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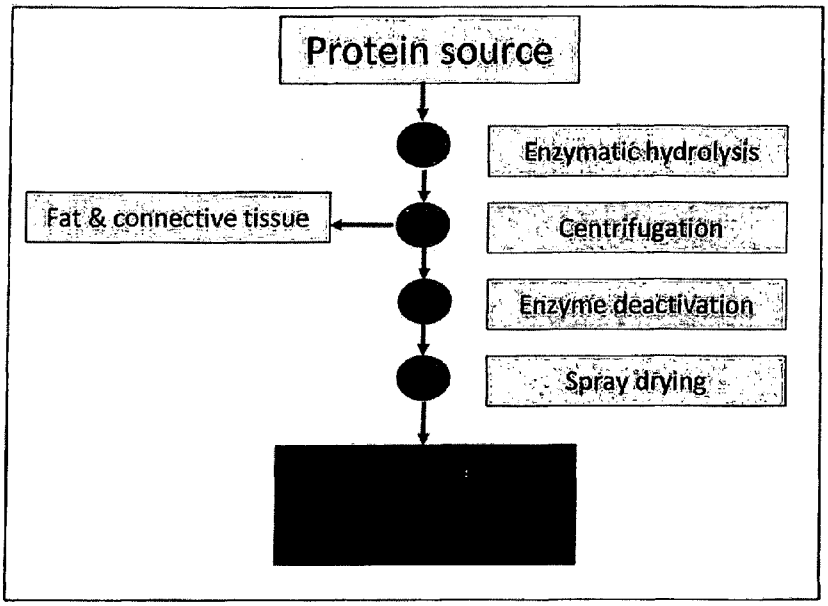
(54) Title: USE OF EDIBLE COMPOSITION

FIGURE 1



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WO 2012/121613 A2

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(57) Abstract: This invention relates to the use of a hydrolysate composition isolated from a meat source and containing 60 to 90% w/w protein, in the manufacture of a medicament, to treat amino acid malnourishment or an associated condition in an animal.

USE OF EDIBLE COMPOSITION

TECHNICAL FIELD

This invention relates to the use(s) of an edible composition.

BACKGROUND ART

The present invention relates particularly to edible compositions in the form of hydrolysates which are hydrolysed biomatter 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, weight control or treating malnutrition 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 demands in these industries have resulted in an increased price of these commodities. This can lead to an increased cost of the product for the end-consumer.

Furthermore, the nutrients (e.g. amino acids) available from these sources often do not effectively match the nutrient requirements of the human body. This is particularly so in the 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 amount 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 undesirable food components (e.g. fat, salt, sugars, flavourings or preservatives) can also be consumed, potentially leading to their side effects. .

Some nutrients (e.g. essential amino acids) that are particularly advantageous to humans are not well provided by some of the currently available hydrolysates (e.g. those from vegetable sources). Amino acids play an important role in human body by acting as building blocks for tissues. Besides, 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 nutritional value of the final product.

In addition, 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 upon consumption by the user. Furthermore, many hydrolysates can have undesirable flavour profiles. Often the flavour level can be overbearingly salty or bitter, or the hydrolysate is bland. In order to overcome unpalatable flavours of the hydrolysates, synthetic flavouring is often added. This can be a further disadvantage as people prefer natural products lacking synthetic additives.

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 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 mistrust in 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.

Often, nutrients within the hydrolysates can be poorly absorbed by the body, or are absorbed at the inappropriate position 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 is a major concern 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 different amino acid positions, the choice of enzymes utilised depends on the level of hydrolysis and amino acid profile required in the hydrolysate.

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 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. hydrolysis 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 unwanted components. This can take up valuable time, equipment and/or can lead to a reduction of nutrients lost in the additional hydrolysis step

It is therefore important to improve the efficiency of preparing the hydrolysate, 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 all other purposes. It has been a goal to develop a well-rounded hydrolysate that may 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 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 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.

DISCLOSURE OF THE INVENTION

According to the present invention there is provided the use of a hydrolysate composition isolated from a meat source and containing 60 – 90% w/w protein, in the manufacture of a medicament, to treat amino acid malnourishment or an associated condition in an animal.

According to a further aspect of the present invention there is provided a method of treating an amino acid malnourishment or an associated condition in an animal using a hydrolysate composition,

wherein the hydrolysate composition is isolated from a meat source and the hydrolysate composition contains 60-90% w/w protein.

Preferably the hydrolysate composition is provided in a form wherein in the order of 30 g of protein can be consumed in a single time added into any serving size of food material.

According to a further aspect of the present invention there is provided a method of using a hydrolysate composition, isolated from a meat source and containing 60 – 90 % w/w protein,

characterised by the step of providing the hydrolysate composition in a nutritional

supplement to an animal.

According to a further aspect of the present invention there is provided a method of using a hydrolysate composition,

wherein the hydrolysate composition is isolated from a meat source and containing 60 – 90 % w/w protein;

characterised by the step of

providing the hydrolysate composition in a medicament for the treatment of an amino acid malnourishment or an associated condition in an animal.

According to a further aspect of the present invention there is provided a hydrolysate composition isolated from a meat source and containing 60 – 90% w/w protein,

for the treatment of amino acid malnourishment or an associated condition in an animal.

A method of improving flavouring of a food stuff characterised by the step of

adding to the food stuff a hydrolysate composition wherein the hydrolysate composition is isolated from a meat source and;

wherein the hydrolysate composition 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 utilisation. The latter is determined by digestion and absorption processes in the small intestine. The digestion and absorption processes are affected by many factors which include changes in taste and smell,

poor oral health resulting in difficulties with chewing and/or swallowing, decreased appetite and reduced food intake. This reduced food intake is an important concern 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 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, a current method 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).

In preferred embodiments, the nutritional supplement is geared at boosting health, promoting muscle growth and enhancing wellbeing. For example, the inventors envision that the present hydrolysate could be used for body builders, athletes and individuals with special requirements like the elderly and pregnant woman.

Throughout this specification the term amino acid malnourishment should be taken as meaning any disease or condition in the animal body which leads to a lower than acceptable level of at least one amino acid used in a human or other animal.

The amino acid malnourishment or an associated disorder may more easily affect the people with reduced immunity like those suffering from cancer, conditions associated with elderly population, starvation or poor diet in impoverished countries, AIDS patients, or post operative patients.

Preferably, the composition or medicament of the present invention is provided in a soup, savory snack, cereal bar, drink, or any other form of food or nutraceutical easily consumed by the human or other animal. The inventors acknowledge that alternative forms of delivery could be provided including a bolus or injectable material.

High Protein content

A novel feature of the hydrolysate is that it includes a protein level of between 60 and 90 % w/w. The inventors have identified many sources from meat which are able to provide this level of protein for human consumption.

Preferably the meat source is mechanically separated (MS) meat from lamb, however the source of meat is not restricted to MS meat, nor just lamb and can include any form of meat. The inventors acknowledge that different meat sources may have different amino acid profiles which are geared towards treating alternative conditions or diseases.

MS meat is generally not used for consumption by humans because of its poor flavour and texture characteristics can be utilized to produce a protein hydrolysate which has superior flavour profile and is rich in amino acids. The current invention thus helps to provide added value to the MS meat (e.g. the inventors have found they may convert a by-product costing 80 cents/kg MS meat into a product which may be sold for $25/kg hydrolysate)

For example, different meat sources may be used, such as cattle or sheep. Furthermore, different parts of an animal may be used. Each component may have variations in the amino acid profile, which may be tailored to a given condition to be treated. The hydrolysate of the present invention may still maintain a high protein level (60-90%), however subtle differences in amino acid balance may be altered to suit different needs by using different meat sources.

Amino acid profile

One advantage of utilising a meat source for the protein is that the amino acid profile of the resulting hydrolysate may be well matched to the requirements of human growth and development, and overall well being e.g. vegetable sources like cereals are deficient in sulphur containing amino acids. Other types of hydrolysates e.g.those from vegetable sources may have high levels of proteins upwards of 90% w/w. However due to the composition of the starting material, the amino acid profile may not be well balanced to match the requirements of humans and other animals.

Even if one amino acid is at low levels in the composition, and hence the absorption into the body, it may affect protein synthesis in the human body which requires a range amino acids.

This can mean that recipients receiving currently available hydrolysates may not be able to effectively synthesize proteins in body. This may result in many of the disorders associated with amino acid malnurishment. Similarly, in humans which are undergoing rapid growth and development, such as in body building or pregnancy, it may be necessary to have excess amounts of each type of amino acid at appropriate levels for such growth.

It is especially important that the essential amino acids are included in our diet. If the recipient is malnourished and is not receiving adequate amounts of these essential amino acids, many complications can arise. As such, the present hydrolysate may provide these essential amino acids in appropriate amounts, which may prevent or treat the complications. This is why an animal e.g. meat source is particularly advantageous over non-meat sources like vegetable sources of protein hydrolysates.

Absorption/Digestability

A further important feature of a hydrolysate is its level of absorption and digestibility.

Protein hydrolysates typically have undergone enzymatic digestion of the protein from the sourced material. The hydrolysate of the present invention preferably has undergone extensive hydrolysis such that the average polypeptide is only two amino acids in length (discussed further in the best modes). This helps to ensure that the hydrolysate may be highly absorbed after consumption. In cases where proteins are poorly digested by the human body e.g. due to reduced diet intake in elderly, having a highly absorbable hydrolysate may be particularly advantageous.

The inventors consider it may be particularly advantageous to have highly hydrolysed protein in the hydrolysate. This means that a large percentage of the peptide links are severed by the hydrolysis to result in a mixture of small peptides or individual amino acids.

To improve absorption characteristics, the inventors consider it may be beneficial to:

- have more than 40% digestion of the peptide links in the hydrolysate,
- have a high proportion of di- or tri- peptides or single amino acids in the hydrolysate,
- have approximately 78% or more of the peptides, which are less than 10 amino acids in length and/or

have the average size of the peptide to be two amino acids in length.

The inventors have found that the hydrolysate from a meat source in accordance with the present invention is highly absorbed in the gastrointestinal track of pigs

and mice. Studies showed that the true ileal digestibility is up to 98%. Furthermore, each type of amino acid is highly absorbable ensuring that each type of amino acid is effectively utilized by the human body. The inventors expect similar results in other animals including humans.

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 even between individual milk proteins, for example beta-lactoglobulin and casein. The true Ilial digestibilities for different protein sources studied include, 94.1% for Casein, 91.5 % for soy, 95% for milk protein (casein and whey proteins), 92.3% for casein hydrolysate, where as the true ilial digestibility for lamb protein hydrolysate in accordance with the present invention was found to be 98.4%.

The high digestible amino acid content indicates that the hydrolysate of the present invention 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 muscle mass.

Therefore the inventor's data also indicates that the absorption level of the present hydrolysate sourced from MS meat is exceptionally better than hydrolysates sourced from vegetable sources.

Palatability

Many hydrolysates currently available can have a relatively high level of fat. This can often be due to difficulty in removing the fats from the protein source.

The present invention is particularly advantageous as the fats may be effectively removed without losing the good palatability characteristics of the meat hydrolysate. Also, the fats may be selectively removed while maintaining a high protein level and an excellent amino acid profile.

Hydrolysates sourced from vegetable sources for example are claimed to be high in protein content, low in fat content, and be palatable – similar to the features in the present invention. However, a disadvantage of hydrolysates sourced vegetable sources is they do not have an amino acid profile that closely matches human requirements, i.e. meat.

In summary, some of the advantages and uses of the hydrolysate include:

- Treatment of those malnourished and/or have underlying deficiency in protein/amino acid levels (e.g in third world regions);
- Treatment of those susceptible to infection (due to unbalanced amino acid profiles);
- Treatment of those with disorders which may otherwise lead to loss of protein/muscle (e.g muscle wastage);
- Treatment/supplement for those who do not wish to, or cannot eat large amounts of solid protein yet require amino acid supplements in small volumes (e.g. the elderly or those in rest homes, or those recovering or convalescing from illness);
- Providing a high level protein source (with excellent amino acid profile) that is palatable without the need to retain or add components for improved flavour;

- preferably low fat;
- The preferred hydrolysate sourced from NZ meat is a verified source of protein (e.g. body builders or infant formulators);
- represents a natural and/or organic supplement;
- the ability to differentiate the product from those of competitors (e.g. by providing a "functional soup").
- Disclose the invention, as claimed, in such terms that the technical problem (even if not expressly stated as such) and its solution can be understood, and state the advantageous effects, if any, of the invention with reference to the background art.

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:

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 at four different temperatures for a period of 24 weeks.

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 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;

Figure 10 Serum insulin concentrations after the ingestion of a mixed meal containing ^{15}N-labelled lamb hydrolysate or casein in older adult humans;

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;

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;

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.

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 can be an indicator 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%.

The hydrolysate has a degree of hydrolysis in the order of 40%. 90% of the protein in the hydrolysate has a molecular weight of less than 1000 Daltons. Furthermore, 90% 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. However this should not be considered limiting – the applicant acknowledges that other means of providing the hydrolysate may include a slurry, tablet, injecteable liquid or drink, bolus, etc or any other form of food or nutraceutical.

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	

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 is heated to 95°C to deactivate the enzymes.

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

Steam

De-ionised water

Chilled operating room, meeting export standards.

Freezer for storing meat in cartons

Processing Technique

Hydrolysis:

- The minced MS lamb meat was suspended in an equal volume of deionised water.
- The temperature of the mixture was raised to 45°C $\pm$ 2°C and held at that temperature for the duration of the hydrolysis.

- 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 – 6.05 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.

- 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. 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 temperature was controlled at 80°C while the 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 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 %
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
	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

	5%	MW > 10,000 Da
Microbiologic test:	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

Fat analysis

Soxhlet assay (AOAC 991.36)

Total lysine / Available lysine

A procedure described by Moughan and Rutherfurd (1996)

Water solubility measurement:

The measurement is based on a modified AOAC (950.81) method. Approximately 1 g (Ws) 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 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

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 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%. 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.

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

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

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, Rutherfurd 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 the Microbiological Examination of Food. American Public Health Association (APHA), Washington DC, USA.

EXAMPLE 4: Provision of Hydrolysate in a Soup Mix

Flour 2g

Firmtex 2g (modified starch)

SMP	1g
Vege fat	1g
Salt	0.5g
Beef Stock	3g
Beef flavour	1g
Onion Powder	0.8g
Colour	0.06g
Hydrolysate	4.8g
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 numbers (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 ≤ 20°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.

Methods and materials

Source of MS lamb meat and Meat hydrolysate

MS lamb meat was purchased from a New Zealand supplier.

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.

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 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 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 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 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.

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

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 Supelcowax™ 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 Rutherfurd (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 to assess microbial numbers 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 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 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

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 and the results are shown in Table 9, Table 10 and Table 11. As can be seen from 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

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 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 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.

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. 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
Phenylanine	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 deceased 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 probably has 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 the profile of air used as control. 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 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 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

timeframe, except for the -20°C sample at 5.9 min. The volatile components at 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 would appear that both, the number of peaks and height of peaks in the chromatogram 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.

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. At the elution times of 15.8 min and 19.1 min, the two component levels both increased with storage temperatures, making up 7.8%, 14.4%, 40.2% and 79.6% of their respective total components for the samples stored at -20°C, 4°C, 20°C to 37°C. 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 may suggest minor loss of flavour components with increase in 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% to 58.6 % for the samples stored at - 20°C to 37°C by the end of the trial. The respective losses of available lysine were 27.7%, 24.6%, 27.0% and 33.9%. In summary, available lysine decreased with both storage time and storage temperature with storage time having the greater impact on the levels.

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 exact CFU (colony forming unit) count 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 CFU numbers 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 decreased CFU number whilst the samples stored at -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

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	
4°C week 24	0.3032	1.67	0	275	315 ± 49.2

		0.2419	2	0	413		
		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.

** 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 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 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.

- 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 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 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 smelled more oily and meaty than the rest of the samples while the sample stored at 37°C smelled somewhat fishy, 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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EXAMPLE 6: Preliminary Human Metabolic Study

Abstract

The purpose of this project was to evaluate the quality of a hydrolysed meat protein 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 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 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.

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 lamb protein, uniformly and intrinsically labelled with ^{15}N. The source of protein was either lamb 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 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 isonitrogenous providing 320 mmol of nitrogen.

Each meal was prepared immediately before serving. ^{15}N-labelled lamb meat hydrolysate (36 g) or ^{15}N-labelled casein (33 g) was added to maltodextrin (100 g; 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.

Protocol

The volunteers were randomised 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 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. Serum insulin was measured using a double antibody radioimmunoassay method. Serum urea, urinary creatinine and urinary urea were assayed using an enzymatic method. Urinary ammonia was measured using an enzymatic method using glutamate dehydrogenase.

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). 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). 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). After centrifugation (2400 g, 25 min, 4 °C) the pellet containing the 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. Before isotopic determination resins were eluted with $KHSO_4$

(2.5 mol/L). The ^{15}N:^{14}N isotope ratio was determined by isotope-ratio mass spectrometry (Stable Isotopes Laboratory, GNS Science) 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 ($N_{exo\text{-}pool}$, % of ingested nitrogen) was evaluated for each group using the following general equation:

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

where $N_{tot\text{-}pool}$ 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). $N_{exo\text{-}pool}$ was expressed as % of nitrogen ingested. For urinary urea 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 water (TBW) and according to the following equation:

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

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).

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:

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

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

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

$$PBV = (NPPU / TID) \times 100$$

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

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.

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 ± 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 15: Anthropometric measurements for study volunteers.

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±0.3	73.9±0.6	23.5±0.5	50.4±0.4	25.7±0.2	36.1±0.3
Casein	5M/4F	69.1±0.7	72.0±1.3	19.3±1.3	52.7±1.0	24.2±0.4	36.0±0.5

Values are shown as mean ± 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.

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 ($p > 0.05$).

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 μU/ml) and 60 minutes postprandially for the lamb hydrolysate meal (89.7 ± 11.9 μU/ml). 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 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 ± 09 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 14.

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 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 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 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 ± 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 16

Table 16: Comparison of bioavailability and postprandial metabolic utilization of, dietary nitrogen 8 h after ingestion of a single mixed meal containing hydrolyzed lamb protein 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

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

[2]Value determined in pigs (Awati *et al.*2008 unpublished results)

[3]Value 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 ^{15}N 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. 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 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 ± 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 acids in the meat hydrolysate are absorbed almost completely anterior to the end of the small intestine.

True ileal digestibility (± SE) of lamb meat hydrolysate determined in a rat model and a pig model is listed in Table 17 True ileal digestibility (TID), postprandial

biological value (PBV), net postprandial protein utilization (NPPU) values for various proteins are compared in Table 18.

Table: 17:True Ileal digestibilities of lamb meat hydrolysate determined in a rat model and a pig model:

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: 18: 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 *et al.* 2001
Lupin	91.0	81.5	74.0	Mariotti *et al.* 2002
Soy	91.5	80.0	73.5	Bos *et al.* 2003, Gaudichon *et al.* 2002
Wheat	91.5	73.0	66.0	Bos *et al.* 2005
Rapeseed	84.0	84.0	70.5	Bos *et al.* 2007
Milk	95.0	82.0	78.0	Bos *et al.* 2003, Gaudichon *et al.* 2002
Casein intact	94.1	79.0	74.4	Deglaire *et al.* 2007
Casein hydrolysate	92.3	81.2	74.0	Deglaire *et al.* 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 know to clot 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 lower than the meat hydrolysate (Table 18). 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 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 without departing from the scope of the appended claims.

WHAT WE CLAIM IS:

1. The use of a hydrolysate composition isolated from a meat source and containing 60 to 90% w/w protein,

   in the manufacture of a medicament, to treat amino acid malnourishment or an associated condition in an animal.

2. A use as claimed in claim 1 wherein the hydrolysate composition is in a form wherein in the order of 30 grams of protein can be added to a serving size.

3. A use as claimed in either claim 1 or claim 2 wherein the meat source is mechanically separated meat.

4. A use as claimed in any one of claims 1 to 3 wherein the meat source is lamb.

5. A use as claimed in any one of claims 1 to 4 wherein the degree of hydrolysis of the composition is in the order of 40%.

6. A use as claimed in any one of claims 1 to 5 wherein the composition has approximately 78% or more of the peptides less than 10 amino acids in length.

7. A use as claimed in any one of claims 1 to 6 wherein 90% of the peptides in the hydrolysate composition are less than 10 but have molecular weight of less than 1000 Daltons.

8. A method of treating an animal for malnourishment characterised by the step of using a hydrolysate composition manufactured according to any one of claims 1 to 7.

9. A method of treating an animal for susceptible to infection characterised by the step of using a hydrolysate composition manufactured according to any one of claims 1 to 7.

10. A method of treating an animal for muscle wastage characterised by the step of using a hydrolysate composition manufactured according to any one of claims 1 to 7.

11. A method of treating an amino acid malnourishment or an associated condition in an animal using a hydrolysate composition,

wherein the hydrolysate composition is isolated from a meat source and the hydrolysate composition contains 60-90% w/w protein.

12. A method of using a hydrolysate composition, isolated from a meat source and containing 60 – 90 % w/w protein,

characterised by the step of providing the hydrolysate composition in a nutritional supplement to an animal.

13. A method of using a hydrolysate composition,

wherein the hydrolysate composition is isolated from a meat source and containing 60 – 90 % w/w protein;

characterised by the step of

providing the hydrolysate composition in a medicament for the treatment of an amino acid malnourishment or an associated condition in a non-human animal.

14. A hydrolysate composition isolated from a meat source and containing 60 – 90% w/w protein,

for the treatment of amino acid malnourishment or an associated condition in an animal.

15. A method of improving flavouring of a food stuff characterised by the step of

adding to the food stuff a hydrolysate composition wherein the hydrolysate composition is isolated from a meat source and;

wherein the hydrolysate composition contains 60 – 90% w/w protein.

16. The use of 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

17. A method of using 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

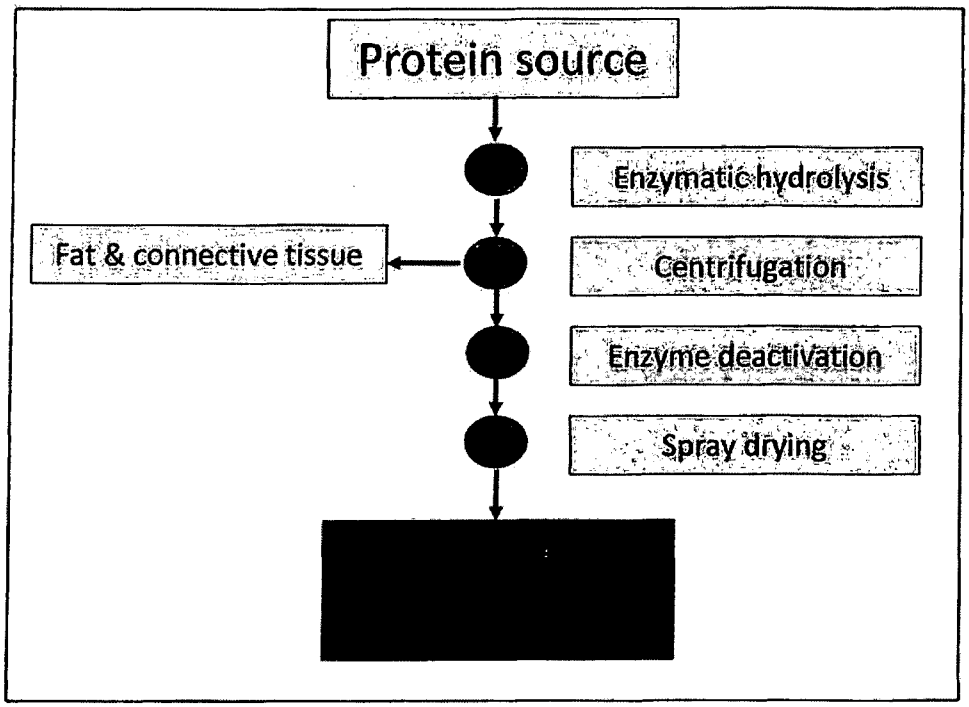

Protein source
Enzymatic hydrolysis
Fat & connective tissue
Centrifugation
Enzyme deactivation
Spray drying

Figure 2

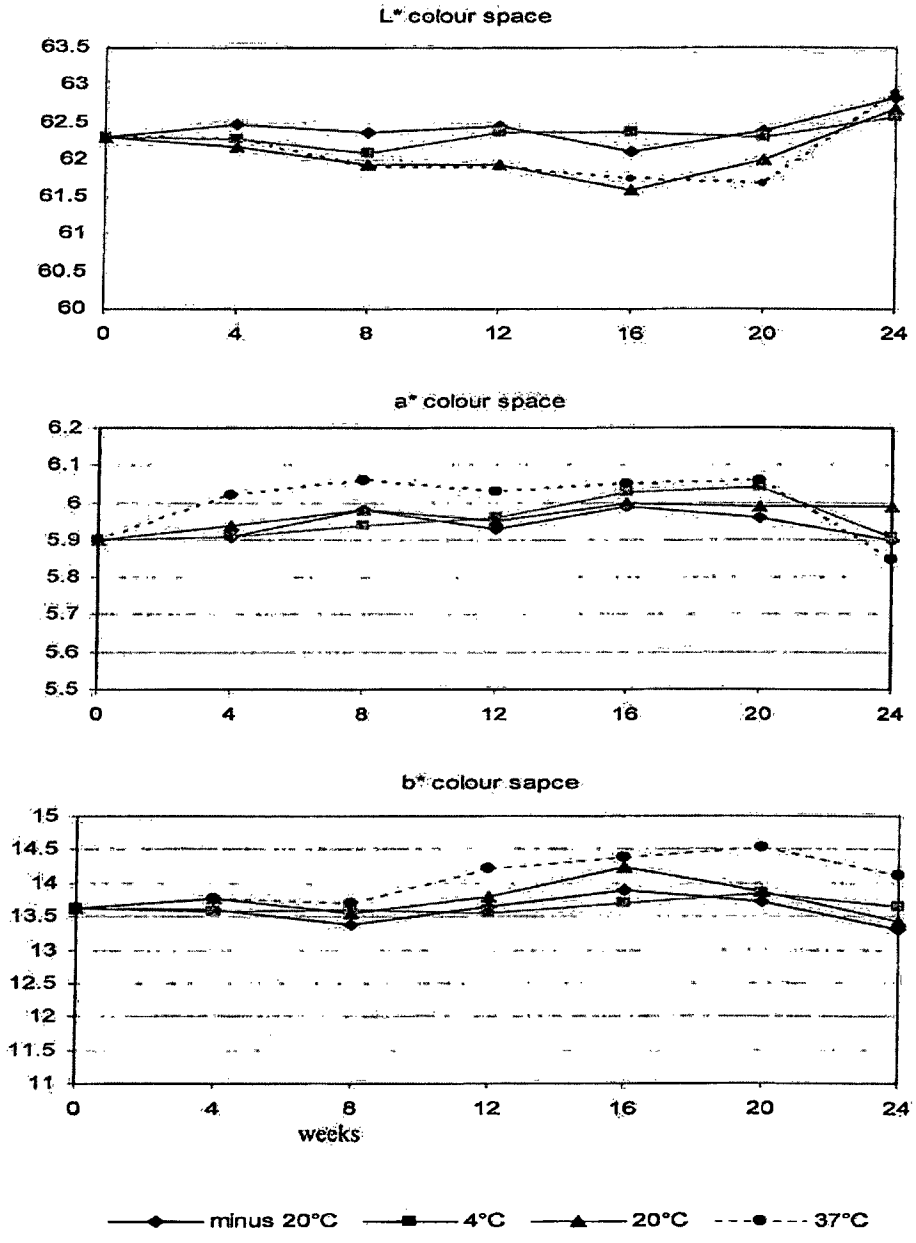

L* colour space
63.5
63
62.5
62
61.5
61
60.5
60
0
4
8
12
16
20
24
a* colour space
6.2
6.1
6
5.9
5.8
5.7
5.6
5.5
0
4
8
12
16
20
24
b* colour sapce
15
14.5
14
13.5
13
12.5
12
11.5
11
0
4
8
12
16
20
24
weeks
minus 20°C
4°C
20°C
37°C

Figure 3

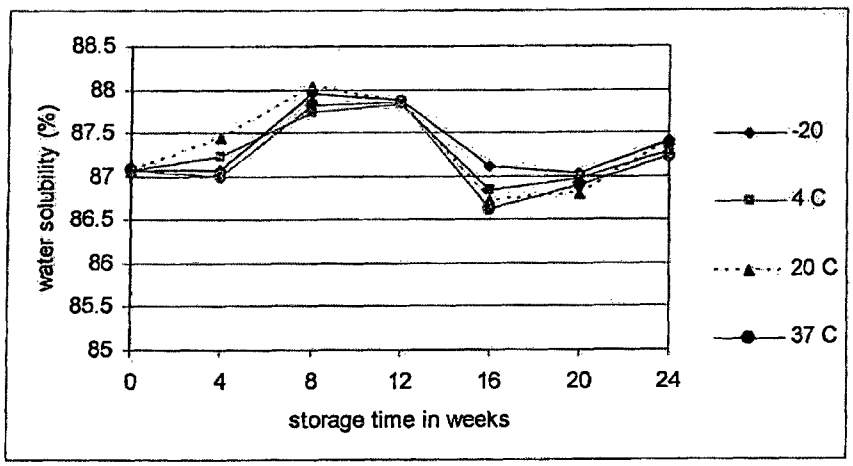

88.5
88
87.5
87
86.5
86
85.5
85
water solubility (%)
0
4
8
12
16
20
24
storage time in weeks
-20
4 C
20 C
37 C

Figure 4

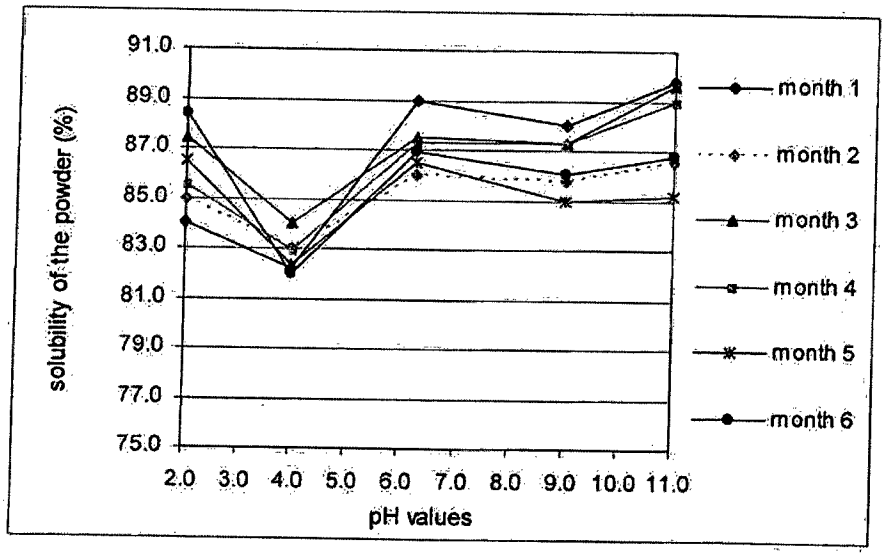

91.0
89.0
87.0
85.0
83.0
81.0
79.0
77.0
75.0
solubility of the powder (%)
2.0 3.0 4.0 5.0 6.0 7.0 8.0 9.0 10.0 11.0
pH values
month 1
month 2
month 3
month 4
month 5
month 6

Figure 5

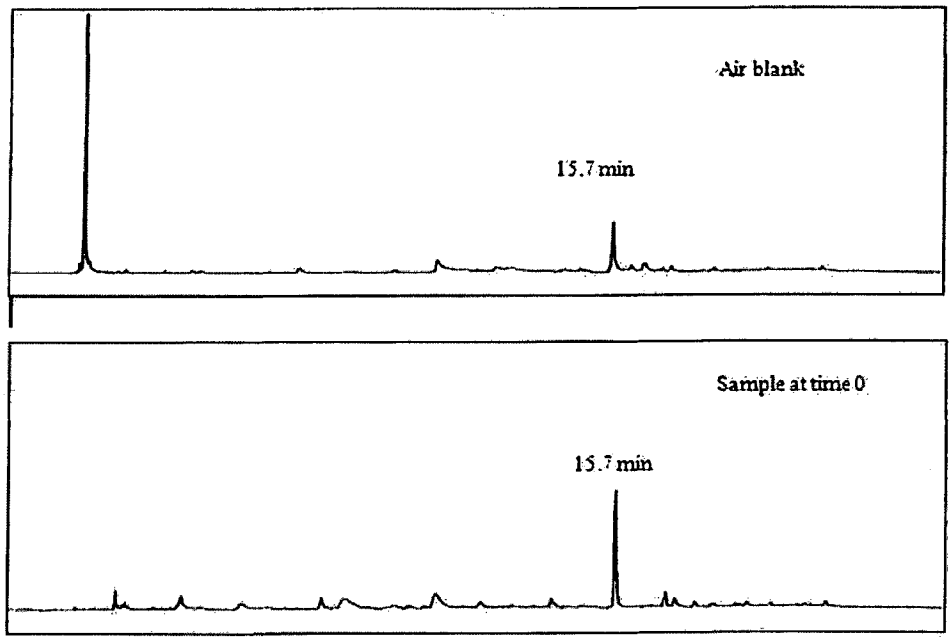


Figure 6

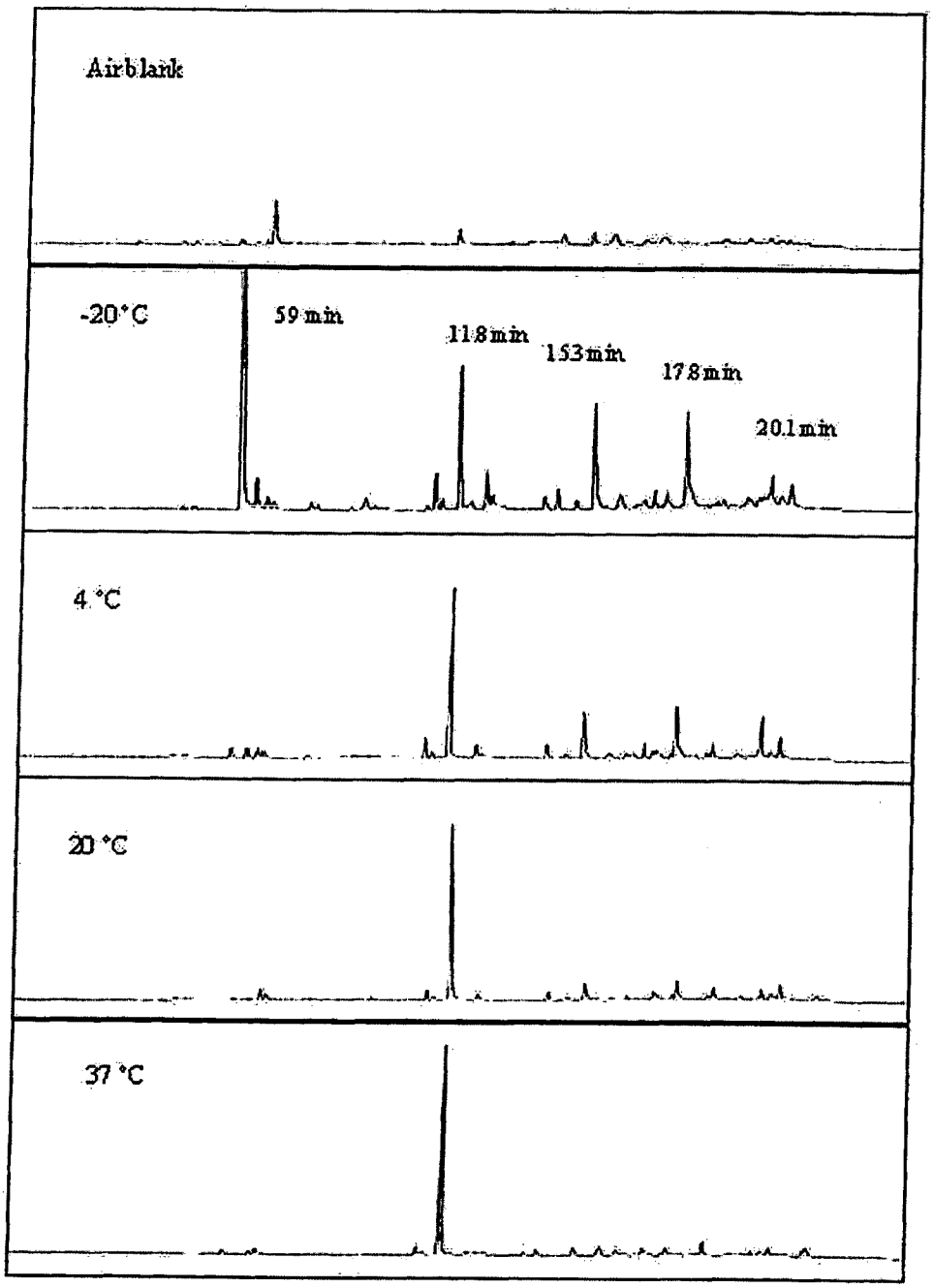


Figure 7

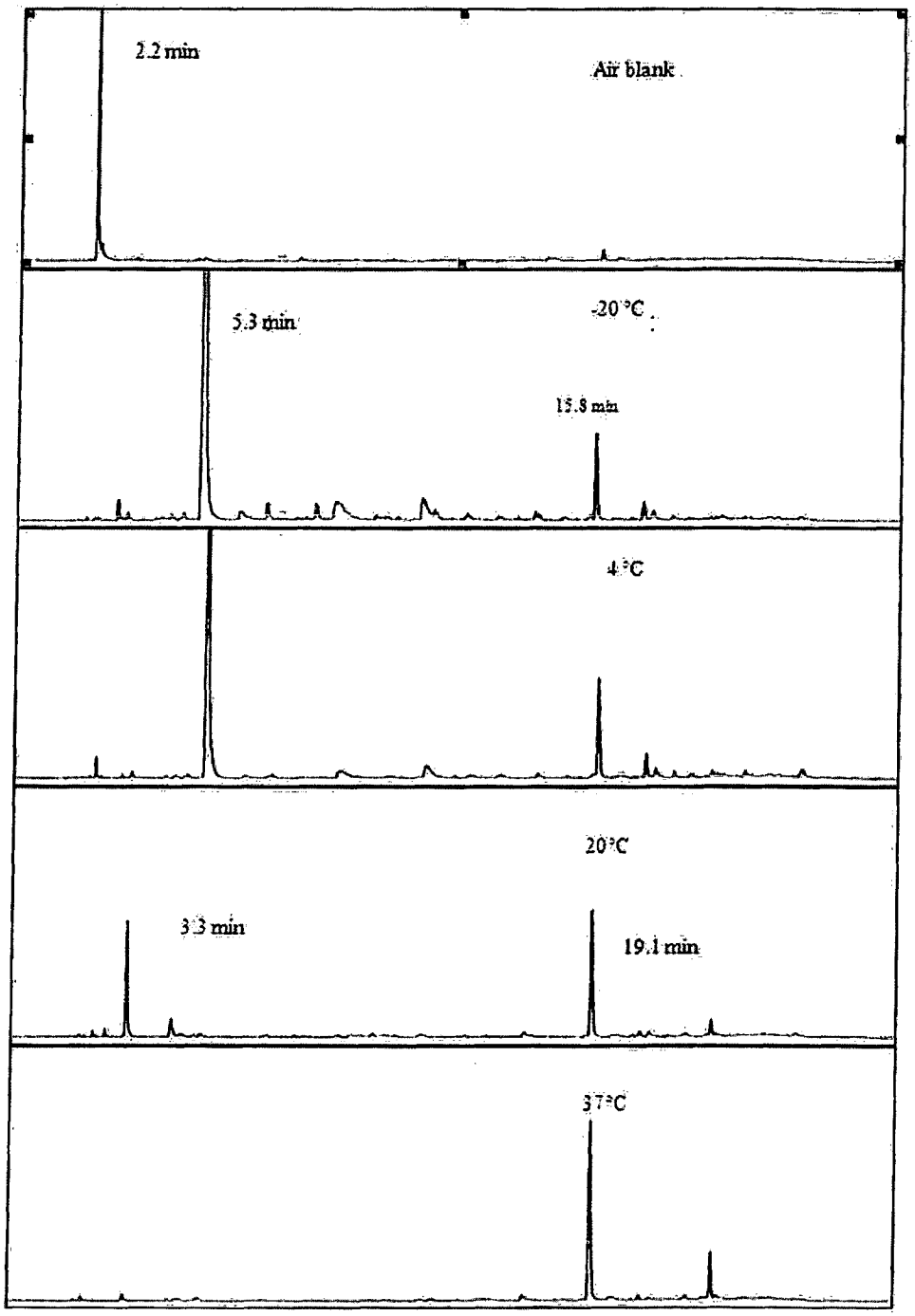
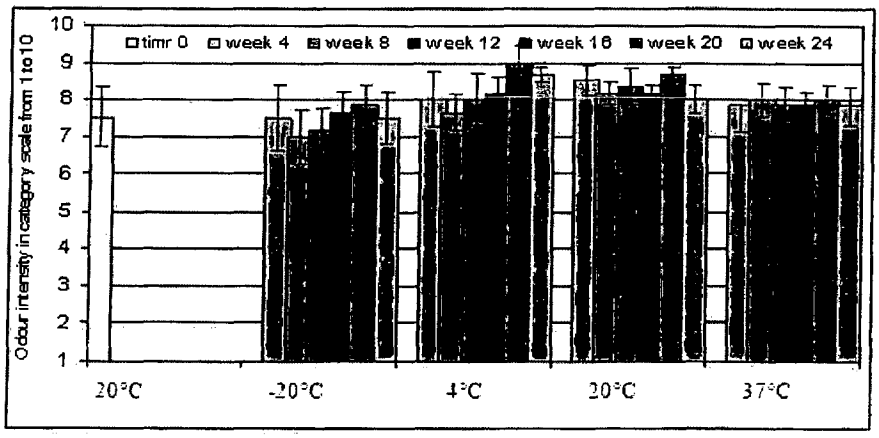
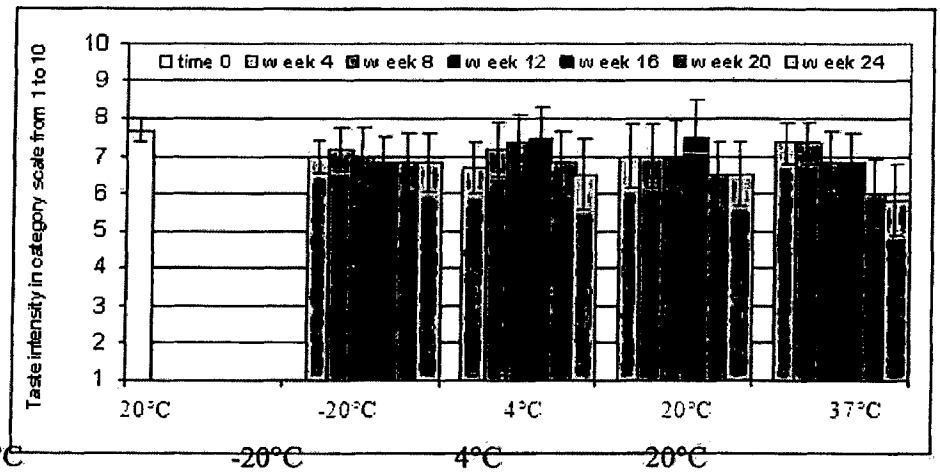


Figure 8



(A)



20°C -20°C 4°C 20°C

(B)

Figure 9

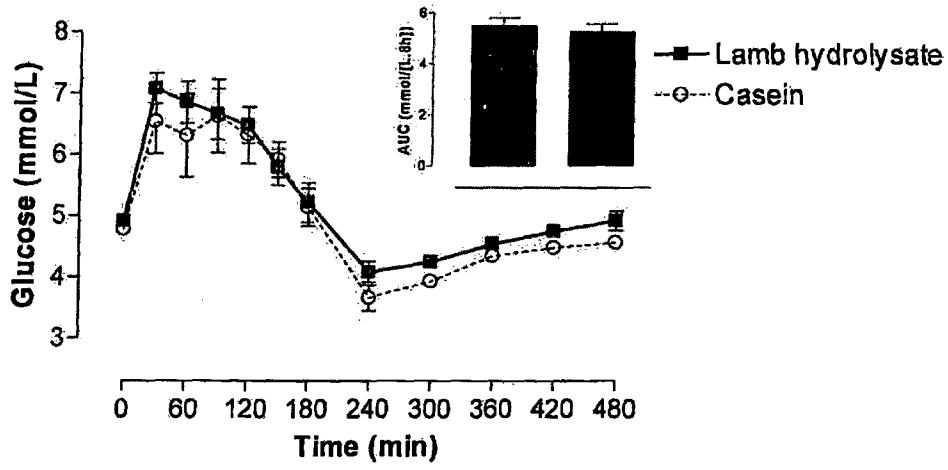

Glucose (mmol/L)
8
7
6
5
4
3
0 60 120 180 240 300 360 420 480
Time (min)
AUC (mmol/[L.6h])
6
4
2
0
Lamb hydrolysate
Casein

Figure 10

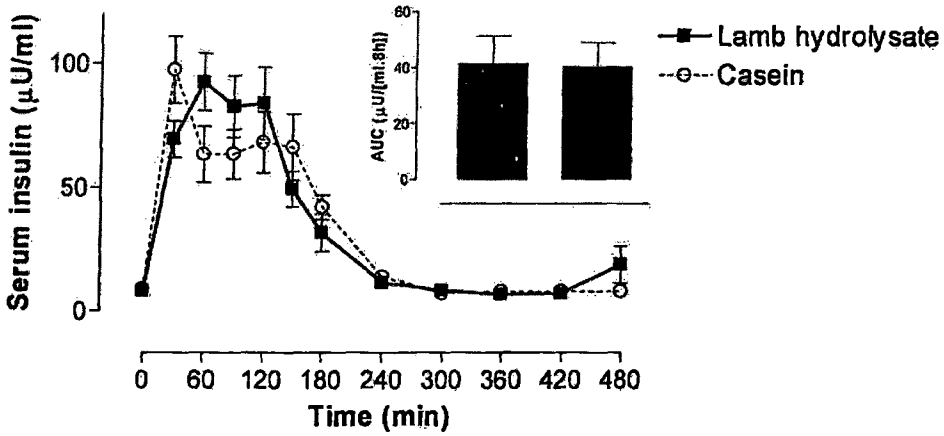

Serum insulin (µU/ml)
100
50
0
0 60 120 180 240 300 360 420 480
Time (min)
AUC (µU/[ml.8h])
60
40
20
0
Lamb hydrolysate
Casein

Figure 11

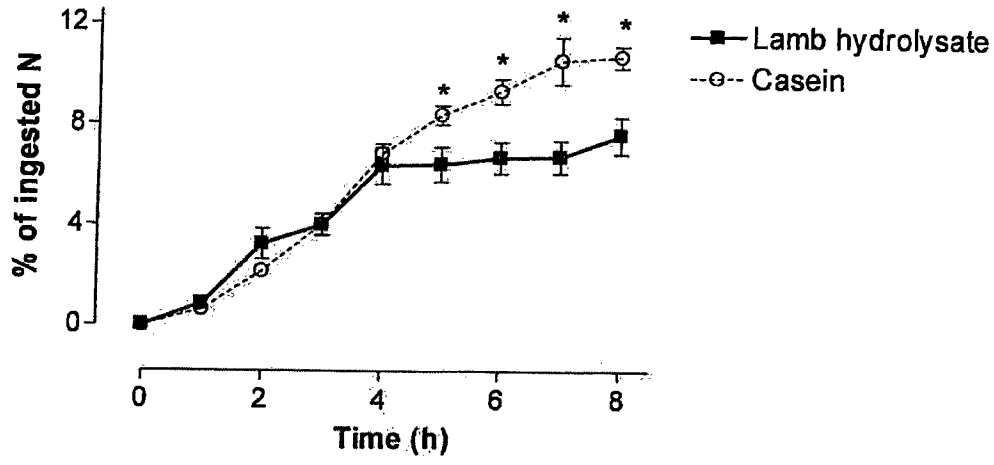


Figure 12

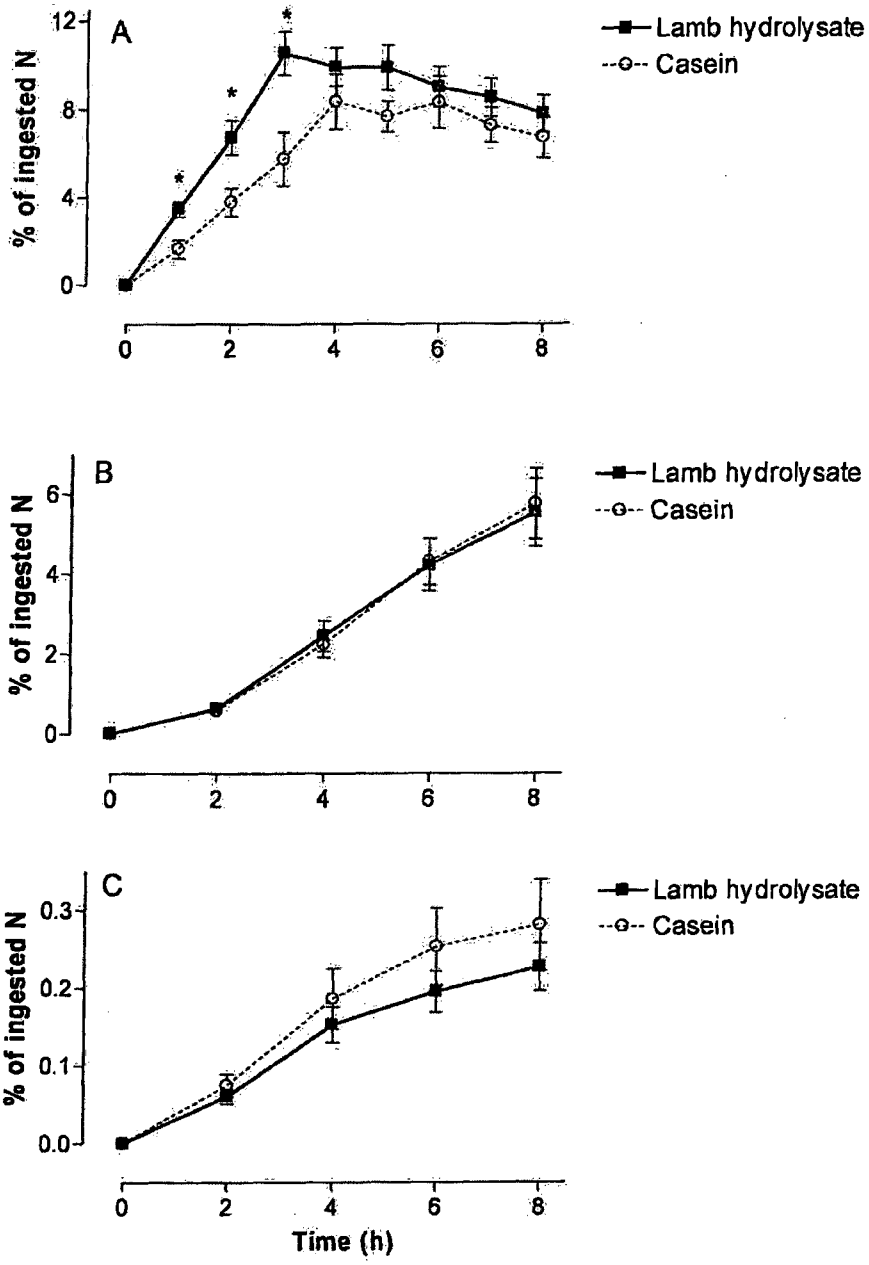
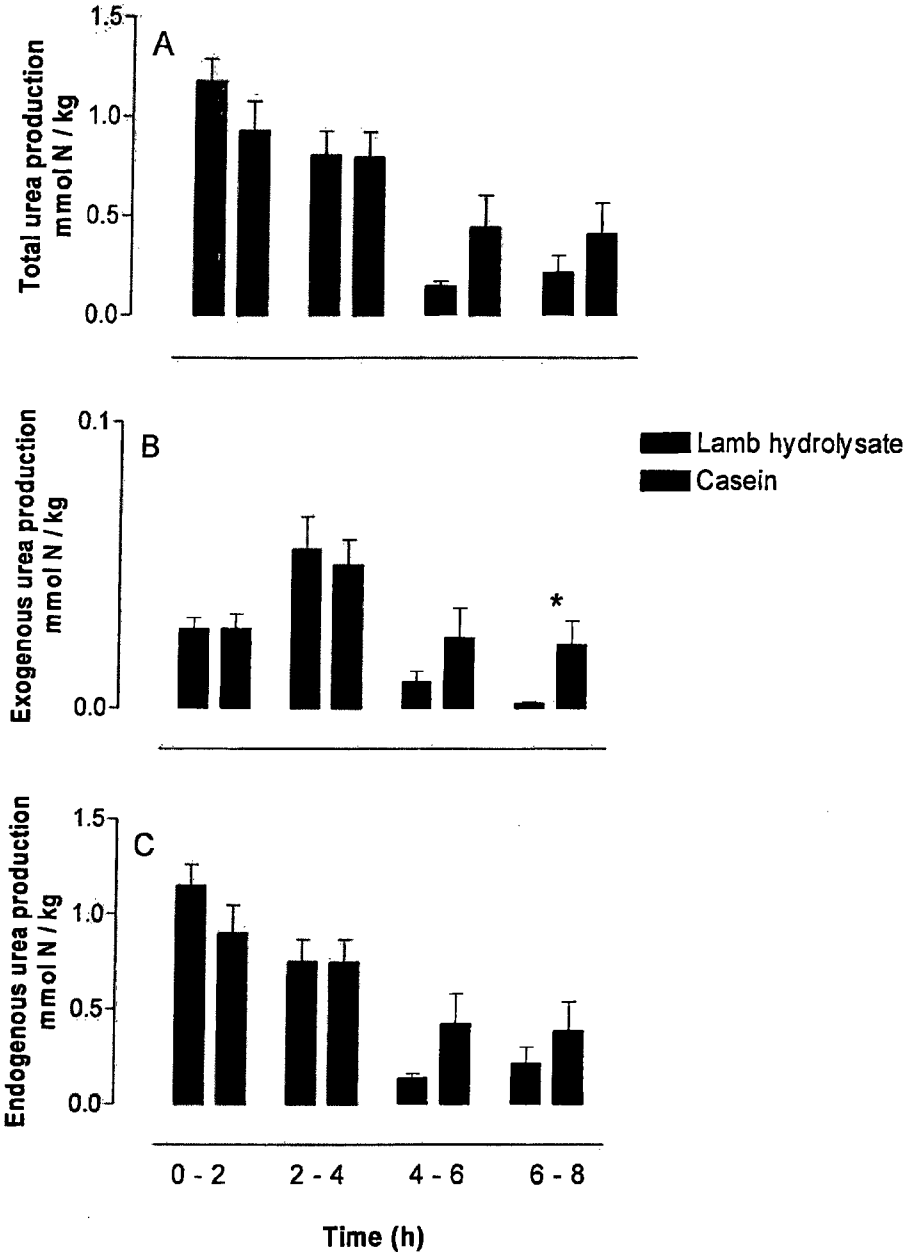

A
% of ingested N
12
8
4
0
0
2
4
6
8
*
*
*
Lamb hydrolysate
Casein
B
% of ingested N
6
4
2
0
0
2
4
6
8
Lamb hydrolysate
Casein
C
% of ingested N
0.3
0.2
0.1
0.0
0
2
4
6
8
Time (h)
Lamb hydrolysate
Casein

Figure 13


A
Total urea production mmol N / kg
1.5
1.0
0.5
0.0
B
Exogenous urea production mmol N / kg
0.1
0.0
*
Lamb hydrolysate
Casein
C
Endogenous urea production mmol N / kg
1.5
1.0
0.5
0.0
0 - 2
2 - 4
4 - 6
6 - 8
Time (h)