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(54) Title (EN): GELATIN DETECTION AND DIFFERENTIATION METHODS THEREOF

(54) Title (FR): DÉTECTION DE GÉLATINE ET PROCÉDÉS POUR SA DIFFÉRENCIATION

(57) Abstract:
(EN): The present invention relates to a method of identification/detection and differentiating bovine from porcine gelatin that could be used as a quality control tool in industry. The methods provides a solution for a long felt need which is to identify and differentiate porcine and non-porcine products in pharmaceutical, nutraceutical, cosmetic and food industry.

(FR): La présente invention concerne un procédé d'identification/détection et de différenciation de gélatines bovines et porcines pouvant être utilisé comme outil de contrôle de qualité dans l'industrie. Ces procédés constituent une solution permettant de remédier à un besoin depuis longtemps ressenti consistant à identifier et à différencier des produits porcins et non porcins dans les industries pharmaceutiques, nutraceutiques, cosmétiques et alimentaires.

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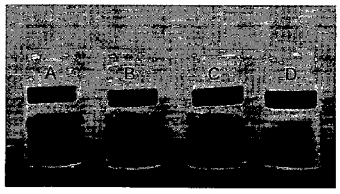

FIGURE 1

(57) Abstract: The present invention relates to a method of identification/detection and differentiating bovine from porcine gelatin that could be used as a quality control tool in industry. The methods provides a solution for a long felt need which is to identify and differentiate porcine and non-porcine products in pharmaceutical, nutraceutical, cosmetic and food industry.

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GELATIN DETECTION AND DIFFERENTIATION METHODS THEREOF

FIELD OF INVENTION

The present invention relates in general to the field of food chemistry. More particularly, present invention provides a method to detect and distinguish between porcine and bovine gelatin powder that is typically used in foods, pharmaceutical and nutraceutical products. Moreover, the method of the present invention furnishes good sensitivity, quick identify and distinguish and cost effective.

BACKGROUND OF INVENTION

Food safety in particular has become a subject of strict scrutiny in recent years, and there is a growing interest in improvement of technology. In food industry, gelatin acts as a stabilizer, thickener, and texturizer. Moreover, gelatin widely used in confections (candies, jellies, and marshmallows), and milk products (yogurts and ice creams) (Venien & Levieux, 2005). In pharmaceutical industry, gelatin is widely used as suspending agent, and in capsule and tablet formulations (Venien & Levieux, 2005). Gelatin is typically manufactured from naturally occurring collagen in bovine and porcine sources, in particular, from hides and bones. In some instances, gelatin can be extracted from, for example, piscine, chicken, or equine sources. Raw materials of typical gelatin production, such as bovine hides and bones, originate from animals subject to government-certified inspection and passed fit for human consumption. Both bovine and porcine gelatins usage in consumed products (both in food and pharmaceutical industry) posted a strong concern to its consumers (Islam forbid the consumption of any pork-related products, while Hinduism forbid the consumption of cow-related products) (Nemati *et al.*, 2004). Fish gelatin is an alternative to both bovine and porcine gelatins, but it is still in its emerging stage (Karim & Bhat, 2009).

Gelatin, a high molecular weight polypeptide derived from animal collagen and is widely used in the food and pharmaceutical industries. Gelatin is obtained by thermal denaturation of collagen. Two types of commonly used gelatin are Type A and Type B. Type A gelatin is

derived from acid pretreatment, while Type B gelatin is derived from alkaline pretreatment. Two main sources of gelatin are derived from porcine skin (using acid treatment) and bovine skin (using alkaline treatment) (Sigma-Aldrich).

Object to be solved by the Present Invention

The present invention responds specifically to the long-felt need heretofore unmet by the prior art, and especially with a view to overcoming the objective of the present invention which relates to a method of identification/detection and differentiating bovine from porcine gelatin that could be used as a quality control tool in industry. The methods provides a solution for a long felt need which is to identify and differentiate porcine and non-porcine products in pharmaceutical, nutraceutical, cosmetic and food industry. Whereby this products are also consumed by Muslim customers.

The present invention has overcome the problem in the art by developing a fast, cost effective, specific assay for identification and differentiation between bovine from porcine gelatin powder, whereby the method is performed by providing Maillard browning between ribose or other reducing sugar such as xylose and glucose with basic amino acids of gelatin derived from porcine or bovine. Gelatin powder of different physicochemical properties, protein content and amino acid composition will yield different extend of the Maillard browning which could be useful for identification, differentiation and quality control purposes.

The present application relates to an improved method of detecting and differentiating bovine gelatin powder from porcine gelatin powder which do not have the drawbacks of prior art techniques, and which further show unexpected effects and advantages.

Moreover, the present application notably provides heating conditions and concentration of gelatins which provides usefulness to the degree of browning in a Maillard system. Indeed, the differences in chemical properties (e.g. protein content, amino acids composition, initial pH value, physicochemical properties) between tested on selected gelatins have further advantage of providing to the differences in the rate of browning. Hence, it is possible to use these properties to differentiate between porcine and bovine gelatin powder.

SUMMARY OF INVENTION

Accordingly, the present invention relates to methods for detecting and differentiating animal (bovine and porcine) gelatin in a sample, wherein the method includes obtaining gelatin from an animal, preparing the gelatin at a concentration between 1 and 5% (w/v),dissolving the gelatin at concentration between 1 and 5% (w/v) in distilled water, obtaining solutions at concentration between 1 and 5% (w/v), heating the solutions for at least 15 minutes; spinning the solutions for at least 30 seconds; obtaining homogenise solutions; cooling the solutions and obtaining suspend containing gelatin.

Also, the above method further includes adding reducing sugar such as ribose into the suspend containing gelatin and obtaining a mixture; spinning the mixture for at least 30 seconds; heating the mixture between 15 and 60 minutes; cooling the mixture; analyzing the mixture by means of absorbance measurement between 420 and 550nm.
Preferably, the sample includes gelatin powder intended for use in food (food product include beverages, dairy products, confectionaries, chocolates, and any application in food formulation/s as an ingredient or for any functional properties), pharmaceutical, nutraceutical and cosmetic product (the cosmetic product includes cream, lotion eye cream, ointment or gel, sun-screen, oral administration, face mask cream, anti-inflammatory medicine, and/or anti-irritant medicine).

In addition the present invention relates to the use of the method by means of detecting and differentiating bovine gelatin from porcine gelatin and for quality control purposes in food or pharmaceutical industries.

BRIEF DESCRIPTION OF DRAWINGS

The accompanied drawings constitute part of this specification and include an exemplary or preferred embodiment of the invention, which may be embodied in various forms. It should be understood, however, the disclosed preferred embodiments are merely exemplary of the invention. Therefore, the figures disclosed herein are not to be interpreted as limiting, but merely as the basis for the claims and for teaching one skilled in the art of the invention

Figure 1: Gelatin powders; (A) Bovine 75, (B) Bovine 225, (C) Porcine 90, and (D) Porcine 300.

Figure 2: Dissolved Bovine 75 (5% w/v) with 10% (w/v) ribose. (A) 0 min (before heating), (B) 15 min, (C) 30 min, (D) 45 min, (E) 60 min.

Figure 3: Dissolved Bovine 225 (5% w/v) with 10% (w/v) ribose. (A) 0 min (before heating), (B) 15 min, (C) 30 min, (D) 45 min, (E) 60 min.

Figure 4: Dissolved Porcine 90 (5% w/v) with 10% (w/v) ribose. (A) 0 min (before heating), (B) 15 min, (C) 30 min, (D) 45 min, (E) 60 min.

Figure 5: Dissolved Porcine 300 (5% w/v) with 10% (w/v) ribose. (A) 0 min (before heating), (B) 15 min, (C) 30 min, (D) 45 min, (E) 60 min.

Figure 6: Comparison on absorbance value at 420*nm between various gelatins at concentrations of (A) 1% (w/v), (B) 3% (w/v), and (C) 5% (w/v) heated with ribose (10% w/v) at 95°C.

Figure 7: pH reduction on various gelatins (5% w/v) with ribose (10% w/v) during 60 minutes heating at 95°C.

Figure 8 shows a graph representing 4 samples from different suppliers.

DETAILED DESCRIPTION OF THE INVENTION

The invention will now be described in more detail by reference to the following Figures and Examples. The following examples are provided for illustrative purposes only and are not intended to limit the invention.

BEST MODE TO CARRY OUT THE INVENTION

Before the present invention is further described, it is to be understood that this invention is not limited to particular embodiments described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims. When a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the invention, subject to any specifically excluded limit in the stated range. When the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

EXAMPLES

Materials and Methods

Chemicals

D-(-)-Ribose (minimum 99%), gelatins type A from porcine skin (300 Bloom and 90 - 110 Bloom) and gelatins type B from bovine skin (225 Bloom and 75 Bloom) were purchased from Sigma Chemical Company. There are various types of gel strength (measured in Bloom number) available in the market to suit different applications. Higher Bloom number reflects stronger the strength of gel formed from gelatin and higher molecular mass of the gelatin. Sigma-Aldrich categorized 3 types of gelatin based on their Bloom number; Low Bloom (Bloom number ranging between 50 and 125), Medium Bloom (Bloom number ranging between 175 and 225), and High Bloom (Bloom number ranging between 225 and 325).

Inducement of Maillard Reaction

Maillard or non-enzymatic browning reaction comprises the reaction between reducing sugar (example; fructose, ribose, and xylose) with amino acids or proteins to form a condensation product. Subsequently, a range of reactions takes place, including cyclisations, dehydrations, retroaldolisations, rearrangements, isomerisations, and further condensations, which ultimately lead to the formation of brown nitrogenous polymers and co-polymers, known as melanoidins (Ames, 1998). This reaction contributes to desirable flavour and colour development (van Boekel, 2006; Tomlinson *et al.*, 1994; Tomlinson *et al.*, 1993), as well as anti-oxidative activity (Osada & Shibamoto, 2006; Yilmaz & Toledo, 2005) and carcinogenic compounds (Stadler *et. al.*, 2002). Browning occurs due to the formation of high molecular weight (more than 12,000 Daltons) polymeric compounds also known as melanoidins. These are generally formed by the reaction of the Amadori product or/and with other dicarbonyls, i.e. the deoxyosuloses, with amino acids (Ames *et. al.*, 1993). The most commonly used indicator for brown pigment detection in Maillard system is spectrophotometric measurement at wavelength of 420 nm (Carabasa-Giribet & Ibarz-Ribas, 2000; Garza *et. al.*, 1999)

The course of the Maillard reaction is strongly affected by factors, which influence the different chemical reactions involved. These are temperature and duration of heating, pH and presence of weak acids and bases, type of reactant, amino acid to sugar ratio, reagent concentration, water activity and glass transition temperature (Gerrard, 2006).

For sugars, the rate of the reaction depends on the rate at which the sugar ring opens to the reducible, open-chained form and this increases with increasing pH. Pentose sugars (such as ribose, and xylose) react more rapidly than hexoses (such as glucose, and fructose). For hexoses, the typical order of reactivity is D-galactose>D-mannose>D-glucose. Reducing disaccharides are considerably less reactive than their corresponding monomers (Davies & Labuza, 1997).

The rate of browning also differed strongly depending the sugar structure. Presence of glycosidic bond at the C4 hydroxyl group of the glucopyranose (for example maltose) was difficult to cleave. Thus, browning was slow in these types of sugar. However, if the

glycosidic bonding is at the C6 hydroxyl group (for example isomaltose) further degradation was not blocked. Thus, the rate of browning was therefore comparable to that of glucose (Kato *et. al.*, 1988).

For amine, basic amino acids are more reactive than neutral or acidic amino acids. The most reactive were lysine, glycine, tryptophan, and tyrosine. Lysine appears to be the most reactive amino acids due to the fact that it has two available amino groups. Melanoidins from peptides exhibited a darker degree of colour than from amino acids (Davies & Labuza, 1997).

The extent of browning varies according to the sugar to amine ratio. The effect of increasing the amino acid concentration show a greater increase in browning than of increasing the sugar content on a molar basis and the increase for both is greater than the relative concentration increase (2 times greater for sugar and 2 to 3 fold times greater for amine) (Davies & Labuza, 1997).

Temperature and pH are believed to play a crucial role in Maillard reaction. The rate of the reaction increases with temperature. The length of heating is important as the formation of melanoidins usually occurs at a rate, which increases in proportion to the square of the reaction time at any given temperature, and different flavours are formed depending on the extent of reaction (Davies & Labuza, 1997).

The open chain form of the sugar and the unprotonated form of the amino group, considered to be the reactive forms, are favoured at higher pH. The lower the pH, the more protonated amino group is present in the equilibrium and therefore, less reactive with the sugar (Davies & Labuza, 1997).

Steps involved in gelatin identification and differentiation

Three different/individual solutions were prepared at gelatin concentrations of 1, 3 and 5% (w/v). The gelatin concentrations of 1, 3 and 5% (w/v) were dissolved in distilled water in universal bottles heated in water bath (Memmert shaking water bath WB22-16X2501) at 70°C for 15 minutes (Typical concentration of gelatin used for good differentiation is 5%).

The solutions were then vortex/spin (IKA MS 3 Digital) for 30 seconds. This is to ensure the solutions were homogenised before cooling it under running tap water.

Later, 0.5g (also includes 0.5 ± 0.001) of reducing sugar such as ribose was added into 5mL of dissolved gelatin. Then, the dissolved gelatin containing ribose was transferred into a plastic test tube and spin/ vortex for 30 seconds or longer. Obtaining mixture from the vortex step and the samples were later submerged in water bath at 95°C. The mixture is removed from the water bath at selected heating time intervals (15, 30, 45, and 60 minutes) and cooled under running tap water prior to absorbance measurement (420nm and 550nm are recorded using UV spectrophotometer) (Typical heating times that yield good absorbance values for differentiation are 45 and 60 minutes).

Measurement of absorption at 420nm and 550nm

The degree of browning is measured via absorbance at 420nm using Minolta CM-3500d model spectrophotometer. Samples are transferred into a glass cuvette to measure the absorbance at least twice from both front and rear sides. Absorbance at 550nm is measured as well to correct the absorbance reading at 420nm for any presence of turbidity in the extracts.

$$\text{Abs } 420^*\text{nm} = \text{Abs } 420\text{nm} - \text{Abs } 550\text{nm} - \text{Abs } 420^*\text{nm at } 0 \text{ min}$$

where,

420^* = corrected wavelength at 420nm

Statistical Analysis

Statistical analysis on the data was performed using SPSS 16 for mac. One-way ANOVA test was performed on the absorbance measurement. This statistical test is used to check for significant difference ($P>0.05$) between the browning derived from gelatins with heating time intervals. Data were reported as means ± standard deviation.

Table 1 shows the abbreviates of the 4 types of gelatin used in this study.

Table 1: Abbreviates of the gelatin samples.

Types of Gelatins	Source	Abbreviates
Bovine (75 Bloom)	Sigma Aldrich (M) Sdn. Bhd.	Bovine 75
Bovine (225 Bloom)	Sigma Aldrich (M) Sdn. Bhd.	Bovine 225
Porcine (90 - 110 Bloom)	Sigma Aldrich (M) Sdn. Bhd.	Porcine 90
Porcine (300 Bloom)	Sigma Aldrich (M) Sdn. Bhd.	Porcine 300

Figure 2 to Figure 5 show the colour of the dissolved gelatins (5% w/v) heated with 10% (w/v) ribose at different heating time interval at 95°C. Based on visual observation on the heated samples, the followings could be inferred:

(1) At longer heating time, the degree of browning was higher and therefore offer better differentiating potential.

(2) At higher concentration of gelatin, the degree of browning was higher.

(3) Gelatins obtained from the same species yield similar colour intensity.

(4) In general, bovine gelatins yield higher degree of browning compared to porcine gelatins.

Based on these observations, it is preferred to use ribose-induced Maillard browning as a quantitative chemical marker to differentiate between porcine and bovine gelatin powder. Indeed, powder is widely available form for bovine and porcine gelatins.

These could be developed as an in-house Quality Control method to identify and authenticate the source of gelatin raw materials used for capsule or food production. The absorbance of gelatin powder to be controlled is first established using the Maillard reaction and set as a specification. Subsequent batches are then controlled using the established specifications.

For quality control purposes, quantitative confirmation of the colour could be carried out using spectrophotometry analysis. During our trials, absorbance for the brown polymer compounds (melanoidins) in the samples was measured at 420nm. Absorbance at 550nm was

also measured. A correction was made by subtracting the absorbance value obtained at 550nm from the absorbance value obtained at 420nm. The correction was made to eliminate the error obtained from the absorbance read at 420nm due to the presence of any turbidity in the extracts.

Figure 6 (A) (B) (C) shows the increments of absorbance at 420*nm throughout the 60 minutes heating time. These increments are due to the accretion of brown polymer compounds in all the gelatins due to Maillard reaction. Evidences of the Maillard reaction during heating are the formation of brown polymers compounds and the reduction in pH value of the samples as shown in Figure 7. The pH reduction on 5% (w/v) gelatins was displayed as an example. Similar trend was observed on 1 and 3% (w/v) gelatins (graph not shown). Similar responses of the absorbance (browning) increase and pH decrease are observed at lower levels of ribose or when ribose is replaced with less reactive reducing sugars such as xylose or glucose.

Table 2 represents Mean absorbance at 420*nm for gelatin samples at concentrations of (A) 1%, (B) 3%, and (C) 5% heated with 10% ribose at 95°C.

(A) 1% gelatin

Samples	**Heating Time (min)**			
	15	30	45	60
Bovine 75	0.0013±0.0001^{a}	0.0059±0.0005^{a}	0.0138±0.0004^{a}	0.0248±0.0021^{a}
Bovine 225	0.0007±0.0005^{a}	0.0052±0.0010^{ab}	0.0136±0.0003^{a}	0.0246±0.0005^{a}
Porcine 90	0.0012±0.0006^{a}	0.0045±0.0005^{b}	0.0107±0.0006^{b}	0.0186±0.0013^{b}
Porcine 300	0.0015±0.0005^{a}	0.0049±0.0004^{ab}	0.0101±0.0002^{b}	0.0181±0.0003^{b}

(B) 3% gelatin

Samples	Heating Time (min)			
	15	30	45	60
Bovine 75	0.0027±0.0004[a]	0.0225±0.0008[a]	0.0623±0.0004[a]	0.1184±0.0030[a]
Bovine 225	0.0010±0.0014[ab]	0.0211±0.0009[a]	0.0631±0.0003[b]	0.1157±0.0053[a]
Porcine 90	0.0018±0.0003[ab]	0.0162±0.0003[b]	0.0448±0.0004[c]	0.0855±0.0008[b]
Porcine 300	0.0035±0.0007[b]	0.0157±0.0004[b]	0.0412±0.0003[d]	0.0803±0.0015[b]

(C) 5% gelatin

Samples	Heating Time (min)			
	15	30	45	60
Bovine 75	0.0068±0.0005[a]	0.0457±0.0017[a]	0.1287±0.0071[a]	0.2341±0.0110[a]
Bovine 225	0.0063±0.0025[a]	0.0458±0.0023[a]	0.1306±0.0032[a]	0.2418±0.0054[a]
Porcine 90	0.0043±0.0015[a]	0.0340±0.0026[b]	0.0949±0.0064[b]	0.1779±0.0087[b]
Porcine 300	0.0044±0.0010[a]	0.0316±0.0011[b]	0.0894±0.0008[b]	0.1658±0.0014[b]

1. Mean ± Standard deviation of 4 replicates.
2. Values followed by the same letter in the same column at the same heating time are not significantly different (P>0.005).

The inventors in the present invention have noted that the differences in chemical properties (e.g. protein content, amino acids composition, initial pH value, and physicochemical properties) between tested gelatins could be a suitable reason for differences in the rate of browning. Hence, it is possible to use these properties to differentiate between porcine and bovine gelatin powder. Also, the concentration range between 1% to 5% of dissolved gelatins and heating time of 45 minutes and 60 minutes, or longer, are suitable conditions for the identification and differentiation between bovine and porcine gelatin.

Example of results

Table 3: Confidence Intervals for known (control) gelatin powder samples (5% (w/v) at 60 min heating time) and absorbance value for unknown gelatin powder sample (5% (w/v) at 60 min heating time).

Types of gelatin	Mean Abs 420*nm	Confidence Intervals (α=0.05)	
		Lower Limit	Upper Limit
Bovine 75	0.2341	0.2232	0.2449
Bovine 225	0.2418	0.2365	0.2471
Porcine 90	0.1779	0.1694	0.1865
Porcine 300	0.1658	0.1645	0.1671
Unknown	0.2407		

1. Abs 420*nm = Abs 420nm - Abs 550nm - Abs 420*nm at 0 min

The absorbance for unknown (5%, w/v) at 60 minutes heating time is 0.2407. This value falls within the confidence intervals of bovine gelatin powder, which is between 0.2232 and 0.2471. Thus, the unknown can be identified as gelatin derived from bovine skin.

Verification of the identification methods

Four commercial grade gelatin powders and one commercial clear hard gelatin capsule were used to verify the proposed method. Table 4 shows the abbreviates of the unknown samples used in this verification.

Types of gelatins	Source	Abbreviates
Bovine	Sim Company Sdn. Bhd.	Unknown 1
Porcine 250	Halagel (M) Sdn. Bhd.	Unknown 2
Bovine 225	Halagel (M) Sdn. Bhd.	Unknown 3
Bovine 250	Halagel (M) Sdn. Bhd.	Unknown 4
Bovine	Ee Koh Enterprise Sdn. Bhd.	Unknown Capsule

All the unknown samples underwent the same procedure as the known gelatin (control) obtained from Sigma Aldrich.

Table 5: Confidence Intervals for known (control) gelatin powder samples (5% (w/v) at 60 min heating time) and absorbance value for unknown gelatin samples (5% (w/v) at 60 min heating time). Represented by Figure 8.

Types of gelatin	Mean Abs 420*nm	Confidence Intervals (α=0.05)	
		Lower Limit	Upper Limit
Bovine 75	0.2341	0.2232	0.2449
Bovine 225	0.2418	0.2365	0.2471
Porcine 90	0.1779	0.1694	0.1865
Porcine 300	0.1658	0.1645	0.1671
Unknown 1	0.3305	**Most likely Bovine**	
Unknown 2	0.1748	**Porcine**	
Unknown 3	0.2299	**Bovine**	
Unknown 4	0.2407	**Bovine**	
Unknown Capsule	0.2314	**Bovine**	

1. Abs 420*nm = Abs 420nm - Abs 550nm - Abs 420*nm at 0 min

The absorbance for Unknown 2 (5%, w/v) at 60 minutes heating falls within the confidence intervals of porcine gelatin. Meanwhile, the absorbance for Unknown 3, 4 and Unknown Capsule (5%, w/v) at 60 minutes heating falls within the confidence intervals of bovine gelatin. The results obtained matched with the confirmation from the gelatin powder and gelatin capsule manufacturer.

Unknown 1 showed a much higher absorbance value as compared to the absorbance obtained from control (bovine). This sample is bovine gelatin of low quality derived from unreliable supplier. This shows that the method could also be used for controlling quality of gelatin powder.

CLAIMS

1. A method for detecting and differentiating animal gelatin in a sample, wherein the method comprising the steps of:

a)obtaining gelatin from an animal,

b) preparing the gelatin from step (a) at a concentration between 1 and 5% (w/v);

c) dissolving the gelatin from step (b) at concentration between 1 and 5% (w/v) in distilled water,

d) obtaining solutions at concentration between 1 and 5% (w/v) from step (c),

e) heating the solutions from step (d) for at least 15 minutes;

f) spinning the solutions from step (e) for at least 30 seconds;

g) obtaining homogenise solutions from step (f);

h) cooling the solutions and

i) obtaining suspend containing gelatin.

2. The method for detecting and differentiating animal gelatin in a sample according to claim 1, wherein the method further comprising the steps of:

a) adding ribose into the suspend containing gelatin;

b) obtaining a mixture from step (a);

c) spinning the mixture from step (b) for at least 30 seconds;

d) heating the mixture from step (c) between 15 and 60 minutes;

e) cooling the mixture from step (d);

f) analyzing the mixture from step (e) by means of absorbance measurement between 420 and 550nm.

3. The method for detecting and differentiating animal gelatin in a sample according to claim 1 and 2, wherein the animal is bovine and porcine.

4. The method for detecting and differentiating animal gelatin in a sample according to claim 3, wherein the method comprises detecting and differentiating of bovine gelatin from porcine gelatin.

5. The method for detecting and differentiating animal gelatin in a sample according to claim 1 and 2, wherein the sample includes gelatin powder intended for use in food, pharmaceutical, nutraceutical and cosmetic product.

6. The method for detecting and differentiating animal gelatin according to claim 5, wherein the cosmetic product includes cream, lotion eye cream, ointment or gel, sun-screen, oral administration, face mask cream, anti-inflammatory medicine, and/or anti-irritant medicine.

7. The method for detecting and differentiating animal gelatin according to claim 5, wherein the food product include beverages, dairy products, confectionaries, chocolates, and any application in food formulation/s as an ingredient or for any functional properties.

8. Use of the method claim 1 to 7, is by means of detecting and differentiating bovine gelatin from porcine gelatin and for quality control purposes in food or pharmaceutical industries.

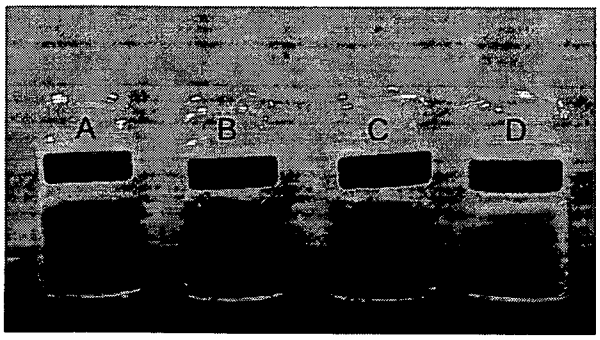

FIGURE 1

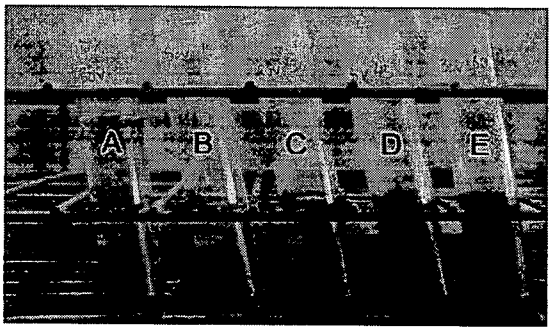

FIGURE 2

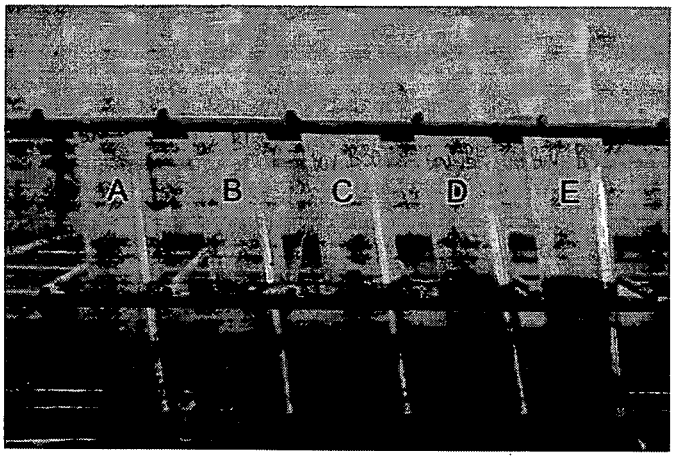

FIGURE 3

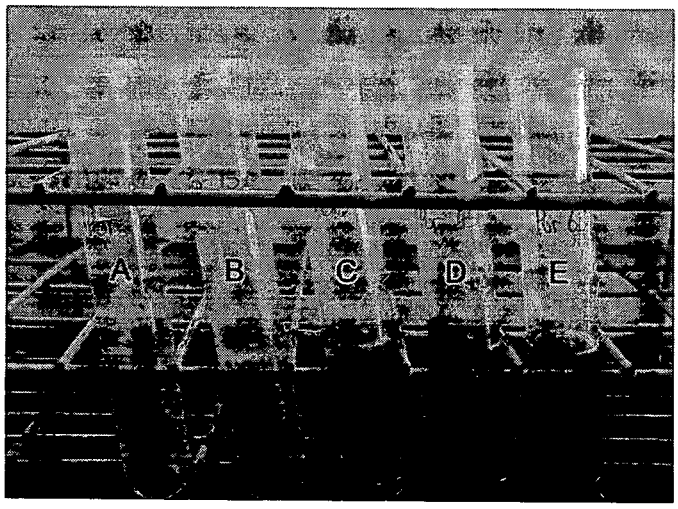

FIGURE 4

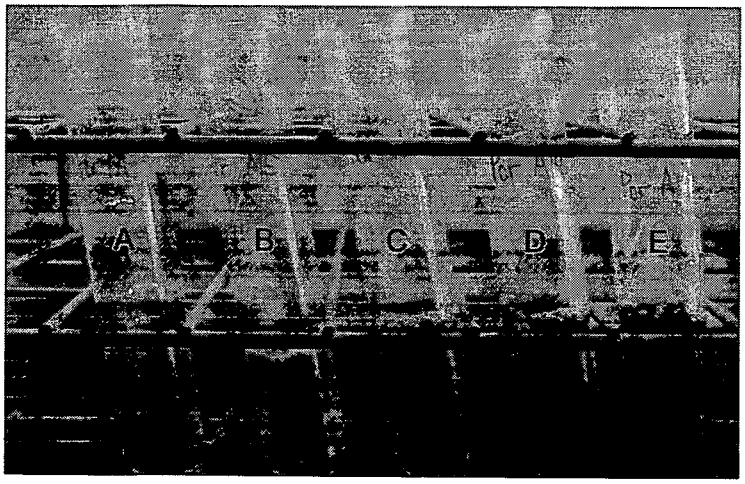

FIGURE 5

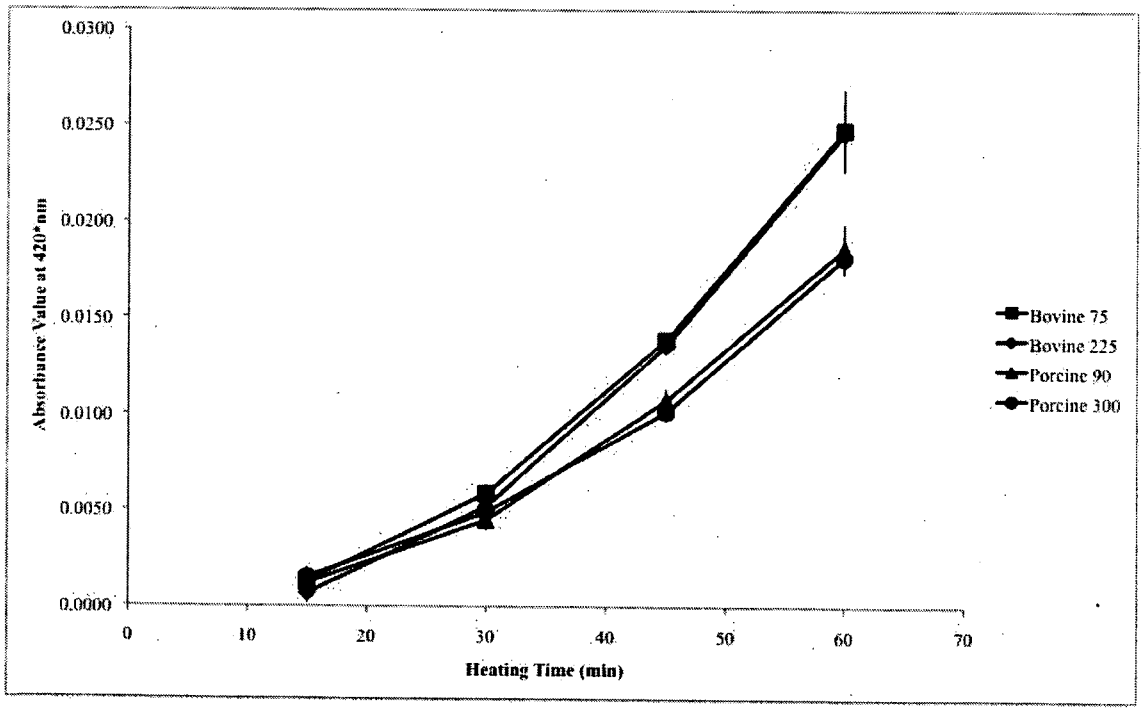

FIGURE 6 (A)

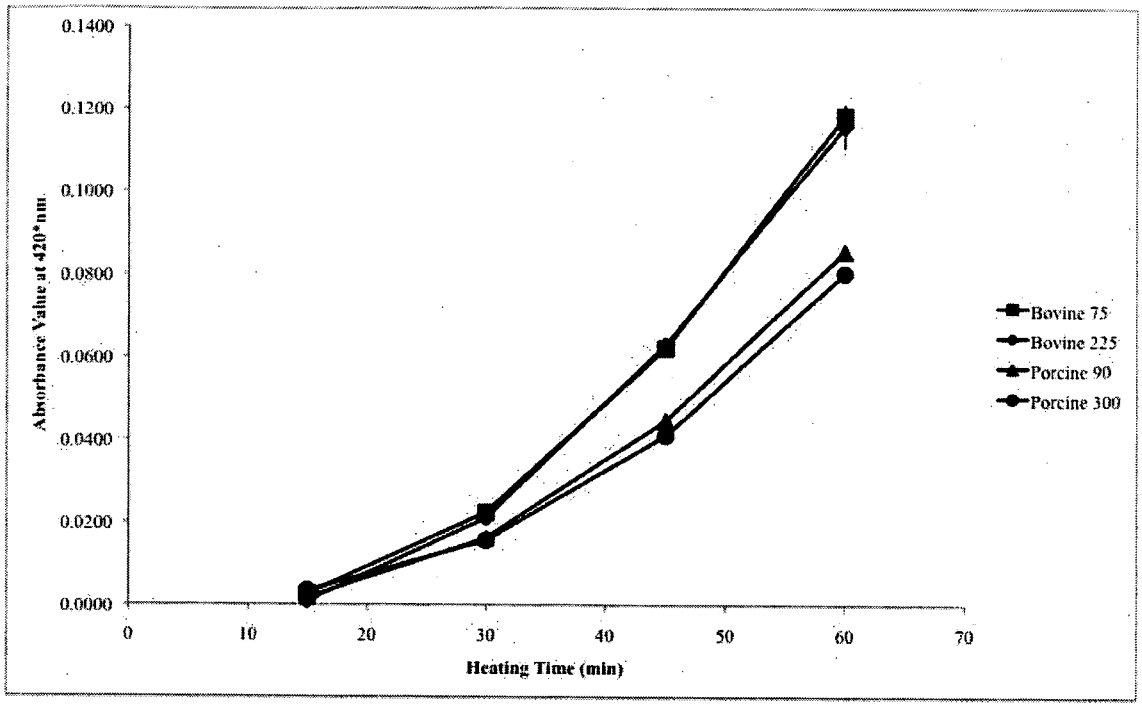

FIGURE 6 (B)

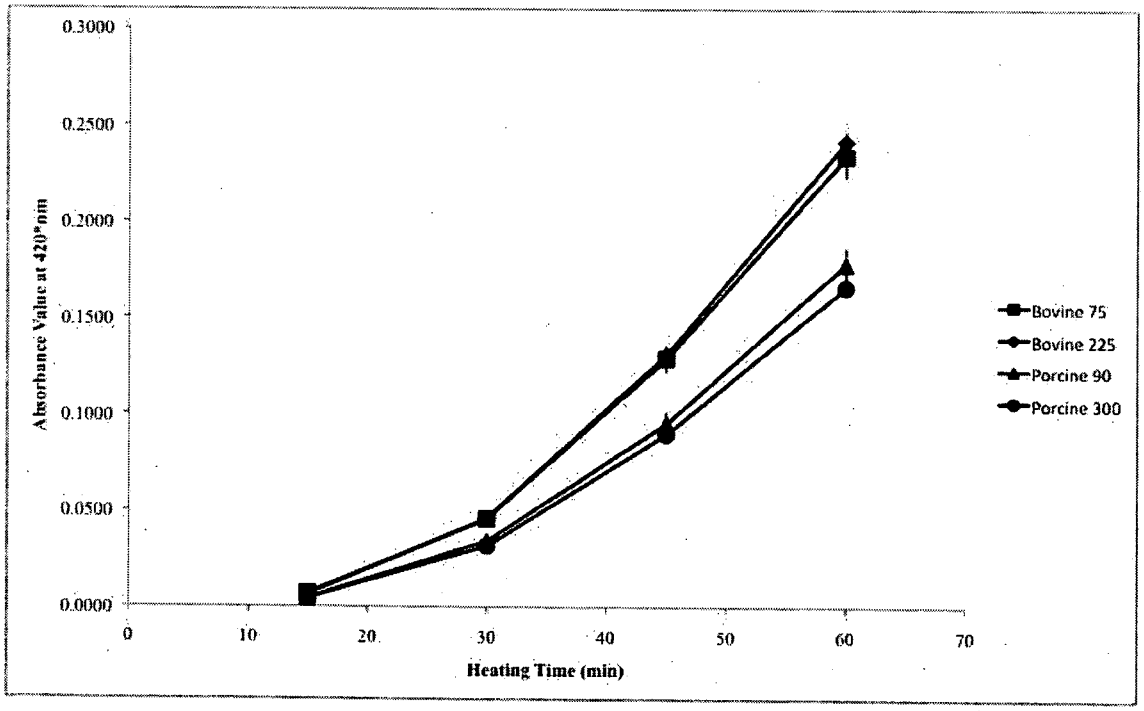

FIGURE 6 (C)

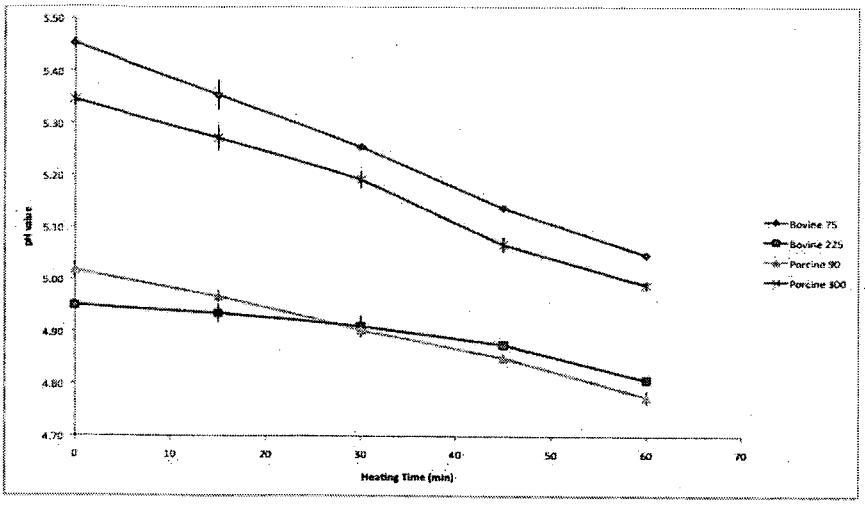

FIGURE 7

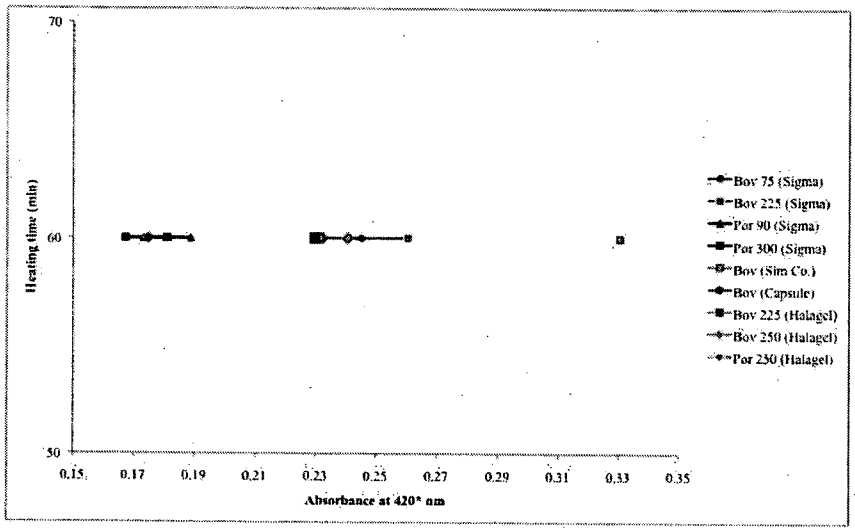

FIGURE 8

INTERNATIONAL SEARCH REPORT

International application No.
PCT/MY2010/000213

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.

G01N 21/31 (2006.01) *G01N 33/12* (2006.01) *G01N 33/68* (2006.01)
G01N 33/02 (2006.01) *G01N 33/52* (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPI, EPODOC, FSTA, BIOSIS, MEDLINE, CAPLUS, AGRICOLA. Keywords: gelatin, collagen, porcine, pig, pork, bovine, cow, cattle, beef, type A, type B, Maillard, reaction, browning, spectro+, absorbance, ribose, xylose, glucose, reducing sugar, quality control, halal, kosher.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	COURTINE R.J. (Ed.) (1990) "Larousse Gastronomique: The World's Greatest Cookery Encyclopaedia." English Edition. Mandarin Paperbacks, London. See page 52, col 1, lines 13-17 & 29-32.	1
X --- A	HASHIM D.M. *et al.* "Potential use of Fourier transform infrared spectroscopy for differentiation of bovine and porcine gelatins." Food Chemistry, (1 February 2010) vol. 118, pages 856-860. See abstract, p856 col 1 line 10- col line 5, p857 col 1 lines 1-19.	1,8

[X] Further documents are listed in the continuation of Box C [ ] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 January 2011	- 3 FEB 2011
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. +61 2 6283 7999	Authorized officer **JIVE BELLHOUSE** AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No : +61 2 6283 2959

INTERNATIONAL SEARCH REPORT

International application No.
PCT/MY2010/000213

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	SHIN S.Y. & SONG K.B. "Comparison between bovine hide and pigskin gelatins and preparation of gelatin hydrolysates." Journal of Food Science and Nutrition (1999) Vol. 4, No.1, pages 14-17.	
A	See abstract, p14 col 2 paragraph 1, Figure 1.	
	VENIEN, A. & LEVIEUX, D. "Differentiation of bovine from porcine gelatines using polyclonal anti-peptide antibodies in indirect and competitive indirect ELISA." Journal of Pharmaceutical and Biomedical Analysis (2005) Vol. 39, pages 418-424.	
A	See abstract, page 418 col 2 line 5 – p419 col 1 line 25.	
	ZHANG G. *et al.* "Mass spectrometric detection of marker peptides in tryptic digests of gelatin: A new method to differentiate between bovine and porcine gelatin." Food Hydrocolloids (October 2009) Vol 23, Issue 7, pages 2001-2007.	
A	See abstract.	
	HIDAKA S. & LIU S.Y. "Effects of gelatins on calcium phosphate precipitation: a possible application for distinguishing bovine bone gelatin from porcine skin gelatin." Journal of Food Composition and Analysis (August 2003) Vol. 16, Issue 4, pages 477-483.	
A	See abstract.	
	COLE, C.G.B. & ROBERTS, J.J. "The fluorescence of gelatin and its implications." Imaging Science Journal (1997), Vol.45 No.s3/4, pages 145-149.	
A	See abstract, p145 cols 1- p146 col 1, p147 col 2 par 2- p148 col 1 last par, p149 col 1 lines 1-4.	
	LIN, Y.K. & LIU, D.C. "Comparison of physical-chemical properties of type I collagen from different species." Food Chemistry (2006) Vol. 99, Issue 2, pages 244-251.	
L A	See abstract, p245 col 2 lines 4-16 & 24-40, p246 col 1 par 4, p248 par bridging cols 1-2, Figure 2, p250 col 1 lines 46-49.	

Box No. VIII (ii) DECLARATION: ENTITLEMENT TO APPLY FOR AND BE GRANTED A PATENT

The declaration must conform to the standardized wording provided for in Section 212; see Notes to Boxes Nos. VIII, VIII (i) to (v) (in general) and the specific Notes to Box No. VIII (ii). If this Box is not used, this sheet should not be included in the request.

Declaration as to the applicant's entitlement, as at the international filing date, to apply for and be granted a patent (Rules 4.17(ii) and 51*bis*.1(a)(ii)), in a case where the declaration under Rule 4.17(iv) is not appropriate:

IN RELATION TO THIS INTERNATIONAL APPLICATION,UNIVERSITI SAINS MALAYSIA (U.S.M) IS ENTITLED TO APPLY AND BE GRANTED A PATENT BY VITRUE OF THE FOLLOWING:
UNIVERSITI SAINS MALAYSIA IS ENTITLED AS AN EMPLOYER FOR THE FOLLOWING INVENTORS:

MAT EASA,Azhar
TAN,Thuan Chew
F.M ALKARKHI,Abbas

UNIVERSITI SAINS MALAYSIA IS A UNIVERSITY INCORPRATED UNDER THE LAWS OF MALAYSIA.

☐ This declaration is continued on the following sheet, "Continuation of Box No. VIII (ii)".