

Production of artificial sausage casings from whey proteins

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Abstract: This research and product development work is a contribution to both the Dairy and Meat Industry. The work involved the production of artificial sausage casings from whey proteins. The main objective was to utilize whey, a by-product from cheese production and produce an edible film which can be utilized as an alternative sausage casing material. The work was based on the whey proteins film forming capabilities. The formation of whey protein-based films mainly involved heat denaturation in aqueous solution at 75 -100°C, which produced intermolecular disulfide bonds, which were responsible for film structure. A plasticizer was added to impart flexibility and extensibility of the edible polymeric film. Addition of a cross-linking agent was for the formation of chemical links between molecular chains to form a three-dimensional network of connected molecules. Harvesting of the edible films was done via the casting method.

Keywords: Whey, Whey Protein, Film, Casingscollagen, Cross Linking Agents, Moisture Content

1. Introduction

Whey is a slightly acid, yellow-green liquid which is the residue obtained from the coagulation of milk by rennet or by the lowering of its pH. A considerable volume of whey is produced in the world, and it has continued to increase in recent years. Whey is a strong pollutant when discharged into streams, its high organic matter content leading to a biochemical oxygen demand ranging from 30 to 40 g of oxygen per litre. As a result, the pollution load from a cheese factory processing 100000 litres of milk per day would be equivalent to the pollution of a city of 60 000 inhabitants (Petillot, 1976). Given the large quantity of whey produced worldwide each year, the risks of pollution are therefore extremely high.

Whey was discovered 3000 years ago when calves stomachs were used to transport milk. Through the action of the naturally occurring enzyme chymosin found in calves' stomachs, the milk coagulated during storage and transport, resulting in curds and whey, and as such spawned the start of the cheese industry [78]. Despite being considered as a medicinal agent in the 17th and 18th

century, whey came to be regarded as waste of the dairy industry that was disposed of as effluent or as animal feed [84]. In the late 20th century, restricted environmental regulations prevented disposal of untreated whey.

Simultaneously, the dairy industry realized that whey represents an excellent source of functional and nutritional proteins and peptides, lipids, vitamins, minerals and lactose, which have helped to transform whey from a waste material to a valuable dairy stream [78].

There are various methods of utilizing or disposing of whey. It can be dumped at the production site, provided that the land area is large enough and the soil permits the absorption of the mineral elements and the organic matter. The principal constituents of whey can also be separated either by precipitation or by passing through an ultra-filter [40]. The resulting proteins can be utilized for the manufacture of certain types of cheese; the lactose obtained by the crystallization of the raw or concentrated product could be utilized for human food or by the pharmaceutical industry. Whey is also an excellent substrate for the cultivation of yeasts; there are various procedures which make it possible to obtain large quantities of protein, lactic acid, ethyl alcohol and vitamins by this technique.

However, animal feed is by far the principal outlet for whey [75]. It has been used in liquid form, principally for pigs, when the available quantity was not large and could be used nearby. However, for practical reasons of transport and storage this pattern of use has been progressively superseded by drying for animal feed. This involves increased production costs which limit its use to the feeding of young animals (calves or young pigs) or fattening pigs. Even though recycled in several ways, too much liquid whey is still wasted in the environment and consequently there is a significant interest in finding new applications to avoid the pollution mostly due to the whey proteins [47].

Protein-based edible films have received considerable attention in recent years because of their advantages, including their use as edible packaging materials, over the synthetic films [7]. In addition, protein-based edible films can also be used for the individual packaging of small portions of food, particularly products that are not currently individually packaged for practical reasons, such as beans, nuts and cashew nuts. Protein-based edible films can also be applied inside heterogeneous foods at the interfaces between different layers of components. They can be tailored to prevent the deterioration of inter-component moisture and solute migration in foods such as pizzas, pies and candies [31].

Numerous studies demonstrated that whey proteins have interesting nutritional value and possess several functional properties important for biofilm formation. Whey proteins are by-products of cheese manufacture and contain two major protein types, α -lactalbumin and β -lactoglobulin. A huge amount (20 106 t) is produced per year, much of which is never used [48] 9). Formerly, this fluid whey was discharged into a river, which represents a risk for the environment.

This study builds on past literature showing that edible coatings made of whey proteins offered good aroma, fat, humidity, and oxygen barriers on, among others, peanuts, salmon, fruits, or cereals. Such coatings helped to improve the shelf life of, for example, peanuts, by retarding the lipid oxidation causing rancidity [44]. In addition, these edible films were reported not to modify the sensory attributes of the coated good or its aspects, while providing some health benefits for the consumer. A number of authors have also reported the good barrier properties of whey protein-based coating on paper, but also plastic substrates [26]

Edible coatings and films can be advantageously used on meat and meat products with the following benefits [28]:

- moisture loss reduction during storage of fresh or frozen meats;
- retention of juices from fresh meat and poultry when packed in plastic trays;
- oxidation/reduction of lipids and myoglobin;
- reduction of spoilage and pathogen microorganisms on the surface of coated meats;
- restriction of volatile flavor loss and foreign odor pick up.

Casings are soft cylindrical containers used to contain

sausage mixes. Casings can be of natural origin or artificial. Natural casings are obtained from animal intestines derived from slaughtering. Manufactured artificial casings are made of cellulose, collagen or synthetic materials. Sausage fillings are mostly minced or comminuted meat mixes held together by the casings during further processing steps such as smoking, boiling, frying or roasting. In addition, casings also protect products during storage.

To keep up with the ever growing demand in the meat industry, there is the need for alternative forms of casings as the natural casings alone are not sufficient for the growing consumer demand. The consumer has become health conscious, requiring healthier products. When compared with the natural casings, the whey protein casings have negligible microbial contamination [74]. The whey protein casings offer less health risks than the synthetic ones. In fact, Polyvinylidene chloride (PVDC)-based casings have been found to contain phthalates that are harmful for the endocrine system especially in children [41]. Increased use of synthetic packaging materials has led to the serious environmental problems due to their non-biodegradability. Whey proteins have an interesting nutritional value and possess several functional properties important for biofilm formation. Whey proteins are of high quality, since they have all the essential amino acids and a biological value higher than egg or casein proteins and also some functional properties of interest to the food industry, such as solubility, emulsification, foaming, gelation, and viscosity development. These functional properties are important for biofilm formation. There is therefore the need to fully utilize these whey proteins rather than just allowing them to go to waste.

The discarding of whey was witnessed by the researchers during a plant tour at Kefalos Dairy Products, Mubaira, Harare. The average daily whey output was around 15 000l but only a fraction of that was retained for ultrafiltration. Most the whey was disposed due to lack of storage space since ultrafiltration was not a daily operation. Ultrafiltration brought about whey concentrate that was used in ice-cream production. Seeing so much high volumes of whey proteins go to waste pushed the researchers to brainstorm on means of utilizing whey before it is disposed.

1.1. Proteins as a Film Forming Material

The formulation of films and coatings requires the use of at least one component capable of forming a structural matrix with sufficient cohesiveness [17]. Biopolymeric materials, which provide such a cohesive matrix include: cellulose, starch, chitosans and protein. These biopolymers can produce a film or coating that is both biodegradable and edible. Proteins are one of the natural biopolymer materials that have most successfully used in film and coatings [14].

The monomers of proteins are amino acids, which in addition to amino (-NH₂) and a carboxyl (-COOH) groups, also have different side groups attached to the central carbon. The side groups lend unique character to individual

amino acids and may be hydrophobic, hydrophilic, positively or negatively charged. The individual amino acids are linked together by peptide bonds in a sequential order specific to each protein species forming a polypeptide chain. Depending on the sequential order of the amino acids in the polypeptide chain, the protein will assume different structures. The secondary structure is the spatial structure that the polypeptide chain assumes a native structural conformation that is thermodynamically stable. The native conformation is related to the polarity, hydrophobicity and steric hindrance of the amino acid side chains.

The interactions and linkages involved in the formation and stabilizing the secondary and tertiary protein structures include steric interactions, van der Waals interactions, hydrogen bonding, electronic interactions, hydrophobic interactions, and disulphide cross-links. It is these interactions and linkages, which stabilize the native protein that are taken advantage of in the formation and stabilization of the protein network in a protein-based film. The successful use of proteins in film and coating production is due to their polymeric structure arising from the different amino acid monomers [14].

The mechanical properties of films depend on the type of film forming material especially on its structural cohesion, which in turn depends on the structure of the polymer and especially its molecular strength, geometry and the type of and position of its lateral functional groups [7]. Regularly branched polymer molecules like proteins attend to have greater cohesive strength than the more uniform non-branched polymers like polysaccharides [14]). Also, because proteins are made up of different amino acid monomers, unlike polysaccharides, which have the same monomer, their complex structure offers more functional properties [53], 2002). Thus proteins have potential for forming intermolecular bonding and interactions at numerous positions [30]. This could be instrumental in the modification of the functional properties of protein films.

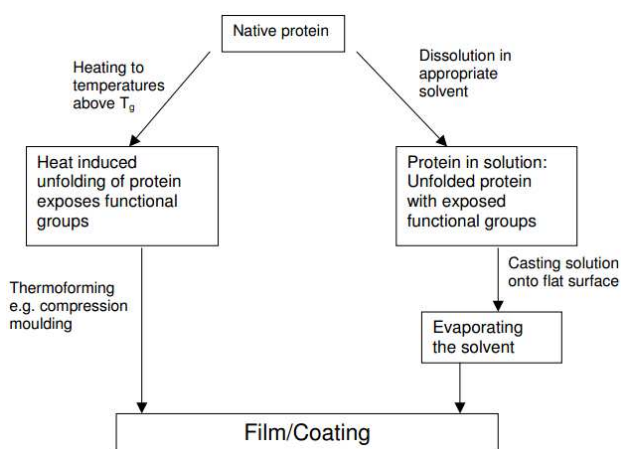


Fig. 1. Protein film/coating forming procedures - adapted from Krotcha (2002)

In the making of films, the native protein structure is

disrupted either by dissolution in an appropriate solvent or heating the protein to temperatures above its glass transition temperature (T_g). Proteins are formed into a film by either casting the protein solution onto a flat surface followed by evaporating off the solvent or by thermoforming e.g. extrusion, compression or injection moulding. Figure 1 is a schematic presentation of the two film making procedures. As the film is formed, the protein interactions and linkages that stabilize the native protein are re-formed. New protein interactions resulting from process treatments may be formed to stabilize the film network and confer its properties [53].

Table 1. Advantages and disadvantages of casing types

	Natural	Collagen	Cellulose
Cost	Most expensive	Less expensive	Least expensive
Refrigeration storage	Yes	Yes	No
Degree of tenderness	Most tender	Less tender	Peeled
Break during processing	Most likely	Less likely	Least likely
Casing preparation cost	Most expensive	None	None
Soaking and flushing before use	Yes	No	Sometimes soaking
Ease of smoke penetration	Most penetration	Less penetration	Least penetration
Best machinability	Least	Less	Best
Best product yield per foot of casing	Least	Less	Best
Finished product yield per foot of casing	Least	Less	Best
Finished product uniformity	Least	Less	Best
Cost of casing removal	None	None	Most
Printability	None	Limited	Best
Old World appearance	Best	Less	None
Ease of plant storage	Least storage	Less storage	Best storage

Source: Ockerman 1996, Chemistry of Meat Tissue. The Ohio State University, Columbus, USA

1.2. Cross-Linking Agents

Crosslinking is the process of chemically joining two or more molecules by a covalent bond. Crosslinking reagents contain reactive ends to specific functional groups (primary amines, sulphhydryls, etc.) on proteins or other molecules. Because of the availability of several chemical groups in proteins and peptides that may be targets for reactions, proteins and peptides are readily conjugated and otherwise studied using crosslinking methods. Cross linkers also are commonly used to modify nucleic acids, drugs and solid

surfaces. Crosslinking reagents have been used to assist in determination of near-neighbor relationships, three-dimensional structures of proteins, solid-phase immobilization, hapten-carrier protein conjugation and molecular associations in cell membranes. They also are useful for preparing antibody-enzyme conjugates, immunotoxins and other labeled protein reagents.

Cross linkers (CL) are either homo- or hetero-bifunctional reagents with identical or non-identical reactive groups, respectively, permitting the establishment of inter- as well as intra- molecular cross linkages. Conformational changes of proteins associated with a particular interaction may be analyzed by performing crosslinking studies before and after the interaction occurs. Comparing cross linkers with different arm lengths for success of conjugation can provide information about the distances between interacting molecules.

2. Methodology

2.1. Sources of Data

Primary data was obtained from the following: Kefalos Dairy Industry, Standards Association of Zimbabwe (SAZ), Arg Laboratories and Laboratory experiments and analysis

Secondary data was obtained from the following: Internet, books, journals, publications, oral interviews and lecturers

2.2. Film Formulation

One of the objectives of this study is to evaluate the composition and characteristics of the whey concentrate. This is of importance as it gives a measure of the whey protein content after ultrafiltration. This in turn allows for the adjustment of the protein content to levels that facilitate for the formation of a film.

Whey concentrate composition analysis was carried out by Arg Laboratories using the Bentley 2000.

2.3. Materials

Whey protein concentrate (WPC) with a protein content of (10%) by Kefalos Dairy Products (Mhondoro, Zimbabwe) was used to produce the whey protein based films. To overcome film brittleness glycerol (98% purity) as plasticizer (Labchem, South Africa) was used. Formaldehyde (Labchem, South Africa) and CaCl_2 were used as additives.

2.4. Method

Aqueous solutions of 10% (wt/wt) whey protein concentrate were prepared and heated to 75 °C for 1 h with constant agitation using a stirrer. Solutions were cooled to room temperature (below 30 °C) and the coagulant that was formed was filtrated using a mutton cloth. The pH of the solution was adjusted before and after heat treatment to pH 7 using 0.1 M HCl, 0.1M NaOH, or both. The appropriate

weights of glycerol were then added to plasticize the films (40 g/100 g of DM), and formaldehyde (9 g/100 g of DM) and CaCl_2 (at 0.05 g/100 g of DM) were added to crosslink the films. The film-forming solutions were cast on a plate and evenly spread out. The thin layer of film-forming solution was dried in a ventilated oven at 30 °C for 18 to 24 h.

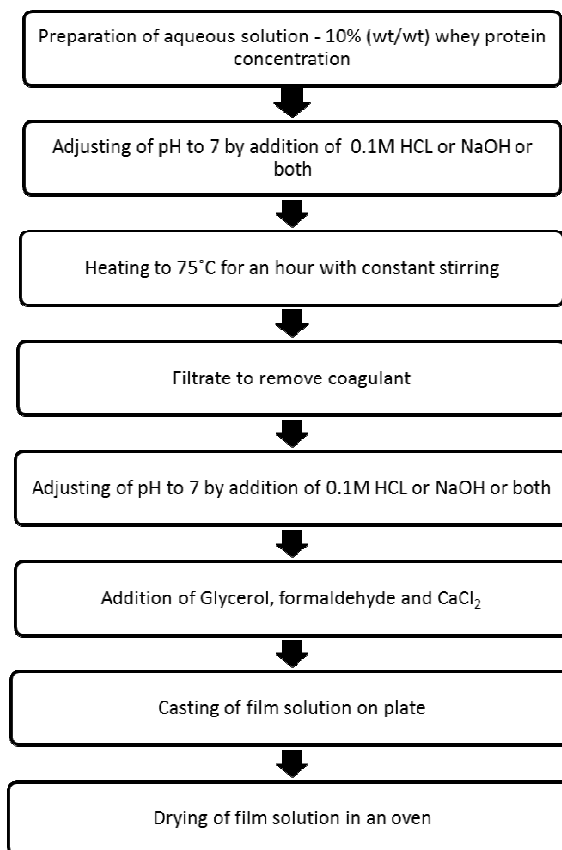


Fig. 2. Diagrammatic Representation of Procedure

2.5. Film Characterization

2.5.1. Thickness

The film thickness was measured using a micrometer (Model M120, Braive Instruments), to the nearest 0.001 mm; the thickness was calculated as the average of five measurements, taken at different locations on each film sample.

2.5.2. Density

The film density (P^s) was calculated directly from the film weight and dimensions, according to:

$$P^s = m/A \times \delta$$

where:

A is the film area (12.8cm² in this case),

δ the thickness (cm),

m the dry mass (g) and

P^s the dry matter density (g cm⁻³).

The film density was expressed as the average of five

independent determinations.

2.5.3. Moisture Content (MC)

The moisture content (MC) of the protein films was determined after drying in an oven at 105 °C, under forced air circulation for 24h. Small specimens (0.200g) of films were cut after adequate conditioning, and placed on Petri dishes. These were weighed before and after oven drying. MC values were determined as a fraction of initial film weight lost (ASTM, 1994) during drying, and reported on a wet basis.

2.5.4. Solubility (S)

The water solubility was determined According to Irissin-Mangata *et al.* (2001). The Samples of biofilm were weighted and the value of its mass was named (*idm*), subsequently the samples were immersed in 100 mL of distilled water and the system maintained under mild agitation at 25 °C for 24 h. The end dry mass (*edm*) was determined by subjecting this sample to oven drying (105 °C for 24 h). The solubility of the biofilm (S) was expressed in terms of the initial dry mass (*idm*) of the biofilm through the Eq (1).

$$S = [(idm - edm) / (idm)] \times 100$$

2.5.5. Biodegradability and Digestibility

Biofilms were incubated in the pancreatin solution, and their weight loss was determined as a function of time (15 min interval). The enzyme solution contained 1% pancreatin 50 mM potassium phosphate buffer (pH 7.5). Each film of 8.5 cm diameter was incubated in 50 mL of enzyme solution at room temperature (23 °C).

2.5.6. Heat Stability

Small pieces of the casing were placed in cooking oil that was kept constant between 160 - 190°C for 5minutes

3. Results and Discussion

3.1. Film Appearance



Fig. 3. A picture of the whey protein based casing

Casings were transparent, flexible and homogenous. The surfaces appeared smooth, without visible pores or cracks. The casing side not facing the casting plate was indeed shiny and the other was dull, which is likely an indication of some phase separation occurring in the solution during drying. The casings were difficult to separate from the casting plates.

3.2. Thickness, Density, Moisture Content and Solubility

Thickness: The whey protein based casings exhibited a thickness of 0.17±0.04 mm for the five measurements taken at different points across the casing. The thickness of edible films and coatings is an important parameter since it directly affects the biological properties and the shelf life of the coated food. The effectiveness of edible films and coatings for protection of food depends primarily on controlling the spreading of the coating solutions, which affect the thickness of the film.

However, films and coatings must not exceed a critical thickness so as not to reduce drastically the internal O₂ concentration and to increase CO₂ concentration from anaerobic fermentation. Coating thickness depends on the physical properties of the solution (such as density, viscosity, and surface tension) but also of the method used in the film formation: dipping, spraying solvent casting, skimming, etc. [13].

Solubility: The plasticized casings were clearly not dispersed after a 24hr immersion in water and had no visual loss of integrity. The solubility was determined to be 38 ± 5% as shown in table 2. This relative insolubility in water is consistent with the observation that whey protein films are largely insoluble [20]. The high interaction density and the presence of intermolecular covalent bonds or physical knots could be responsible for part of the insolubility of these films.

According to results of McHugh and Krochta., 1994 and Fairley *et al.*, 1996, whey protein-based films are partially insoluble in water because of the presence of intermolecular disulfide bondings. The protein polymer network of films resisted solubilization in aqueous buffers.

Moisture: The moisture content of whey protein concentrate casings were determined in to be 33 ± 1,5%. Moisture content of the film is an important property because hydrophilic films absorb moisture more readily at higher humidity levels, thereby increasing the plasticizing effect of water, which influences the other functional properties of the films [10]. Whey protein based films are hydrophilic in nature and have strong protein-protein interactions mainly due to non-covalent attractive forces like hydrogen bonds and hydrophobic interactions [56]

Density: As shown in the table below, the density of the casings was determined to be 1.29 g cm⁻³. Sorbitol, glycerol and polyethylene glycol are low molecular weight hydrophilic molecules that could easily fit into protein chains and establish hydrogen bonding with reactive groups of proteins. Bringing together plasticizers and proteins induced formation protein-plasticizer interactions to the

detriment of protein-protein interactions. As a consequence, the density of intermolecular interaction in material decreased and the free volume between polymer chains increased [14].

Plasticizers are known to reduce the tension of the deformation, hardness, density, viscosity and electrostatic charge of a polymer. At the same time they increase the polymer chain flexibility, resistance to fracture and dielectric constant [85].

Table 2. Results of thickness, density, moisture content and solubility

Thickness(mm)	Density(gcm ⁻³)	Moisture Content (%)	Solubility (%)
0,17 ± 0,04	1.29	33 ± 1,5	38 ± 5

3.3. Biodegradability and Digestibility

The biodegradability and digestibility of the casings was described by the data that is shown in table 2 and figure 4.

Table 3. Results of Yield Recovery vs Time

Yield of Recovery (%)	100	95	90	70	60	40	35	30	22
Time (mins)	0	15	30	45	60	75	90	105	120

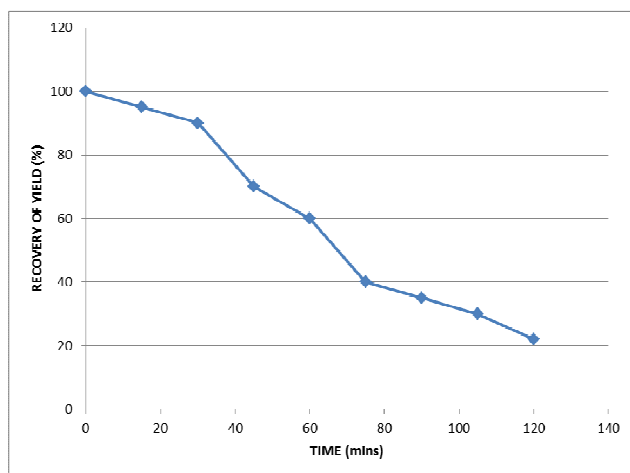


Fig. 4. Graphical representation of Yield of Recovery vs Time

The essential factor in the high stability of whey protein films to pancreatin attack could be the presence in whey protein concentrate of other components such as fatty acids, which can hinder the access to the proteolysis site, reducing thus the catalytic activity of the enzymes.

3.4. Heat stability

When subjected to ideal frying temperatures and time the casing did not disintegrate. The casings exhibited high heat stability. They remained intact indicating that they would be ideal for frying.

4. Conclusion and Recommendations

Samples that were sent to Arg Labs indicated that the amount of protein content in the whey concentrate was 10%. This meant that there was no need to adjust the protein composition when conducting experiments as it met the required levels according to McHugh et al., 1994.

Upon introduction of heat, there is the formation of a white coagulant. The formation of this coagulant is due to keratin proteins that are left-over after removal of casein proteins during cheese production. This coagulant (curd) is used to produce an Italian cheese called ricotta [36]. These were removed by filtration to remain with a cloudy solution.

Heating of the whey concentrate should be done with constant stirring. However, this process has the adverse reaction of introducing air into the film solution. Normally, this air can be eliminated by the use of an ultrasonic water bath or use of a vacuum pump [77]. Neither of these two or alternative equipment could be sourced by the researcher. This will in turn run the risk of affecting the tensile strength of the casing even though it was not tested.

The film solutions were cast in petri dishes instead of the recommended Teflon coated plates. These Teflon coated plates have a non-sticky surface that ensures that when films dry off, they can easily be peeled off [30]. Unfortunately such plates could not be attained by the writer hence resulting in the films becoming difficult to remove from the petri plate surfaces.

Even though the tests that were run on the prototypes indicated that it is possible to use them as casings, they were still not sufficient to give conclusive evidence to warrant them as effective packaging systems. Such properties as water vapour permeability, oxygen permeability and tensile strength were not determined due to lack of adequate resources. If the casing readily allows the passage of gases and water vapour, it might end up being detrimental to the quality of the sausages [21].

It was established that even though it is viable to produce a casing from whey concentrate, it is still not as economical as the production of the non-biodegradable synthetic casings. The amount of film forming solution left at the end is almost half of the original whey concentrate volume at the beginning. Losses in volumes are registered during filtration of the coagulant as well as via evaporation. In as much as these casings may be environmentally friendly, production costs will probably continue seeing the natural and synthetic counterparts still being the first choice options.

To improve the properties of the prototype, the researchers recommend that a vacuum pump or an ultrasonic water bath be used to remove air from the air trapped in the film solution. The presence of this air in the solution has adverse effects on the mechanical properties of the casing [32]. Casting of the films should also be done on Teflon coated plates so as to ensure that harvesting of the films is easier [37]. To improve the tensile strength of the films, they can further be equilibrated in saturated solutions of NaCl or NaBr [8].

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