than 40% of the peptide links in the material being processed are severed).

The hydrolysate may contain a relatively high proportion of di- or tri- peptides or single amino acids.

Most preferably, 70-90% of the peptides in the hydrolysate composition have a molecular weight of less than 1000 Daltons. Typically, amino acids range between
5 75 to 204 Daltons.

Preferably, approximately 78% or more of the peptides in the hydrolysate are less than 10 amino acids in length.

Preferably, the average size of the peptide is two amino acids.

The provision of protein in the form of small peptides as discussed above may
10 enhance both the degree and speed of absorption after consumption.

The hydrolysate composite is as claimed may also include additional beneficial characteristics, as discussed below.

Preferably, the hydrolysate includes between 0.1 to 10% fat. Most preferably, the hydrolysate includes only 0.4% fat (w/w).

15 Preferably the hydrolysate of the present invention has a high ratio of protein to fat.

Many of the currently available hydrolysates, such as that described in US2004/0131750 have relatively high levels of fat in the composition.

Typically, red meat is associated with having not only high levels of protein, but also high levels of fat. Providing a hydrolysate that includes high levels of protein
20 (as in the claimed invention), but inversely low levels of fat may be particularly advantageous especially with consumers that are interested in weight management or improving overall health.

The inventors found that sourcing the protein from meat allowed a hydrolysate not only with very high levels of protein (and an amino acid profile that closely matches human and other animal requirements) but also to be acceptable in taste.

As previously discussed many of the currently available hydrolysates have a
5 limitation of taste, e.g., some protein hydrolysate may impart unacceptable bitterness. Some other types of hydrolysates have good flavour and have been used as flavourings in different products e.g. chicken and meat stalks. However these good tasting hydrolysates have relatively lower protein content.

There is a need for a hydrolysate composition that is rich in protein and is
10 palatable. The current invention is not only rich in protein content having essential amino acids and has high digestibility, but also has an acceptable taste and does not require use of any additives or flavours to mask or alter the flavour profile of the product.

A product currently on the market is Whey Protein hydrolysate 4020. This product
15 is reported as having a protein level of 90% w/w, and a good flavour profile. However the current invention provides an alternative for individuals who are allergic to milk proteins and those who are lactose intolerants.

US20040131750 discloses a pet food prepared from a hydrolysate. Although this is likely to be palatable, it could not be used for the purpose of the claimed
20 hydrolysate. This is because it still has very low levels (i.e. less than 30% w/w) of protein compared to the present invention. There is no discussion in US'750 that a high protein level could be achieved, nor does it state that with a high protein level being achieved flavour is maintained. In fact, it states that additional materials (e.g. fat) can be added to improve the foodstuff. This is contrary to what the

present invention preferably provides, which is high protein with palatable flavouring with no additional components (e.g. fats).

Similarly, a product such as Maggi soup mix has hydrolysates prepared from meat sources (e.g. chicken or beef). However, the protein levels of these hydrolysates are very low (e.g. 4-10% w/w) and are used for imparting flavour to the product. .
5 The current invention is not only rich in protein content having essential amino acids and has high digestibility, but also has an acceptable taste and does not require use of any additives or flavours to mask or alter the flavour profile of the product.

10 Preferably, the hydrolysate has a moisture level below 20%. Most preferably, the moisture level is between 3 to 7%.

Having a low moisture level may allow the composition to be prepared and stored as a powder (a preferred embodiment). It may also help to increase the shelf life of the hydrolysate and avoid the product from breaking down or going rancid.

15 Preferably, the hydrolysate has a pH level between 6-9%. Most preferably, the pH of the hydrolysate is 6.3 (at 1% in water at 23°C). The near neutral pH of the current invention makes it suitable for a range of applications. If the ingredients of the products were acidic, its use would have been restricted to formulations with low pH

20 Preferably, the hydrolysate has a solubility level between 60-100% (at 1% w/v in water). Most preferably, the solubility level is in the order of 87%.

The inventors acknowledge that the hydrolysate when prepared into a composition may include further additives known in the art. These may include preservatives, solubilising agents, gelling agents, flavourings, colouring, etc.

BRIEF DESCRIPTION OF DRAWINGS

Further aspects of the present invention will become apparent from the following description which is given by way of example only and with reference to the accompanying drawings in which:

- 5 Figure 1 Overview of hydrolysate method of preparation;
- Figure 2 Graphic indication of the L*a* b* colour spaces of Meat hydrolysate
 stored at four temperatures for a period of 24 weeks;
- Figure 3 Water solubility of Meat hydrolysate stored under four temperatures
 for a period of 24 weeks;
- 10 Figure 4 Solubility of Meat hydrolysate stored at four different temperatures
 for a period of 24 weeks in different pH aqueous solutions;
- Figure 5 Headspace GC chromatographs of Meat hydrolysate at the initial
 stage of the storage trial;
- Figure 6 Headspace GC chromatographs of Meat Hydrolysate stored at four
15 temperatures for 12 weeks;
- Figure 7 Headspace GC chromatographs of Meat hydrolysate stored at four
 temperatures for 24 weeks;

- Figure 8: (A) Odour intensity of the powders stored at the four temperatures over a period of 24 weeks in the category scale from 1 worst, to 10 neutral (B) Taste intensity of aqueous solutions each containing 2.1% of the powder in the category scale from 1 worst, to 10 neutral. The powder samples were stored at the four temperatures over a period of 24 weeks.
- Figure 9 Plasma glucose concentrations after the ingestion of a mixed meal containing ^{15}N -labelled lamb hydrolysate or casein in older adult humans. Values are means $\pm$ standard errors. Time is given as minutes from ingestion of the test meal;
- Figure 10 Serum insulin concentrations after the ingestion of a mixed meal containing ^{15}N -labelled lamb hydrolysate or casein in older adult humans. Values are means $\pm$ standard errors. Time is given as minutes from ingestion of the test meal;
- Figure 11 Incorporation of dietary nitrogen into serum proteins after ingestion of a mixed meal containing hydrolysed lamb meat or casein in older adult humans. Time is given as hours from ingestion of the test meal. * indicates values that are significantly different from each other at a given time;
- Figure 12 Incorporation of dietary nitrogen into body urea (A), cumulative excretion of urinary urea (B) and cumulative excretion of urinary ammonia (C) after ingestion of a mixed meal containing hydrolysed lamb meat or casein in older adult humans. Values are means $\pm$ standard errors. Time is given as hours from ingestion of the test

meal. * indicates values that are significantly different from each other at a given time.

Figure 13 Production of total (A), dietary-derived (B) and endogenous derived (C) urea after ingestion of a mixed meal containing hydrolysed lamb meat or casein in older adult humans. Values are means $\pm$ standard errors. Time is given as hours from ingestion of the test meal. * indicates values that are significantly different from each other at a given time.

BEST MODES FOR CARRYING OUT THE INVENTION

Preferred embodiments of the present invention are now described with particular reference to the examples provided below.

EXAMPLE 1: A description of the preferred hydrolysate of the present invention.

The hydrolysate composition is isolated from mechanically separated (MS) off-cuts of lamb meat sourced in New Zealand. The hydrolysate has a protein level of 83.4% (w/w), fat level of 0.4%, moisture level of 5.0% and ash level (indicative of mineral content) of 7.3%. The pH of the hydrolysate is 6.3 (at 10% in water at 23°C). The solubility of the hydrolysate is 87% (at 1% w/v in water).

The hydrolysate includes a well balanced amino acid profile (discussed further in the Examples below). The hydrolysate is highly absorbable, where the mean true digestibility for all amino acids is 98%.

78% of the peptides in the hydrolysate have a molecular weight of less than 1000 Daltons. Furthermore, 78% of the peptides in the hydrolysate are less than 10 amino acids in length, and the average size of the peptides is two amino acids.

The hydrolysate is provided in a dry soup mix or powder.

EXAMPLE 2: Method of Preparing the Hydrolysate

Minced MS lamb meat was suspended in deionised water and heated to 45°C. Protamex and Flavourzyme, were simultaneously added to the suspension to allow the hydrolysis at 45°C under stirring for 3.5 hour. At the end of the hydrolysis, the digested meat slurry was centrifuged to remove fat and un-hydrolysed solids (e.g. connective tissue). Following deactivation of enzymes, the hydrolysate liquor was concentrated using a climbing film evaporator and spray dried into a light brown fine powder.

Batch Size

250 kg MS lamb meat is used to generate a 15 kg hydrolysate powder.

Materials

Table 1: List of raw materials used in manufacture of hydrolysate powder from MS lamb meat

Raw Material	Brand	Grade/Specification	Supplier	Quantity
Water	N/A	Preferably deionised to minimise salts	Massey University	200 litres
MS lamb meat	N/A		PPCS Ltd Oringi NZ	200 kg
Flavourzyme	Flavourzyme®	food grade 1000LAPU/g	Nutura NZ Ltd	400 mL
Protamex	Protamex®	food grade 1.5 AU/g	Nutura NZ Ltd	400 g
NaOH	BDH®	AR grade	BDH Lab Supplies, NZ	
Acetic acid	BDH®	AR grate	BDH Lab	

			Supplies, NZ	
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Equipment

Table 2: List of equipments used in manufacture of hydrolysate powder from MS

lamb meat

Equipment	Brand	Model	Specifications/Operating Conditions
Frozen meat block chipper			
Mincer			
Jacked vessel with stirrer*			Automatic temperature controlled
Mono pump			Fitted with variable speed drive
Refrigerated bowl centrifuge	Himac®	R22GII	>12000 ×g
Small disc separator	Westfalia	CTC-03-107	> 12000 ×g
Rising film evaporator	APV		Max temperature 60° concentration to 30-40% solids
Spray drier			Exit temperature 80°C Chamber temperature 180°C
Vacuum packaging machine			Aluminium foil bags

* After the hydrolysis reaction the product was heated to 95°C to deactivate the enzymes.

5

Utilities

Food grade processing area meeting export standards

Heating/cooling for stirred jacketed vessel

Hot water for work area cleaning

Vacuum for rising film evaporator

Compressed air for spray drier

5 **Steam**

De-ionised water

Chilled operating room meeting export standards

Freezer for storing meat in cartons

Processing Technique

10 ***Hydrolysis:***

- The minced MS lamb meat was suspended in an equal volume of deionised water.
 - The temperature of the mixture was raised to $45^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and held at that temperature for the duration of the hydrolysis.
- 15
- Two enzymes were then added simultaneously, i.e., Protamex (0.2% by weight of meat) and Flavourzyme (0.2% by weight by weight of meat).
 - During hydrolysis the pH was constantly controlled to a pH between 6.00 – 6.5 with a 5M molar solution of either NaOH or acetic acid.
 - The mixture was stirred continuously at 12 rpm for the whole 3.5 hours.

Separation of the hydrolysate:

- The hydrolysate was separated using an ultracentrifuge above 12000 ×g for 5 minutes. In an industrial setting the material may need to be centrifuged in a decanter type centrifuge.
- 5 • The hydrolysed meat slurry was heated to 75°C (minimum) for 10 minutes to deactivate the enzymes and to pasteurize the product. This was achieved in the water jacketed vessel. The 3.5 hour time limit for the hydrolysis is critical – as even a delay of fifteen minutes can lead to detectable bitter notes as a consequence of excessive hydrolysis.
- 10 Moreover, the amino acid balance changes reasonably fast with the hydrolysis of the connective tissue.
- A rising film evaporator was used to concentrate the hydrolysate liquor to a solids content of 30-40%. The exit temperature was controlled under 60°C.
- A spray drier was applied to dry the concentrate into the powder. The exit
15 temperature was controlled at 80°C while outlet air temperature was at 180°C.
- The hydrolysate powder was packaged under vacuum and stored at -20°C.

Example documentation and completed runsheets

1. 25 kg minced MS lamb meat was suspended in pre-heated 25 L deionised
20 water in a 70 L jacketed vessel.
2. Raise temperature to 45°C under stirring and adjust pH to 6.0-6.5 with 5M NaOH or 5M acetic acid.

3. Add 50g Protamex and 50mL Flavourzyme into the suspension.
4. Maintain the temperature at 45°C under stirring for 3.5 hour. During the hydrolysis, check and adjust pH within the range of 6.0-6.5.
5. Sieve out solid lumps from the digested meat suspension and separate the hydrolysate liquor using a bowl centrifuge at 12000 ×g.
6. Pass the thin lump-free suspension to a disc centrifuge at 12000 ×g to continuously separate the hydrolysate liquor from fat and un-hydrolysed solids.
7. Combine the centrates (31-34 litres, 8% solids) from two centrifuges and heat the liquor up to 75°C and maintain the temperature for 10 minutes.
8. The hydrolysate liquor was concentrated using a rising film evaporator to a solids content of 30-40% under an exit temperature of 60°C.
9. Spray dry the concentrate into a light brown powder (about 1.7 kg). About 30% of yield was lost in the small scale spray drying chamber.
10. The obtained hydrolysate powder is finally packaged in aluminum foil bags under vacuum and stored at -20°C.

Product Specification

Quantification: Crude protein 83.4% (incl.1.5% collagen)

Fat < 0.4%

Ash 7.3%

		Total lysine / Available lysine:	7.9 / 7.0
		g/100g	
		Moisture	5.1 %
5	Colour:	light brown	
	Odour:	meaty / slightly fishy (pleasant smelling)	
	Palatability	Excellent (not too strong flavor, but not bland)	
	The water solubility:	87.3±0.7 %	at pH 6.8
10		Minimum 82.0%	at pH4
		Maximum 89.8%	at pH11
	Molecular weight profile	78% - MW < 1000 Da	
	(for soluble portion of the powder)	17% - 1000 Da < MW < 10,000 Da	
15		5% - MW > 10,000 Da	
	Microbiological analysis:	SPC (Standard plate count)	400 CFU/g
		Yeast and Moulds	900 CFU/g

Coliforms <3 MPN*/g

Salmonella Negative in 25g sample

* MPN = most probable number

CFU: Colony Forming Unit

Analytical Methods

Protein quantification

- Kjeldahl assay
- Dry weight determination

5 *Fat analysis*

Soxhlet assay (AOAC 991.36)

Total lysine / Available lysine

A procedure described by Moughan and Rutherford (1996)

Water solubility measurement:

- 10 The measurement is based on a modified AOAC (950.81) method. Approximately 1 g (W_s) of MS lamb hydrolysate powder was added to 100 mL (V_s) of water in a 250 mL conical flask and shaken on a 150 rpm rotation shaker for 2 hours. About 30 mL (V_c) of the solution was centrifuged at 15000 rpm for 20 minutes. The supernatant was then dried (W_d) in a 105°C oven overnight. All the data were
- 15 measured in duplicate. WS was calculated based on the following equation.

$$WS (\%) = \frac{W_d \times V_s}{V_c \times W_s} \times 100$$

W_d refers to the dried weight (g) of the supernatant in V_c

W_s refers to the weight (g) of the powder sample

V_s refers to total volume (mL) of the solution

V_c refers to the supernatant volume (mL) from centrifuge

Microbiological test

A conventional aerobic plate count method (Vanderzant and Splittstoesser, 1992)

EXAMPLE 3: Characteristics of the Lamb Hydrolysate

5 Meat hydrolysate specification

This protein hydrolysate is produced by carefully selected enzyme hydrolysis technology, under food grade conditions to accredited standards. The final product is sampled and tested for chemical, sensory and microbial parameters using internationally recognised procedures. Typical values and results are as listed in

10 Tables 3 to 8

Table 3: Amino acid profile per 100g meat protein hydrolysate

Branched chain amino acids		Other amino acids	
Isoleucine	3.6	Alanine	5.1
Leucine	6.9	Arginine	5.5
Valine	4.7	Aspartic acid	8.1
Other essential amino acids		Cysteine	0.9
Lysine	7.9	Glutamic acid	13.9
Methionine	1.7	Glycine	3.8
Phenylalanine	3.5	Histidine	3.0
Threonine	3.4	Proline	3.4
Tryptophan	1.0	Serine	2.6

Table 4: Molecular weight profiles of peptides in meat protein hydrolysate

Molecular weight (Daltons)	Lamb hydrolysate
> 20,000	2 %
20,000 – 10,000	3 %
10,000 – 5,000	1 %
5,000 – 1,000	16 %
1,000 – 800	6 %
800 – 100	66 %
< 100	6 %

Degree of hydrolysis

Meat hydrolysate is highly hydrolysed with a degree of hydrolysis of around 40%.

- 5 This means that more than 40% of the peptide links are severed by the hydrolysis and results in a mixture of small peptides or individual amino acids. This aids absorption of the protein and also contributes to the pleasant beefy flavour of Meat hydrolysate.

Analysis

10 **Table 5:** Typical composition of meat protein hydrolysate (%w/w)

Protein	(As is) 83.4% (Dry basis) 87.8%
Moisture	5.0%
Fat	0.4%
Ash	7.3%

Table 6 : Typical mineral composition of meat protein hydrolysate

Calcium	130 mg/100g
Sodium	1200 mg/100g
Potassium	1800 mg/100g
Phosphorus	810 mg/100g
Iodine	0.11 mg/kg
Iron	13 mg/100g
Magnesium	111 mg/100g
Zinc	59 mg/kg

Table 7: Typical physical properties of meat protein hydrolysate

Colour	Light brown
Flavour	Slightly brothy/meaty
Solubility (1% w/v in water)	87%
pH(1% at 20 degrees C)	6.3

Typical microbiological estimates5 **Table 8:** Microbial profile of the meat protein hydrolysate

Standard plate counts	400 CFU/g
Yeasts & moulds	900 CFU/g
Coliforms	<3 MPN/g
Salmonella	Negative in 25g sample

CFU – colony forming units MPN – most probable number

Bibliography:

Moughan PJ, Rutherford SM. (1996). A new method for determining digestible reactive lysine in foods. J Agric Food Chem. 44:2202–9.

Vanderzant, C. and Splittstoesser, D. F. (1992). The compendium of Methods for
 5 the Microbiological Examination of Food. American Public Health Association
 (APHA), Washington DC, USA.

EXAMPLE 4: Provision of Hydrolysate in a Soup Mix

	Flour	2g	
10	Firmtex	2g	(modified starch)
	SMP	1g	
	Vege fat	1g	
	Salt	0.5g	
	Beef Stock	3g	
15	Beef flavour	1g	
	Onion Powder	0.8g	
	Colour	0.06g	
	<u>Hydrolysate</u>	<u>4.8g</u>	
	Total	16.1g	(This is added to 200ml of water and heated to boiling)

EXAMPLE 5: Storage Stability Trials

Introduction

The aim of the trial was to assess the impact of storage conditions on the storage life and quality of Meat hydrolysate when packaged under vacuum in aluminium-foil laminated pouches. The results are presented in this report.

Abstract

A storage trial on a 15kg batch of lamb meat hydrolysate powder was conducted with the aim of assessing the impact of storing the product at -20°C, 4°C, 20°C and 37°C on overall quality over a 24 week period. Meat hydrolysate contains a high level of protein (83.4%) and low levels of fat (0.4%) and collagen (1.5%). Colour (L, a* and b*) of Meat hydrolysate showed small changes with temperature and time. The water solubility (WS) of Meat hydrolysate (87.3±0.7 %) showed no change with either storage time or storage temperature. GC Headspace analysis released a series of characteristic peaks in the initial stage, week 12 and week 24 depending on the storage time and temperature. The number of volatile peaks in the headspace became fewer with storage time and more peaks disappeared in samples stored at 37°C than at the cooler temperatures. Lysine availability tended to decrease with increasing storage temperature and storage time, with storage time having a greater impact on lysine loss than storage temperature. The lysine availability was 88% at time 0, but reduced to the range from 59 % to 67% at week 24. More *in vitro* available lysine was lost at 37°C than at lower temperatures. Microbial counts (200-400 CFU/gm) showed little change with temperature and time. An informal sensory test showed that the sensory quality of samples stored at temperatures $\leq 20^{\circ}\text{C}$ showed little deterioration with either storage time or

temperature. However, the powder stored at -20°C had a meatier and oilier odour than the samples stored at 4°C and 20°C for all assessments. Samples stored at 37°C deteriorated slightly in terms of the taste of its 2.1% aqueous broth at the end of the trial, but the deterioration was not significant.

5 **Methods and materials**

Source of MS lamb meat and Meat hydrolysate

MS lamb meat was purchased from PPCS's plant, Oringi, New Zealand. Meat hydrolysate was produced and spray-dried by New Zealand Pharmaceuticals Ltd. (NZP) according to a protocol that was developed by the Riddet Centre of Massey University, Palmerston North.

Packaging for storage and sampling

Meat hydrolysate was conditioned for 24 hours prior to packaging to allow the moisture in the spray dried powder to equilibrate. Fifteen grams of the powder were weighed into aluminium foil laminated pouches and sealed under vacuum.

15 The fifteen gram pouches were randomly selected and then allocated to a specific storage temperature. The pouches were stored at the following four temperatures: -20°C (control), 4°C, 20°C (room temperature) and 37°C over a period of 24 weeks. At regular 4 weekly intervals, four pouches were randomly removed from each of the storage rooms and evaluated for a range of physico-chemical and

20 sensory measures.

Proximate analysis

Proximate analysis of the product was conducted according to the following methods: total combustion method for protein content (AOAC 968.06), Soxhlet

extraction for fat content (AOAC 991.36), conventional oven drying at 105°C for moisture content (AOAC 930.15, 925.10), furnace combustion at 550 °C for ash content (AOAC 942.05), Plasma Emission Spectrometry for minerals analysis, hydrochloric acid hydrolysis followed by HPLC separation for amino acids (AOAC 5 994.12) and alkaline hydrolysis followed by HPLC separation for tryptophan analysis.

Measurement of colour spaces of L*, a* and b*

The L*, a* and b* colour values for products stored at the four storage temperatures -20°C, 4°C, 20°C and 37°C were measured using a Minolta Chroma 10 meter CR-200 (Konica Minolta Sensing Inc., Japan). Each reported value was an average of quadruplicate measurements of the hydrolysate powder.

Water solubility (WS) measurement

A modified AOAC (950.81) method was used for water solubility measurement. Approximately 1 g of Meat hydrolysate was added to 100 mL of water in a 250 mL 15 conical flask and shaken on a 150 rpm rotation shaker for 2 hours. About 30 mL of the solution was centrifuged at 15000 rpm for 20 minutes. The supernatant was then dried in a 105°C oven overnight. All the data were measured in duplicate. WS was calculated based on the following equation.

$$WS (\%) = \frac{V_s \times W_d}{V_c \times W_s}$$

20

W_d refers to the weight (g) of dried solubles in V_c

W_s refers to the weight (g) of the powder sample

V_s refers to total volume (mL) of the solution

V_c refers to the supernatant volume (mL) from centrifuge

Headspace analysis

A gas chromatograph (GC) was used for headspace analysis. The SHIMADZU GC-2010 gas chromatograph and a SupelcowaxTM 10 fused silica capillary column (30 m × 0.32 mm × 0.50 µm) were used in the present study. The CAR/PDMS fibre (75 µm) was conditioned before use and transferred into the injector port of the GC by a SHIMADZU AOA-5000 auto sampler. The injector port temperature was set at 250°C, the detector at 250°C and the oven 100°C. The splitless mode was used. The carrier gas used was Helium supplied by BOC Gases (Palmerston North) Ltd. The flow rate of the carrier and fuel gas were controlled at 2.4 mL/min. Each 20 mL glass vial for holding the sample contained 2 g of the spray-dried powder and was sealed with a rubber/aluminium cap. Duplicate injections were required for the GC headspace analysis.

In-vitro available lysine analysis

The O-methylisourea (OMIU)-reactive lysine was determined using a procedure described by Moughan and Rutherford (1996) followed by HPLC separation. All analyses were conducted in duplicate. The samples were analyzed for total lysine and 'reactive lysine' or available lysine.

Plate count test

A conventional aerobic plate count method (Vanderzant and Splittstoesser, 1992) was applied to assess the effect of storage time and temperature on microbial quality of the stored hydrolysate powders. Samples were analysed for microbial counts in duplicates for the four temperature treated samples at each testing time. Approximately 0.5g of the powder was taken from each pack and dissolved and

then the following dilutions were performed at 1: 50, 1: 500 and 1: 5000. The plate count agar used was supplied by Merck Co. of USA.

Sensory analysis

An informal sensory trial was carried out on powders stored at the four
5 temperatures every four weeks throughout the storage period. The objective of the informal sensory test was to follow any changes in odour and taste that might have been occurring in the product over the entire storage period. The sensory assessments were based on a 10 point category scale. A score of 1 indicated that significant changes to the odour or flavour had occurred. A score of 5 suggested
10 that the changes were mildly sensitive and a score of 10 suggested that there had been no change in the attribute under study. A sample of powder from each of the various temperature treatments was removed from the vacuum sealed pouch and placed in a container and immediately evaluated for odour. An approximately 2.1 g sample was made up to 100 mL in a 250 mL beaker with distilled water and then
15 brought to the boil. The aqueous broth was then filtered and a portion was evaluated by each of the informal taste panel members.

Results and discussion

Proximate analysis of the product

A chemical analysis of Meat hydrolysate was carried out at the start of the project
20 and the results are shown in Table 9, Table 10 and Table 11. As shown in Table 9 Meat hydrolysate contained less fat (0.4%) and more protein (83.4%) than the MS lamb meat (21.5% fat and 19.4% protein with connective tissue included).

Table 9: Chemical Composition (g/100g) of Meat hydrolysate:

Sample	Moisture	Ash	Crude Protein	Fat
Meat hydrolysate	5.06	7.25	83.38	0.39

5 **Table 10:** Mineral composition of Meat hydrolysate:

Mineral	Unit	Meat hydrolysate
Calcium	g/100g	0.13
Magnesium	g/100g	0.11
Potassium	g/100g	1.8
Sodium	g/100g	1.2
Phosphorus	g/100g	0.81
Sulphur	g/100g	0.88
Aluminium	mg/kg	2.8
Arsenic	mg/kg	<0.1
Boron	mg/kg	0.73
Chromium	mg/kg	0.15
Cobalt	mg/kg	0.04
Copper	mg/kg	2.5
Iron	mg/kg	130
Iodine	mg/kg	0.11
Lead	mg/kg	0.039
Manganese	mg/kg	22
Mercury	mg/kg	<0.01
Molybdenum	mg/kg	0.22

Nickel	mg/kg	0.38
Selenium	mg/kg	0.28
Strontium	mg/kg	3.3
Tin	mg/kg	<0.05
Zinc	mg/kg	59
Chloride	mg/kg	16000

Approximately 51% of the crude protein in the raw meat was recovered and 98% of the fat was removed through the process. In terms of collagen content, based on the hydroxyproline level shown in Table 11 and the calculation using a conversion factor of 7.813 from hydroxyproline to collagen (Lawrie, 1991), Meat hydrolysate

5 contained 1.5% collagen, eight times lower than that in raw MS lamb meat (11.7%)

About 87% of the collagen-associated connective tissue was removed through the process. Due to the fact that there was such a low level of fat in Meat hydrolysate (0.4%), TBA tests or peroxide analysis were not considered.

The full mineral analysis results show that the total minerals made up 6.6% of the

10 powder, and contained high levels of chloride, potassium, sodium, sulphur, phosphorus, calcium and magnesium, low levels of iron, zinc and manganese, and trace amounts of other minerals. Thus, the maximum sodium chloride level was about 2.6 g/100g of Meat hydrolysate assuming all the chloride (1.6g/100g) was bound to sodium.

15 The table below shows the amino acid contents of Meat hydrolysate and two lamb meats. There were no obvious differences between the samples. The threonine, serine, alanine, tyrosine and arginine levels were approximately 10% lower in Meat hydrolysate than in the lamb leg muscle. However, the methionine was 35% lower in Meat hydrolysate and the valine and histidine contents, on the other hand, were

10% higher in Meat hydrolysate than the lamb leg muscle. When compared to MS lamb meat, the threonine, proline, glycine and methionine levels were about 10% lower in Meat hydrolysate than in raw meat. The glutamic acid, valine and lysine levels in Meat hydrolysate were about 10% greater than in the raw MS lamb meat.

- 5 The hydroxyproline content in Meat hydrolysate was 7.4 times lower than that in MS lamb meat and 1.8 times lower than that in lamb lean leg muscle. This implies that Meat hydrolysate has a low collagen level.

Table 11: Amino acid composition (mg/100mg) of Meat hydrolysate with those of MS lamb meat and lamb lean leg meat as a comparison:

Amino Acid	Meat hydrolysate	MS lamb meat	Lamb lean leg meat
Aspartic acid	8.07	7.46	7.93
Threonine	3.39	3.74	3.85
Serine	2.60	2.72	2.93
Glutamic acid	13.87	12.61	13.35
Proline	3.42	3.75	3.54
Glycine	3.75	4.45	3.61
Alanine	5.13	5.10	5.84
Valine	4.73	4.17	4.18
Isoleucine	3.66	3.39	3.91
Leucine	6.86	6.47	7.14
Tyrosine	2.78	2.74	3.08
Phenylalanine	3.50	3.36	3.44
Histidine	2.99	2.80	2.54
Lysine	7.91	7.14	7.66
Arginine	5.47	5.79	6.07
Cysteine*	0.86		

Methionine*	1.70	1.96	2.30
Tryptophan**	0.95		
Hydroxyproline	0.19	1.40	0.35

Note: * from performic acid oxidation

** from alkaline hydrolysis

Effects of the storage on colour spaces of the product

As shown in Figure 2 The results indicate that colour spaces (expressed as L*, a* and b* values) of the samples stored at the temperatures of -20°C, 4°C, 20°C and 37°C showed no obvious changes with time, L* changing by 2.2% from 61.5 to 62.9, a* by 3.6% from 5.85 to 6.06 and b* by 7.6% from 13.43 to 14.53.

For the samples stored at -20°C and 4°C, the L* values changed within a relatively smaller range compared to those of the samples stored at 20°C and 37°C. The L* values of all the samples were at their maximum values at week 24. The a* values of all the samples increased with time until week 20, with the 37°C sample having higher a* values. The a* values then decreased to their minimum at the end of 24 weeks except for the sample stored at 20°C which remained unchanged after week 20. The b* values of all the samples changed in a similar pattern to a* values. However, the 37°C sample had a higher b* value than the other samples till the end. The higher temperatures (e.g. 37°C) generally caused larger variations in the colour spaces over the storage period than the in the samples stored at the lower temperatures.

Effect of storage on water solubility of the product

Water solubility (WS) of Meat hydrolysate in the test, was defined as the maximum amount of the hydrolysate that could be dissolved in water at room temperature (20-24°C) and atmospheric pressure. The results shown in Figure 3 indicate that the WS of the samples increased over the first 8 weeks of storage with the 20°C stored sample showing the greatest change from 86.5% to 88.0%. The solubility of all samples then remained constant until week 12 and then declined. The WS of the sample stored at 37°C dropped from 88% to 86.6% by week 16. It appeared that the samples stored at 37°C had a lower WS than other samples, but this difference was only slight and had no effect on sample quality.

Based on the same methodology, the WS at pH 2.0, 4.0, 6.3 (natural pH), 9.0 and 11.0 were also determined to observe the effects of aqueous solution pH on the WS of Meat hydrolysate stored at 20°C over a period of 6 months. The results are shown in Figure 4, indicating that the WS of the samples under different pH values changed over a wider range from 82.0% to 89.8% than those solutions made from the unadjusted samples (pH 6.3). All the samples exhibited minimum WS (82.0 %) at pH 4 and were highly soluble at pH11 with the maximum WS at 89.8%. Storage time had effects on the WS under different pH values, but with no regular trend except for the pH 2 samples which increased in solubility with time.

Effects of the storage on volatile components of the product

Headspace analysis of Meat hydrolysate stored at four temperatures for a period of 12 and 24 weeks was characterized using GC to observe what effects temperature and time had on the volatile components of the powder. The peak heights (in arbitrary units) obtained from headspace analysis at times 0, 12 weeks and 24 weeks are shown in Figures 5 to 7.

In the initial stage of the trial, the sample presented a series of specific peaks at 2.9, 4.9, and 8.2 minutes which were absent in blank profile of air. The highest peak of the sample appeared at the retention time of 15.7 min with an arbitrary unit of 87,000, twice as high as obtained from the air blank (38,000 au). This

5 component made up 34.6% and 12.1% of the total volatile components of these two samples respectively. At an elution time of 2.1 min, the air blank sample generated a peak of 190,000 au, making up 61.4% of its total components. No peak appeared at the corresponding time for Meat hydrolysate.

For the samples stored for 12 weeks, the major peak (809,000 au) appeared at an
10 elution time of 5.9 min for the sample stored at -20°C, making up 52.9% of its total components. It gave a characteristic feature to the sample's chromatogram. At the elution time of 11.8 min, the peak height increased with storage temperatures from 116,000 au to 177,000 au. No higher peak than these appeared within the elution
15 11.8 min could be closely associated with the flavour characters of the samples stored for a period of 12 weeks. This peak contributed 7.6%, 29%, 49% and 57% of their respective total components to the samples stored at -20°C, 4°C, 20°C and 37°C. In contrast to the 11.8 min peak, the peaks at 15.3 min, 17.8 min and 20.1 min decreased in height with rising storage temperatures as shown in Figure 6. It
20 would appear that both, the number of peaks and height of peaks in the chromatograms decreased with storage temperature suggesting either that volatiles were being lost from the pouches or being scavenged by the film at the higher temperature or that the volatile components were being absorbed by the bulk powder as a consequence of chemical reactions.

25 The volatile components obtained from samples stored at the four temperatures for 24 weeks became more widely divided (Figure 7). At the elution time of 5.3 min,

the 4°C sample released a large peak (348,000 au) at the same position as the -20°C samples (615,000 au), accounting for 61% and 69% of their respective total components, giving a similar flavour character to the -20°C samples. The sample stored at 20°C produced a peak (93,000 au) at an elution time of 3.3 min, giving an additional character feature to the sample, which made up 32.6% of its total components. These changes are also evidence pointing to a loss of flavour components with increase in storage temperature.

It can be concluded from Figures 5-7 that storage temperature influenced the volatile components of the hydrolysate powder in terms of both level and the type. Longer storage may diversify the volatile components. Some trend patterns in the levels of volatile components could also be clearly observed over the time from the low to high storage temperature.

Effects of storage on lysine availability (*in vitro*) of the product

The content of available lysine is a very powerful determinant of the protein quality of a food product. Under a variety of conditions like heating and long-term storage, the free amino group of lysine in protein foods can react, for example, to form Maillard complexes with sugars that may thereby reduce the availability of lysine (Miller and Gerrard, 2005). In this study, the effects of storing the hydrolysate powder at temperatures of -20°C, 4°C, 20°C and 37°C for a period of 24 weeks on lysine availability were examined.

At time 0, the mean contents of duplicate total lysine and available lysine of the hydrolysate powder were 7.9 and 7.0 g/100g, respectively. The amount of lysine lost (11%) at this stage might be attributed to the spray-drying process used to produce the hydrolysate powder. The total lysine content showed virtually no change over the storage period for any of the four storage temperatures with

values ranging from 7.7-8.0g/100g at the end of the trial and 7.9g/100g at the start of the trial. The effect of storage temperature on lysine availability of meat hydrolysate is shown in Table 12.

Table 12: Effect of storage on lysine availability of Meat hydrolysate:

Sample stage	Total lysine (g/100g)	Available lysine (g/100g)	Ratio of available lysine to total lysine (%)	Loss of available lysine within 24 weeks (%)
20°C time 0	7.9	7.0	88.6	
- 20°C week 24	7.8	5.0	64.1	27.7
4°C week 24	7.8	5.2	66.8	24.6
20°C week 24	7.7	5.0	64.7	27.0
37°C week 24	8.0	4.7	58.6	33.9

However, their corresponding available lysine levels decreased to around 5.0g/100g from an initial concentration of 7.0 g/100g. The ratio of available lysine to total lysine decreased from 88.6% in the initial sample to 64.1% and to 58.6 % for the samples stored at - 20°C to 37°C respectively, by the end of the trial. The respective losses of available lysine for the four temperatures studied were 27.7%, 24.6%, 27.0% and 33.9% respectively.. In summary, available lysine decreased with both storage time and storage temperature with storage time having the greater impact on the levels.

10 **Effect of storage on microbial quantity**

The changes in microbial counts were also followed during the storage trial to ensure that they would not become a shelf limiting factor for the product. Unfortunately, the dilutions used in the experiment according to the recommended rules by Vanderzant and Splittstoesser (1992) led to a low number of counts and so it was not possible to get an exact counts for samples from each test day. The total number of colonies for each sample was < 25. Instead the results were

- expressed as an estimated CFU numbers per gram as shown in Table 13. The results indicated that counts in all samples stored at the four storage temperatures over a period of 24 weeks were within a range from 200/g to 400/g. The sample stored at 37°C showed a decrease CFU number whilst the samples stored at -
- 5 20°C, 4°C and 20°C showed no change in the CFU numbers with time. The plate count result indicates that the samples met "the microbiological reference criteria for food" in New Zealand (Ministry of Health of New Zealand, 1995). Clearly, microbial quality was not an issue for this product.

Effects of storage on tastes/odours of the product

- 10 An informal sensory trial was carried out on the MS lamb meat hydrolysate stored at the temperatures of -20°C, 4°C, 20°C and 37°C over a period of 24 weeks. The average score of the 6 panellists was determined for each attribute and these average scores with associated standard deviations are shown in Figure 8.

Table 13: Plate counts of Meat hydrolysate stored at four temperatures for a periods of 12, 24 weeks:

Samples	Sample weight (g)	Mean colony numbers of three plates of each sample from two dilutions		Estimated * CFU in each gram sample	Mean CFU in each gram sample
		1: 50	1: 500		
20°C time 0	0.6269	6	1	478	397
	0.3171	2	0	315	
-20°C week 12	0.5598	2.75	1	246	363
	0.4951	4.75	1	480	
4°C week 12	0.4589	3.5	0.67	381	395
	0.3985	3.25	1.33	408	

20°C	week 12	0.3861	1.25	0.67	162	254
		0.4587	3.25	1	354	
37°C	week 12	0.4206	1.5	0	178	262
		0.5051	3.5	1.33	346	
-20°C	week 24	0.2476	3.33	0.33	672	405 ± 133.8**
		0.243	1.33	0.33	274	
		0.3112	1.33	0	268	
		0.3032	1.67	0	275	
4°C	week 24	0.2419	2	0	413	315 ± 49.2
		0.3241	1.67	0	258	
		0.3139	1.67	0	266	
20°C	week 24	0.2494	1.33	0	267	326 ± 59.8
		0.2614	2.33	0	446	
		0.2895	0.33	0	57	
37°C	week 24	0.2695	0.67	0	124	207 ± 118.4
		0.2642	2.33	0	441	

* According to rule e from 'The compendium of Methods for the Microbiological Examination of Food, p86" (Vanderzant and Splittstoesser, 1992), if plates from all dilutions yield fewer than 25 colonies, record the actual number of colonies on the lowest dilution and report count as estimated CFU per g.

5 ** The number refers to the standard deviation from three raw data of CFU/g.

The statistical analysis of the sensory data using a general linear model procedure of the SAS package (SAS Institute, Cary, NC, USA) showed that there were no significant differences ($P < 0.05$ using Duncan's test) among the samples stored at the temperatures of -20°C, 4°C, 20°C and 37°C over a 24 week period

when compared to the time 0 sample in terms of both tastes and the odours. In general, the samples stored at 20°C and 4°C generally smelled fewer offensives than those stored at -20°C (*i.e.* they were not as oily or meaty smelling than the cold control, Figure 8). The sample stored at 37°C smelled slightly fishy, but the
5 smell was still pleasant. The results indicated that the samples became more palatable with storage time. The headspace analysis indicated that volatile compounds were gradually lost with storage time and these sensory results seem to confirm this change as well.

The taste panel results for the aqueous broth made from the stored hydrolysate samples (2.1 g of powder to 97.9 g of water) based on the sample level in the beef
10 soup prepared for a formal sensory trial suggested that the flavour of the stored samples gradually deteriorated with time (Figure 8). This trend was particularly evident in the sample stored at 37°C. However, the degree of deterioration was not practically significant.

15 Overall conclusions

- Meat hydrolysate contained 0.4% fat and 83.4% crude protein (includes 1.5% collagen), 7.3 % ash and 5.1% water. The sodium chloride level (assuming all the chloride was bound to sodium) was estimated to be 2.6 g/100g of the hydrolysate powder, which
20 explains why the powder was deemed to be 'salty'. Amino acid composition of Meat hydrolysate was slightly different from those for the lamb leg muscle and MS lamb meat. However, the hydroxyproline content of Meat hydrolysate was 1.8 times lower than that in lamb leg muscle and 7.4 times lower than that in MS lamb
25 meat.

- Colour spaces of all the powder samples stored at the four temperatures for a 24 week period changed with time within a narrow range, L* changing by 2.2% from 61.5 to 62.9, a* by 3.6% from 5.85 to 6.06 and b* by 7.6% from 13.43 to 14.53. The samples stored at 20°C and particularly 37°C showed greater colour changes with time than the samples stored at 4°C or less.
- The WS of Meat hydrolysate showed little change with either storage time or storage temperature, and was within a narrow range of 86.5% to 88.0%.
- pH manipulation of the samples had a much greater impact on WS than temperature with the WS ranging from 82% to 90%. All the samples exhibited a minimum WS at pH 4 and were highly soluble at pH11.
- The number of volatile peaks in the headspace above the various powders became less complex with storage time (*i.e.* there were fewer peaks with time and/or the peak heights decreased with time). Longer storage could diversify the volatile components. The complexity appeared to be also influenced by storage temperature with more peaks disappearing in samples stored at 37°C than at the cooler temperatures.
- Total lysine was neither affected by storage time nor temperature. However, available lysine decreased with both storage time and storage temperature with storage time having the greater impact. The available lysine content decreased from about 88% at time 0 to

between 58.6% – 66.8 depending on storage temperature. The higher the temperature the greater was the loss of available lysine.

- There was little difference in the CFU numbers for the samples stored at -20°C, 4°C and 20°C for a period of 24 weeks. For the same period of storage at 37°C, the sample gave a CFU number almost 50% less than that at low temperature storage. Since the CFU numbers of all the samples in the trial were within the range from 200 to 400/g, the microbial quality was satisfactory for this product.
- The sample stored at -20°C smelt more oily and meaty than the rest of the samples while the sample stored at 37°C had somewhat fishy odour, but not offensive. The aroma profiling suggested that the samples improved with storage time and in particular at the higher temperatures. These results tend to corroborate the headspace results which suggested the same trend and indicate that volatile notes were either being lost from the pouches (unlikely given the fact that the powder was packaged in aluminium foil laminate pouches) or being absorbed by the polythene lining film in the pouches. However, the informal tasting panel results of the aqueous broth made from the powders suggested that the flavour deteriorated slightly with time, especially at storage temperature of 37°C. However, none of the above changes were statistically significant.

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AOAC:

EXAMPLE 6: Preliminary Human Metabolic Study

Abstract

The purpose of this project was to evaluate the quality of a hydrolysed meat protein
15 in older adult subjects by measuring its nitrogen utilization in the human body.
Lamb meat was enriched with 15nitrogen (or 15N), the stable isotope of nitrogen and the meat hydrolysed into a powder, incorporated into a meal as the sole source of protein and fed to participants. Blood and urine samples were collected for 8 hours following the meal and analyses of these samples determined how
20 much nitrogen from the meat hydrolysate was transferred into the body's nitrogen pool and excreted in the urine.

Calculations performed using this information determined the utilization of the hydrolysate and were compared with information obtained from volunteers who received a meal containing a marked reference protein prepared from milk.

The results indicate a very high degree of amino acid utilization of the lamb meat hydrolysate amino acids, when they were fed to human subjects in the form of a soup.

The objective of this study was to determine the postprandial (after meal) nitrogen utilization of a meat hydrolysate in older adults by measuring dietary nitrogen intake and absorption. The study employed a state-of-the-art isotope tracer methodology.

Materials and Methods

The study population comprised 26 older (60 - 81 years of age), community-dwelling adults. Volunteers were recruited from the Palmerston North area through advertisements in the local media and selection from the electoral role. Volunteers with a history of diabetes mellitus, bleeding disorders, cancer (any form), any gastrointestinal, hepatic or hormonal disorders or disturbances were excluded as were smokers and people who drank more than 2 units of alcohol per day. Volunteers who used medication known to influence digestion were excluded and any use of multivitamin supplements on a regular basis was stopped one week before the trial. Other exclusion criteria were vegetarians/vegans, allergies to dairy products and significant weight change during the past six months.

Before study entry the volunteers provided urine and fasting blood samples that were screened for hematologic, liver and kidney function and for electrolytes. Volunteers with blood values outside the recorded reference ranges for the

laboratory were excluded. Food intake was recorded for one week and protein intake calculated to ensure that protein intake was within normal guidelines for general health for an older New Zealand adult (> 51 years, NHMRC 2005). People who consumed more than the recommended daily protein intake were excluded.

5 Anthropology

Anthropometric measurements were taken during an initial screening visit. Height was measured by using a stadiometer to the nearest 1 cm and body mass was measured by using the BOD POD calibrated electronic weighing scale to the nearest 0.01 kg. Body composition was determined using air-displacement plethysmography (BOD POD Composition System, Life Measurement, Inc).

Test meals

The meals were designed to be balanced providing one third of the daily recommended dietary energy intake (approximately 700 kilocalories) for an older New Zealand adult (> 51 years, NHMRC 2005). The meals were formulated to provide 30 g of protein, uniformly and intrinsically labeled with ¹⁵N. The source of protein was either lamb protein hydrolysate or casein. The carbohydrate and fat sources (maltodextrin and canola oil respectively) were identical for both meals. The composition and energy values of the ingredients used in each meal are presented in the table below.

Table 14: Composition and energy values of ingredients used to prepare the meals.

Ingredient	Weight (g)	Energy (kcal)	Carbohydrate (g)	Fat (g)	Protein (g)
Hydrolysate	36	121.3	0	0.1	30
Casein	33	123.2	0	0.4	30

Canola oil	20	180.0	0	20.0	0
Maltodextrin	100	400.0	100	0.0	0

The total energy content of the meal prepared with the lamb hydrolysate as the meat source was 701.3 kilocalories of which 17 % was protein, 26 % was fat and 57 % was carbohydrate (36 g hydrolysate, 20 g canola oil and 100 maltodextrin).

The total energy content of the meal prepared with the casein as the meat source
 5 was 703.2 kilocalories of which 17 % was protein, 26 % was fat and 57 % was carbohydrate (33 g casein, 20 g canola oil and 100 maltodextrin). The meals were iso-nitrogenous providing 320 mmol of nitrogen.

Each meal was prepared immediately before serving. ¹⁵N-labelled lamb meat hydrolysate (36 g) or ¹⁵N-labelled casein (33 g) was added to maltodextrin (100 g;
 10 UNIDRI 20, Penford). For the meat hydrolysate the dry ingredients were then added to a saucepan containing cold water (400 ml) and canola oil (20 g). The mixture containing the hydrolysate was blended with a whisk over heat and served at 60 °C whilst the mixture containing the casein was blended and served at room temperature.

15 Protocol

The volunteers were randomized into two groups; one group received the meat protein meal and the other received the casein protein meal. The subjects arrived at 0750 in a fasted state. Following baseline collections of blood and urine, the volunteers ingested the test meal. The study was performed while the participants
 20 were resting in a semi-recumbent position and no other food was ingested until the end of the study period. Water was given bi-hourly. Blood was collected from each subject every 30 minutes for three hours and then every hour for the following

five hours. Blood was collected into Vacutainers with no anticoagulant (serum) or into Vacutainers containing oxalate/fluoride (plasma). Between blood draws the cannula was flushed with sterile physiological saline. Serum samples were left to stand at room temperature for 30 minutes and plasma samples were immediately placed on ice. All samples were centrifuged for 10 minutes at 3 000 RPM at 4 °C, aliquoted and frozen within one hour of collection and stored at – 20 °C until processing. Total urine was collected every two hours throughout the eight hour period. Urine samples were stored at – 4 °C with thymol crystals and paraffin added as preservatives or were immediately frozen at - 20 °C depending on the chemical analysis.

Analytical methods

Plasma glucose was measured using a hexokinase method (Nutrition Laboratory, Massey University, Palmerston North, NZ). Serum insulin was measured using a double antibody radioimmunoassay method (Nutrition Laboratory, Massey University). Serum urea, urinary creatinine and urinary urea were assayed using an enzymatic method (Nutrition Laboratory, Massey University). Urinary ammonia was measured using an enzymatic method using glutamate dehydrogenase (Nutrition Laboratory, Massey University).

For isotopic determinations urea and ammonia were isolated from urine on a Na⁺ form of a cation exchange resin (Biorad Dowex AG50-X8, Sigma-Aldrich, Auckland, NZ). For urinary ammonia extraction, urine (7 ml) was mixed with resin (2 ml) for 20 minutes. The supernatant was kept and the resin containing urinary ammonia was washed 5 times with distilled water. The supernatant (2 ml) was mixed with resin (2 ml) and incubated for 2 hours at 30 °C in the presence of urease (20 µl; Sigma-Aldrich, Auckland, NZ). The resin containing urinary urea-

derived ammonia was then washed with distilled water and stored at 4 °C for isotopic determination. For serum urea extraction, the serum proteins were precipitated by mixing serum (2 ml) with 5-sulpho-salicylic acid (Sigma-Aldrich, Auckland, NZ). After centrifugation (2400 g, 25 min, 4 °C) the pellet containing the
5 serum proteins was freeze-dried and stored until analysis. The supernatant was kept and buffered at pH 7. The urea was isolated from free amino acids on 2 ml of resin in the presence of urease (8 µl). After incubation for 2 hours at 30 °C the supernatant containing free amino acids was removed. The resin containing urea-derived ammonia from serum was washed with distilled water and stored at 4 °C.
10 Before isotopic determination resins were eluted with KHSO₄ (2.5 mol/L). The ¹⁵N:¹⁴N isotope ratio was determined by isotope-ratio mass spectrometry (Stable Isotopes Laboratory, GNS Science, Lower Hutt, NZ) in the urinary urea and ammonia, serum protein, free nitrogen and urea.

Calculations

Incorporation of nitrogen into nitrogen body pools

The time course of dietary nitrogen incorporation into the pools of serum protein, body urea, urinary urea and ammonia ($N_{\text{exo-pool}}$, % of ingested nitrogen) was
 5 evaluated for each group using the following general equation:

$$N_{\text{exo-pool}} = 100 \times [N_{\text{tot-pool}} \times (E_s - E_0) / E_{\text{meal}} - E_0] / N_{\text{ingested}}$$

where $N_{\text{tot-pool}}$ is the nitrogen content of the pool (mmol N) at the collection time, E_s the ^{15}N enrichment of the sample and N_{ingested} is the nitrogen ingested within the meal (mmol). $N_{\text{exo-pool}}$ was expressed as % of nitrogen ingested. For urinary urea
 10 N_{tot} was calculated as the product of the urinary urea nitrogen concentration and the volume of urine excreted. N_{tot} in the serum protein pool was determined as the serum concentration of protein nitrogen multiplied by the serum volume estimated to be 5 % of body weight (Ganong, 2005). The nitrogen in the body urea pool was calculated assuming that urea was uniformly distributed throughout the total body
 15 water (TBW) and according to the following equation:

$$N_{\text{body urea}} = C_{\text{urea}} \times \text{TBW} / 0.92$$

where C_{urea} is the urea nitrogen concentration in the serum sample at the collection time and 0.92 is the correction factor for the water content of serum. TBW was determined according to the equation of Watson et al. (1980).

20 *Net postprandial protein utilization and postprandial biological value*

At the end of the 8 hour experimental period the amount of dietary nitrogen retained in the body or net postprandial protein utilization (NPPU; % of ingested nitrogen) was calculated as follows:

$$\text{NPPU} = [\text{N}_{\text{ingested}} - \Sigma \text{N}_{\text{exo-ileal}} - \Sigma \text{N}_{\text{exo-urinary}} - \Sigma \text{N}_{\text{exo-body urea}} (8\text{h})] / \text{N}_{\text{ingested}}$$

- 5 where $\Sigma \text{N}_{\text{exo-ileal}}$ is the cumulative recovery over 8 h of dietary nitrogen collected in ileal digesta, $\Sigma \text{N}_{\text{exo-urinary}}$ is the cumulative recovery over 8 h of dietary nitrogen incorporated into urinary ammonia and urea and $\Sigma \text{N}_{\text{exo-body urea}} (8 \text{ h})$ is the dietary nitrogen incorporated into body urea at 8 h.

- The postprandial biological value (PBV; % of ingested nitrogen) was calculated as
10 the relative amount of dietary nitrogen absorbed that was not deaminated during the postprandial period:

$$\text{PBV} = (\text{NPPU} / \text{TID}) \times 100$$

- where TID is the true ileal digestibility (% of ingested nitrogen) determined from the cumulative recovery of dietary nitrogen in ileal digesta ($\text{TID} = 100 \times [\text{N}_{\text{ingested}} -$
15 $\Sigma \text{N}_{\text{exo-digesta}}] / \text{N}_{\text{ingested}}$).

Area under the curve

The area under the curve (AUC) was computed for the concentrations of plasma glucose and serum insulin determined from 0 to 8 hour postprandially and referred to as the area between the curve and the baseline.

20 **Statistical analysis**

Statistical analyses were performed using Systat (SPSS Inc) and Prism (GraphPad Software Inc). Data were transformed to logarithms. Levenes tests were

conducted for all variables to check for homogeneity of variances. Differences between participant anthropometric measurements and values recorded at 8 h postprandial (nitrogen concentrations in body urea and serum proteins, exogenous nitrogen excretion, total exogenous nitrogen deaminated, total nitrogen losses, NPPU and PBV) were tested using two sample t-tests (parametric data) or Kruskal-Wallis one way analysis of variance (non-parametric data). General linear model repeated measures analysis of variance were used to compare differences between meals over time for hormones and dietary nitrogen kinetics. The areas under glucose and insulin curves were determined in Prism using the trapezoid rule. Differences in AUC between meals were then determined using two sample t-tests. Data are presented as means $\pm$ standard error. A p value < 0.05 was considered to be statistically significant.

Results

Anthropometric measurements

The anthropometric measurements for study participants are provided in the table below. There were no statistically significant differences among subjects fed either of the two test meals for any of the measured characteristics.

Table 14: Anthropometric measurements for study participants.

Group	n	Age (years)	Weight (kg)	Fat weight (kg)	Lean weight (kg)	Body mass index (kg/m ²)	Total body water (L)
Hydrolysate	5M/11F	71.0 $\pm$ 0.3	73.9 $\pm$ 0.6	23.5 $\pm$ 0.5	50.4 $\pm$ 0.4	25.7 $\pm$ 0.2	36.1 $\pm$ 0.3
Casein	5M/4F	69.1 $\pm$ 0.7	72.0 $\pm$ 1.3	19.3 $\pm$ 1.3	52.7 $\pm$ 1.0	24.2 $\pm$ 0.4	36.0 $\pm$ 0.5

Values are shown as mean $\pm$ standard error

M = male, F = female

Kinetics of plasma glucose and serum insulin

The plasma glucose concentrations in older adult humans fed a mixed meal containing either ^{15}N -labelled lamb hydrolysate or casein are shown in Figure 9.

- 5 The plasma glucose concentrations increased significantly after ingestion of both meals and peaked at 30 minutes postprandially (7.0 ± 0.2 and 6.6 ± 0.6 mmol/L for the lamb hydrolysate and casein meals respectively). There were no significant differences in overall glucose concentrations, peak glucose concentrations or the AUC between the two meals ($p > 0.05$).
- 10 The serum insulin concentrations in older adult humans fed a mixed meal containing either ^{15}N -labelled lamb hydrolysate or casein are shown in Figure 10.

Serum insulin concentrations increased significantly after ingestion of both meals and peaked at 30 minutes postprandially for the casein meal (97.5 ± 13.6 $\mu\text{U/ml}$) and 60 minutes postprandially for the lamb hydrolysate meal (89.7 ± 11.9 $\mu\text{U/ml}$).

- 15 There were differences of borderline significance between the two meals at 60 minutes ($p = 0.059$) but there were no significant differences in overall insulin concentrations or the AUC between the two meals ($p > 0.05$).

Dietary nitrogen incorporation into serum protein pools

- Time courses of isotopic ^{15}N concentration enrichments were measured in serum
- 20 protein and urinary nitrogen pools which allowed for the quantification of dietary nitrogen in each pool. Dietary nitrogen incorporation into the serum protein pool was determined following the ingestion of the two ^{15}N -labelled meals and the results are shown in Figure 11.

The incorporation of dietary nitrogen into the serum protein pool increased during the first 4 hours after the ingestion of the lamb hydrolysate meal reaching a maximum of 7.6 ± 0.7 % at 8 hours. The incorporation of dietary nitrogen into the serum protein pool reached a plateau after 7 hours with 10.7 ± 0.4 % of the ingested nitrogen from the casein meal present in the serum proteins 8 hours after meal ingestion. The amount of dietary nitrogen incorporated into the serum protein pool 8 hours after meal ingestion was significantly higher for the casein meal compared to the lamb hydrolysate meal ($p < 0.05$).

Dietary nitrogen deamination and urea production

Dietary nitrogen incorporation into body urea (Figure 12) increased during the first 3 hours and reached a quasi-plateau from 3 to 5 hours following the ingestion of the lamb hydrolysate meal, peaking at 10.5 ± 1.0 % of the ingested nitrogen and then declining slowly for the last three hours to 7.8 ± 0.8 % of the ingested nitrogen. Dietary nitrogen from the casein meal was transferred to the body urea pool more slowly to reach a maximum of 8.3 ± 1.3 % at four hours and then declined to 6.7 ± 0.9 % of the ingested nitrogen at 8 hours. The level of dietary nitrogen recovered in the body urea pool was not different between the two meals at 8 hours ($p > 0.05$).

The amount of dietary nitrogen excreted in the urine in the form of urea and ammonia was not different between the lamb hydrolysate and the casein meals at 8 hours (5.5 ± 0.9 and 5.8 ± 0.9 %, $p > 0.05$; 0.2 ± 0.03 and 0.3 ± 0.1 %, $p > 0.05$ respectively).

Postprandial production rates of total, dietary-derived and endogenous-derived urea were computed for the four 2-hour periods following meal consumption and are presented in Figure 13.

Total overall urea production (A) was not different following the consumption of either meal with the rate of urea production being highest during the first four hours. Urea production of direct dietary origin (B) was similar between the meals for the first six hours but was significantly less between 6 – 8 hours for the lamb
5 hydrolysate meal (0.001 ± 0.001 mmol N/kg body weight). The endogenous urea production (C) was not different between the two meals over the 8 hour collection period.

Postprandial nitrogen retention and biological value

The metabolic utilization of dietary nitrogen after the ingestion of the lamb
10 hydrolysate meal was characterised by losses of dietary nitrogen not retained after 8 hours of 15.1 % (1.6 % ileal losses and 13.5 ± 1.3 % deamination losses) and by a NPPU of 84.5 ± 1.4 %. The metabolic utilization of dietary nitrogen after the ingestion of the casein meal was characterised by losses of 18.9 % (5.9 % ileal losses and 13.0 ± 1.3 % deamination losses) and by a NPPU of 74.8 ± 1.3 %. The
15 NPPU of the lamb hydrolysate was significantly higher ($p < 0.05$) than that of casein. PBV representing the retention of absorbed nitrogen reached 86.2 ± 1.5 and 85.2 ± 1.5 % for the lamb and casein meals respectively and was not significantly different between the two. Cumulated total (dietary and endogenous) nitrogen losses were significantly less following the lamb hydrolysate meal ($15.5 \pm$
20 1.4 % or 49.6 mmol N / 8h) compared to the losses following the casein meal (25.2 ± 1.3 or 80.64 mmol N / 8h).

Summary of the bioavailability and postprandial metabolic utilization of dietary nitrogen 8 h after ingestion of a single mixed meal containing hydrolysed lamb or casein in older adult humans is as listed in Table 15

Table 15: Comparison of bioavailability and postprandial metabolic utilization of dietary nitrogen 8 h after ingestion of a single mixed meal containing hydrolyzed lamb or casein in older adult humans.

	Percentage of ingested nitrogen		Significance ¹
	Hydrolysed lamb	Casein	
Ileal losses of dietary nitrogen	1.6 ²	5.9 ³	
Real ileal digestibility	98.4 ²	94.1 ³	
Deamination of dietary N in			
Body urea	7.8 ± 0.8	6.7 ± 0.9	NS
Urinary urea and ammonia	5.8 ± 0.9	6.3 ± 0.7	NS
Total loss of dietary N at 8 h	15.5 ± 1.4	25.2 ± 1.3	S
Net postprandial protein utilization	84.5 ± 1.4	74.8 ± 1.3	S
Postprandial biological value	86.2 ± 1.5	85.2 ± 1.5	NS

¹Statistical significance; NS = not significant, S = significant

5 ²Value determined in pigs (Awati *et al.* 2008)

³Value determined in humans (Deglaire *et al.* 2007)

Discussion and conclusions

During the postprandial phase, nitrogen and amino acids of dietary origin are submitted to sequential metabolic processes including gastrointestinal digestion and amino acid absorption, amino acid deamination, subsequent transfer to ammonia and urea or incorporation into organs. Labelling the dietary protein with ¹⁵N made it possible to follow the metabolic fate of the dietary nitrogen and

determine the postprandial nitrogen utilization of a lamb meat hydrolysate by measuring dietary nitrogen intake and absorption in older adults.

The digestive stage of protein utilization was previously determined in our laboratory using two animal models suitable for studying amino acid digestion.

5 Rats and pigs are often used as models for determining true ileal digestibility of proteins. The pig is a validated animal model for the determination of protein digestibility to the end of the small intestine for humans (Moughan & Rowan 1989, Rowan *et al.* 1994). It is appropriate to determine digestibility at the end of the small intestine because the digestion of protein and subsequent absorption of
10 amino acids occur mainly in the upper small intestine and are effectively completed by the end of the ileum (Moughan *et al.* 2005). The calculated true ileal digestibility of the lamb hydrolysate amounted to 97.2 ± 1.1 using the rat model and $98.4 \pm 0.8\%$ using the pig model. These values are high particularly when compared to the true ileal digestibility of milk and plant proteins demonstrating that the amino
15 acids in the meat hydrolysate are absorbed almost completely anterior to the end of the small intestine.

True ileal digestibility of lamb meat hydrolysate determined in a rat model and a pig model is listed in Table 15 True ileal digestibility (TID), postprandial biological value (PBV), net postprandial protein utilization (NPPU) values for various proteins are
20 compared in Table 16

Table: 16: True ileal digestibilities ($\pm$ SE) of lamb meat hydrolysate determined in a rat model and a pig mode:

Amino Acid	True ileal digestibility (%)	
	Rat model	Pig model
Aspartic Acid	94 ± 0.6	96.9 ± 1.7

Threonine	98 ± 0.7	101.3 ± 1.9
Serine	102 ± 0.7	100.7 ± 2.2
Glutamic Acid	92 ± 0.7	96.2 ± 1.9
Proline	94 ± 0.7	98.8 ± 6.3
Glycine	86 ± 2.8	87.1 ± 6.0
Alanine	97 ± 0.5	99.1 ± 1.3
Valine	100 ± 0.4	99.9 ± 1.3
Methionine	104 ± 0.6	99.5 ± 0.7
Isoleucine	101 ± 0.5	100.8 ± 1.0
Leucine	101 ± 0.4	100.0 ± 1.2
Tyrosine	100 ± 0.5	99.4 ± 1.3
Phenylalanine	102 ± 0.4	99.1 ± 1.4
Histidine	92 ± 0.9	98.0 ± 1.4
Lysine	97 ± 0.4	98.4 ± 1.2
Arginine	99 ± 0.5	99.8 ± 1.1
Cysteine	94 ± 1.0	
True ileal digestibility	97.2 ± 1.1 %	98.4 ± 0.8 %

Table 16: True ileal digestibility (TID), postprandial biological value (PBV), net postprandial protein utilization (NPPU) values for various proteins.

Meat source	TID (%)	PBV (%)	NPPU (%)	Reference
Pea	90.0	79.0	71.0	Mariotti <i>et al.</i> 2001
Lupin	91.0	81.5	74.0	Mariotti <i>et al.</i> 2002
Soy	91.5	80.0	73.5	Bos <i>et al.</i> 2003, Gaudichon <i>et al.</i> 2002

Wheat	91.5	73.0	66.0	Bos <i>et al.</i> 2005
Rapeseed	84.0	84.0	70.5	Bos <i>et al.</i> 2007
Milk	95.0	82.0	78.0	Bos <i>et al.</i> 2003, Gaudichon <i>et al.</i> 2002
Casein intact	94.1	79.0	74.4	Deglaire <i>et al.</i> 2007
Casein hydrolysate	92.3	81.2	74.0	Deglaire <i>et al.</i> 2007
Lamb hydrolysate	98.4	86.2	84.5	This study
Casein intact	94.1	85.2	74.8	This study

Data from all studies except for the current study were performed in young, healthy volunteers (age < 40 years).

The sharp appearance of dietary nitrogen in the serum following the lamb hydrolysate meal induced a transient higher deamination rate and a transient faster incorporation of dietary nitrogen into liver exported protein compared with the casein meal. This is in line with previous observations for intact casein which is known to clot at acidic pH in the stomach and hence has a likely slower gastric emptying (Mahe *et al.* 1996). Ingestion of the lamb hydrolysate resulted in remarkably little deamination of dietary nitrogen (13.5 % of the ingested dose 8 h after the meal) indicating that once absorbed the catabolism of amino acids derived from the diet was minimal.

NPPU is an appropriate measure of the nutritional value of a protein as it takes into account both bioavailability and the efficiency of the utilization of protein nitrogen. In this context the NPPU method allows for the discrimination of nutritional quality between proteins. In this study the NPPU for the lamb hydrolysate was 84.5 %. In

previous studies using the same experimental design as in the present experiment the NPPU of both plant and milk proteins were considerably less (Table 16). The difference in protein quality of 10.0 % between the lamb hydrolysate and casein was highly significant as this difference was not obvious when ileal digestibility or
5 postprandial biological values were compared.

In conclusion these results show that a lamb meat hydrolysate has a high digestibility and given as part of a balanced meal to healthy older adult humans can be used efficiently for postprandial protein deposition without promoting specific endogenous nitrogen losses either in the ileum or through protein catabolism.

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- Aspects of the present invention have been described by way of example only and
it should be appreciated that modifications and additions may be made thereto
15 without departing from the scope of the appended claims thereof.

WHAT WE CLAIM IS:

1. A hydrolysate composition isolated from a meat source characterised in that, the hydrolysate contains 60 to 90% w/w protein which has a moisture level of below 20%.
- 5 2. A hydrolysate composition as claimed in claim 1 wherein the hydrolysate contains 83-88% w/w protein.
3. A hydrolysate composition as claimed in either claim 1 or claim 2 wherein the level of hydrolysis is 80% or greater.
4. A hydrolysate composition as claimed in any one of claims 1 to 3 wherein
10 70-90% of the peptides in the hydrolysate composition have a molecular weight of less than 1000 Daltons.
5. A hydrolysate composition as claimed in any one of claims 1 to 4 wherein 78% or more of the peptides in a hydrolysate are less than ten amino acids in length.
- 15 6. A hydrolysate composition as claimed in any one of claims 1 to 5 wherein the hydrolysate composition includes between 0.1 to 10% fat w/w.
7. A hydrolysate composition as claimed in claim 6 wherein the composition contains approximately 0.4% fat w/w.
- 8.. A hydrolysate composition as claimed in any one of claims 1 to 7 which has
20 moisture level of between 3 to 7%.
9. A hydrolysate composition as claimed in any one of claims 1 to 8 which has a pH level of 6 to 9%.
10. A hydrolysate composition as claimed in claim 9 which has a pH level in the order of 6.3%.
- 25 11. A hydrolysate composition as claimed in any one of claims 1 to 10 which has a solubility level in the order of 60 to 100%.

12. A hydrolysate composition as claimed in claim 11 which has solubility level in the order of 87%.
13. A hydrolysate composition substantially as herein described and illustrated by the examples in the Best Modes section excluding the prior art disclosed within the specification.
14. A method of preparing a hydrolysate composition substantially as herein described and illustrated by the examples in the Best Modes section excluding the prior art disclosed within the specification.

FIGURE 1

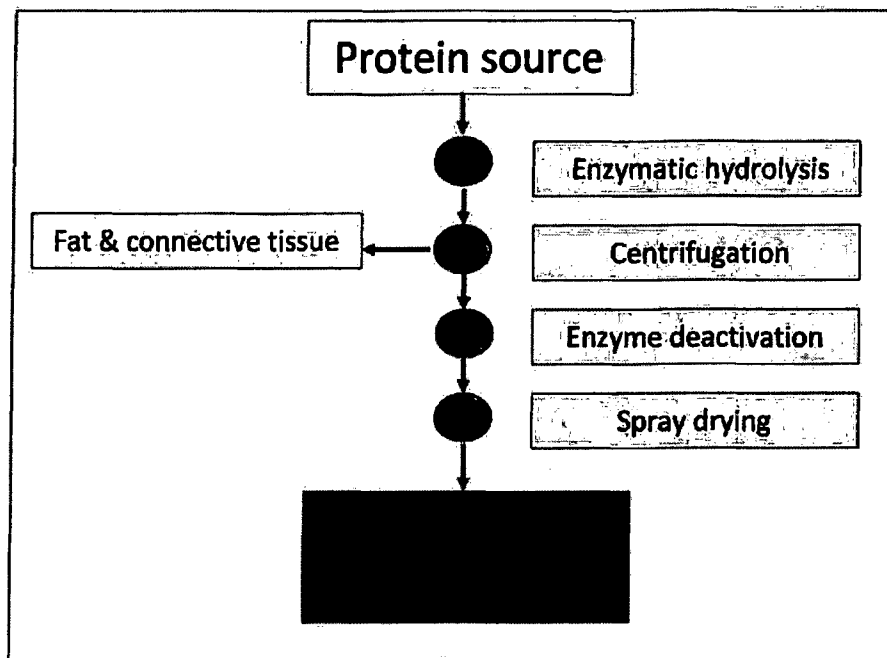


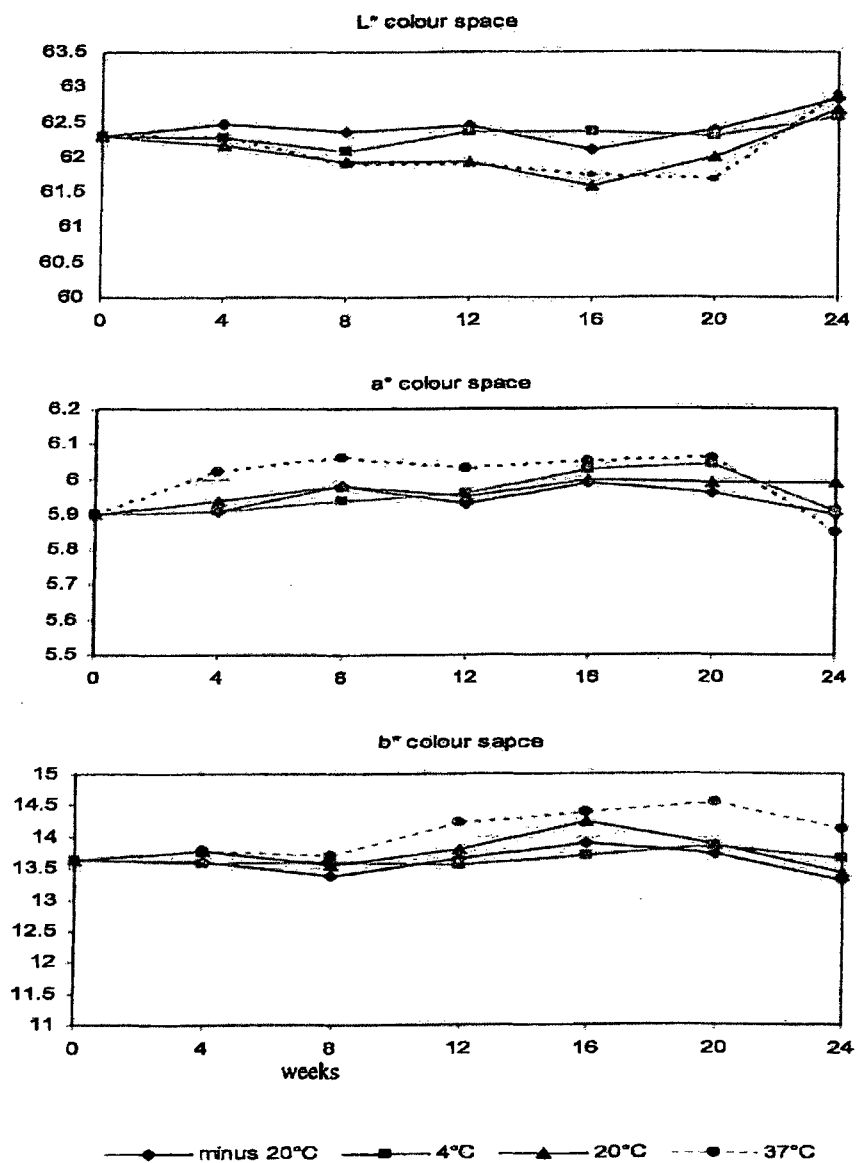
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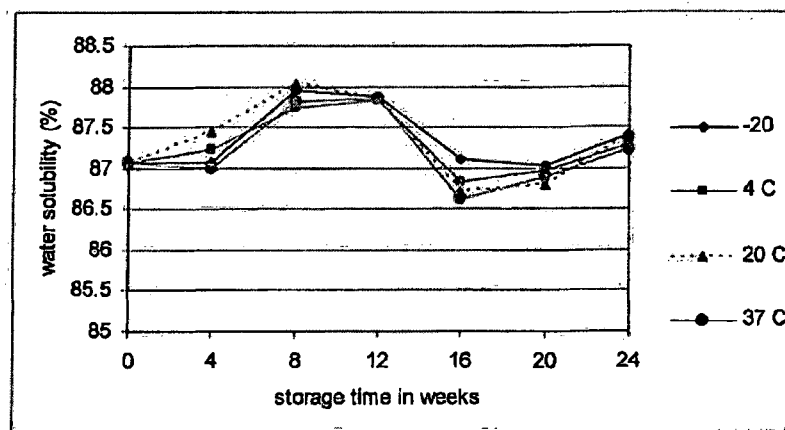
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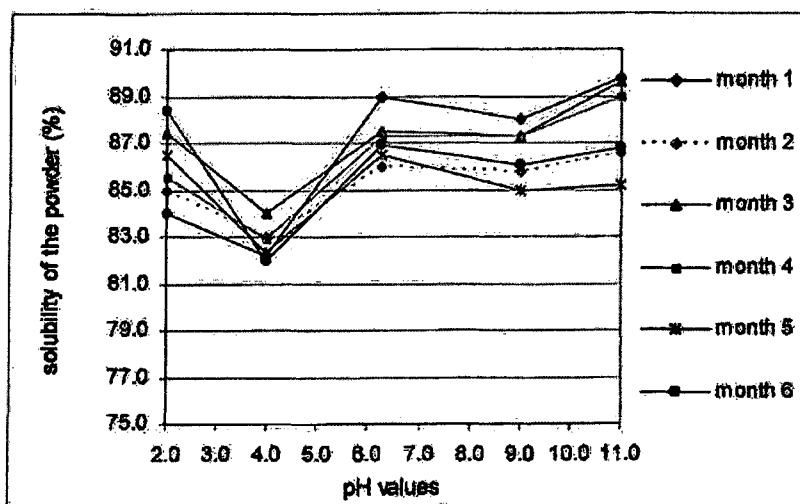
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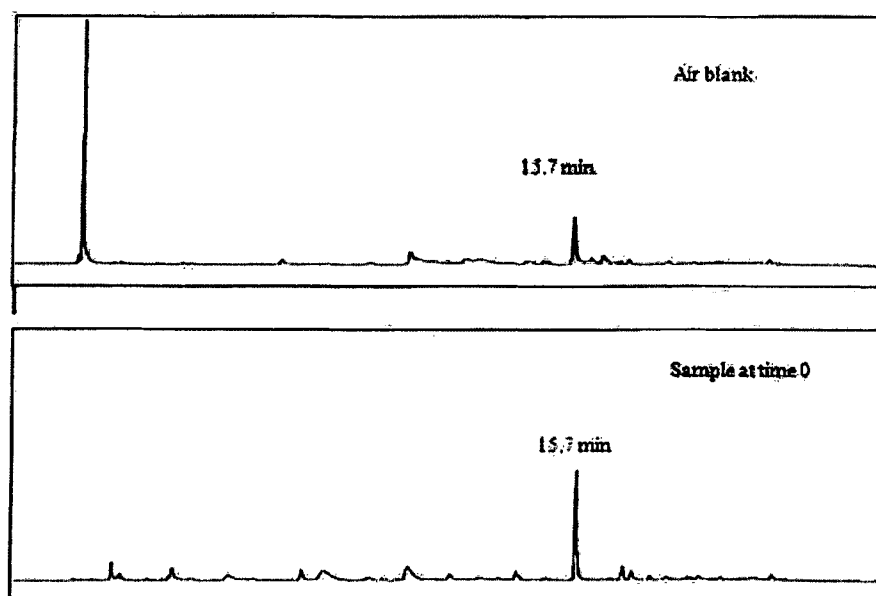
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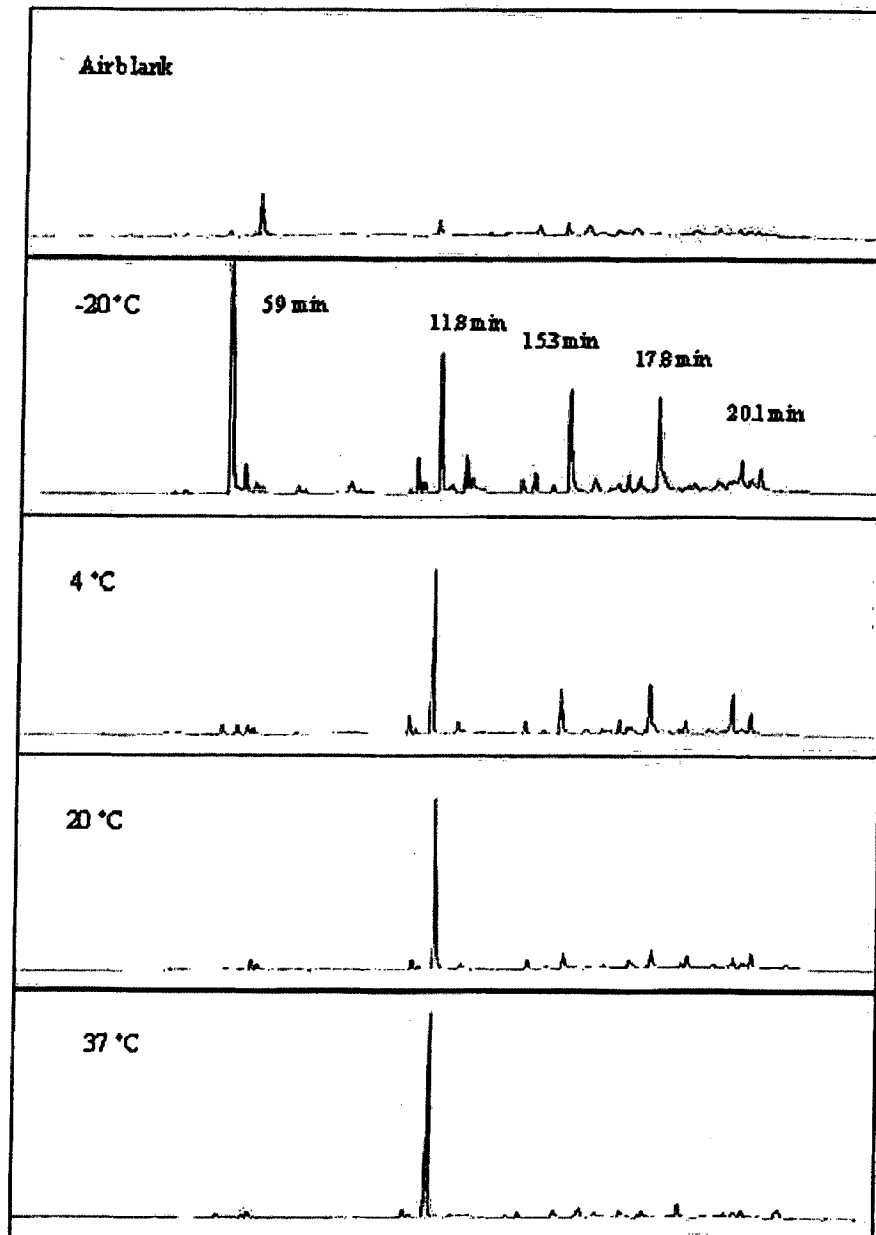
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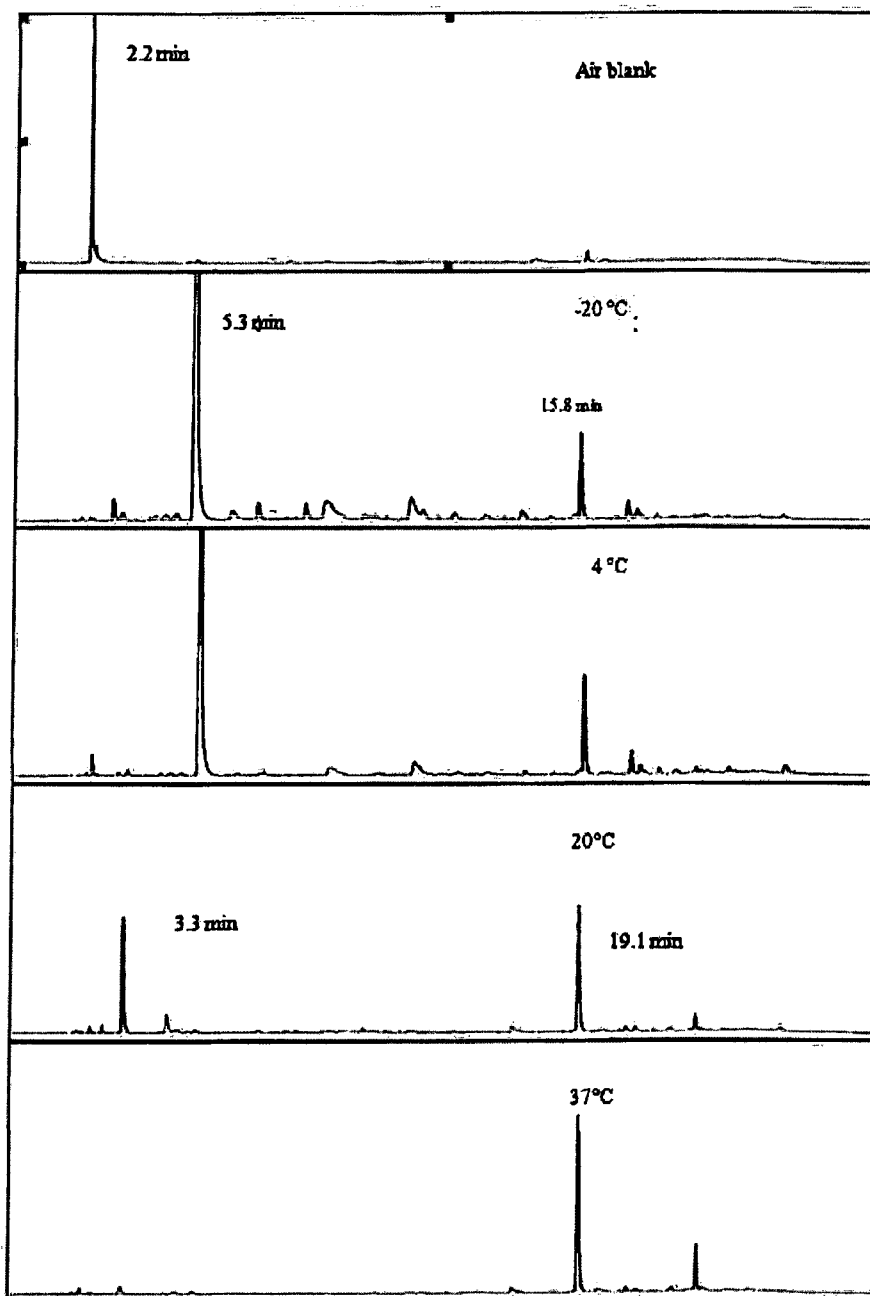
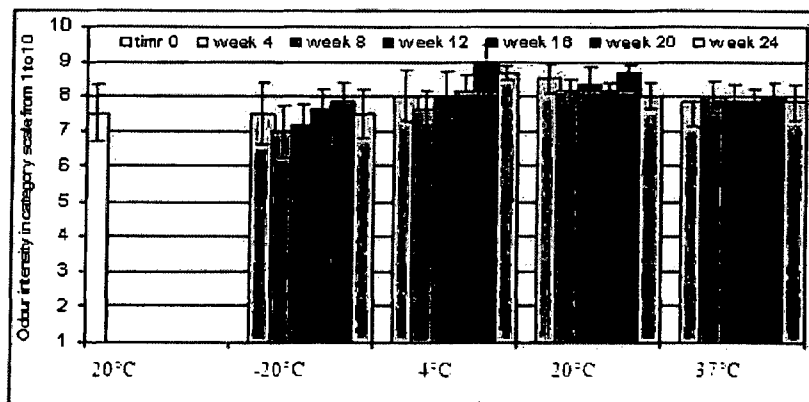
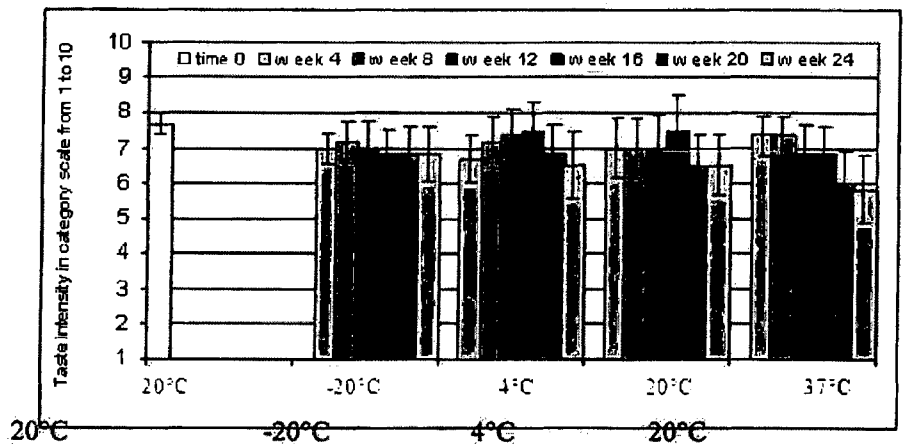
Figure 7

Figure 8



(A)



(B)

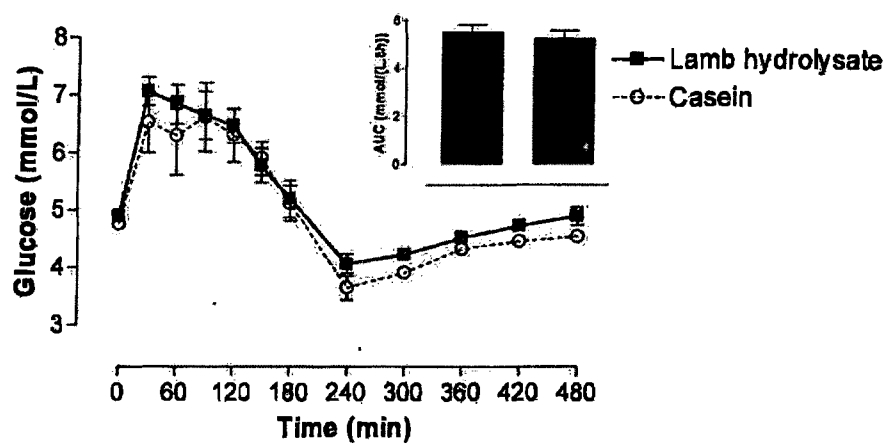
Figure 9

Figure 10

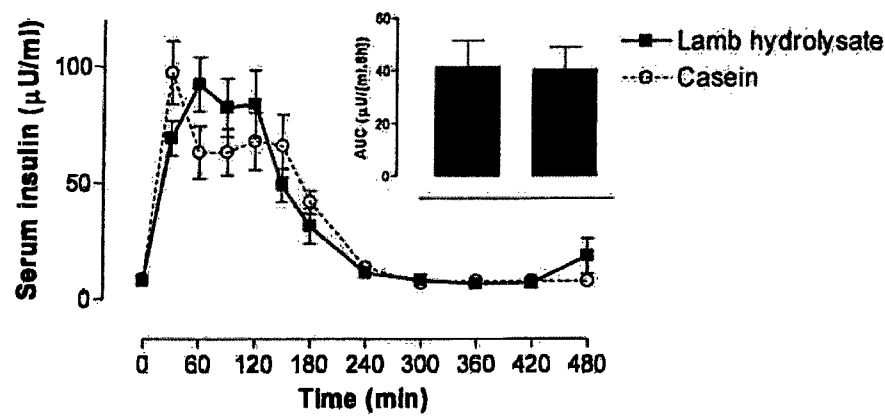


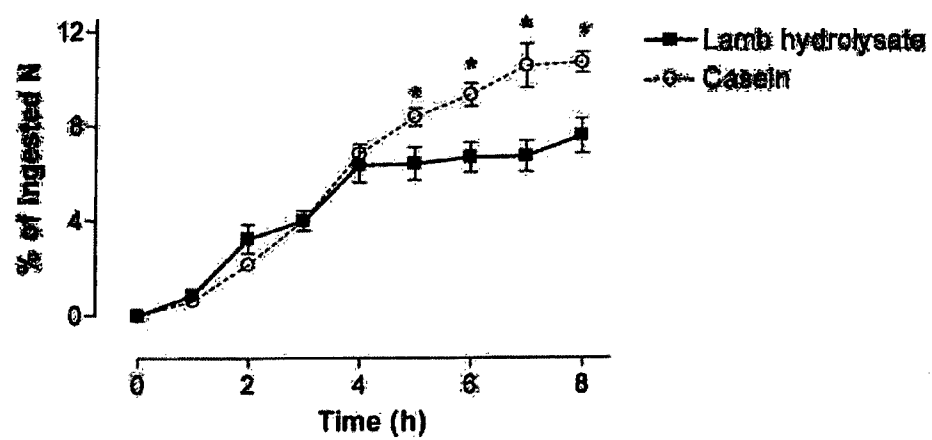
Figure 11

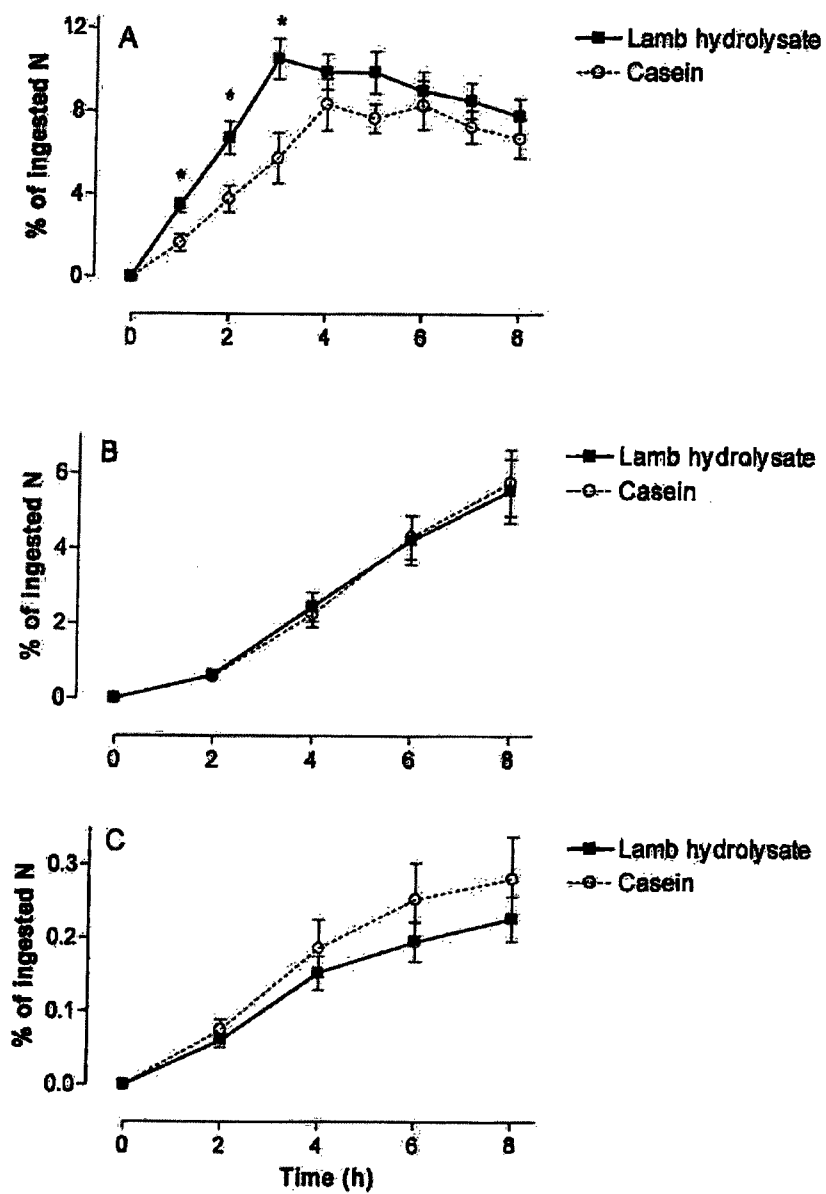
Figure 12

Figure 13

