SOYMILK-BASED DAIRY ANALOGUES

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ABSTRACT

Soymilk based dairy analogues; cheese (Cheddar, Mozzarella, Brie) and yoghurt were prepared using traditional cheese and yoghurt making procedures. Commercial mesophilic, thermophilic and mesophilic bacteria/mould *Penicillium candidum* used to prepare soy Cheddar, Mozzarella and Brie cheese respectively. Soy oil and okara have been added to soymilk at two different concentrations (6 and/or 30%) and their effect on the chemical and textural parameters of soy cheese analogues prepared were evaluated.

Commercial yoghurt starter culture containing *Bifidus* and *Acidophilus* were used to prepare yoghurt. Tropical fruit "*Carica papaya*" have been added to yoghurt samples at two different concentrations (5 and 10%) and their effect on chemical and textural characteristics of soy yoghurt analogues were evaluated.

KEYWORDS:

Dairy milk, Soymilk, Dairy analogues, Cheese, Cheddar, Mozzarella, Brie, Yoghurt.

SUMMARY

In view of the potential health benefits of soybeans and the increasing world wide annual production, the applicability of cheese and yoghurt making procedures has been investigated in order to prepare soymilk based dairy analogues. Soy cheese analogues were prepared in which soymilk was inoculated with mesophilic bacteria (*Lactococcus lactis subsp lactis*) to prepare Cheddar cheese analogue and thermophilic bacteria (*Streptococcus thermophilus, Lactobacillus delbrueckii subsp lactis, Lactobacillus helveticus*) used to prepare Mozzarella analogue and two microorganisms (mesophilic bacteria and mould; *Penicilium candidum*) used to prepare Brie cheese analogue. Growth of these different starter cultures in soymilk did not give rise to three different products as (Cheddar, Mozzarella and Brie cheese) although there were some chemical and textural differences such as the sour taste "pH and lactic acid content" as recorded in soy Cheddar and the white surface as in soy Brie. Soy oil and okara have been used as a means to improve chemical and textural characteristics of cheese analogue samples. Soy oil and okara were added at two different concentrations in soymilk (6 and/or 30%), however, such fortification did not improve the overall quality of cheese analogues compared with control cheese analogues (without fortification).

Soy yoghurt analogue was prepared with yoghurt starter culture containing *Bifidus* and *Acidophilus.* Soymilk supported the growth of yoghurt starter culture and give rise to "soy dessert" like product more than a yoghurt product. Tropical fruit "*Carica papaya*" was added to soymilk at two different concentrations (5 and 10%) and/or $CaCl_2$ at 0.5%. Samples fortified with $CaCl_2$ showed syneresis and were excluded as acceptable product. However, the rest of

yoghurt analogues prepared were not acceptable as products due to textural and organoleptic defaults. Therefore, further research needs to be carried out to improve textural and organoleptic properties of soy dairy analogues prepared.

I . INTRODUCTION

In 1999 Food and Drug Administration has announced that soy protein included in a diet low in saturated fat and cholesterol may reduce the risk of coronary heart disease by lowering blood cholesterol levels. Based on this, soy is considered a new hero in the health food industry and the nutrition world.

Soy is a versatile ingredient for food service professionals and desirable for a growing number of consumers. To facilitate use of soy in food industry, soy-based food products should be prepared, studying and analysing these products is required to assess suitability and edibility of these products. Some of the soy-based products; meat alternatives (meat analogues) and dairy analogues. In this study, dairy-like products (dairy analogues); cheese and yoghurt will be prepared using soymilk as a base.

I . 1) Milk

Milk is a unique food, forming an integral part of the diet of much of the world's population. Milk can be defined as a fluid secreted by the female of all mammals, for the primary function of meeting the complete nutritional requirements of the neonate of the species. It must supply energy (mainly from fat and sugar [lactose]), amino acids from proteins, vitamins and minerals as presented in Table 1. Since milk is a rich source of nutrients, it can spontaneously support bacterial growth. Bacteria can utilize milk sugar (lactose) as a source of energy producing lactic acid as a byproduct, these bacteria, known as Lactic acid bacteria (LAB) are used in the production of a wide range of fermented milk products which are generally considered to have health benefits. Cheese and yoghurt are among the most important dairy fermented products (Fox *et al* 2000).

Cheese is the generic name for a group of fermented milk-based food products throughout the world present in a great diversity of flavours, textures and forms. It is believed that cheese first evolved in the Mediterranean area some 8000 years ago (Fox *et al* 2000). There are more than 1000 varieties of cheese world wide summarized in Figure 1. Cheese making is a simple process in itself, but it involves complex chemical and physical phenomena (Law 1999). Cheese making is a form of food preservation in which milk protein (casein) and fat are concentrated approximately ten-fold, and the milk sugar (lactose) is fermented into lactic acid by lactic acid bacteria. However the production of all varieties of cheese involves several interrelated operations, namely coagulation, acidification, water removal (syneresis, pressing), salting and ripening, (Wood 1998). A general cheese manufacturing protocol is presented in Figure 2.

Table 1: Milk nutrient composition. USDA National Nutrient Database for Standard Reference. Available at: www.nal.usda.gov (NDB NO: 01077)

Nutrient	Units	Value per 100 grams of edible portion	Number of Data Points	Std. Error
Proximates				
Water	g	88.32	12	0.096
Energy	kcal	60	0	0
Protein	g	3.22	15	0.015
Total lipid (fat)	g	3.25	0	0
Ash	g	0.69	12	0.003
Carbohydrate, by difference	g	4.52	0	0
Fiber, total dietary	g	0.0	0	0
Minerals				
Calcium, Ca	mg	113	0	0
Magnesium, Mg	mg	10	0	0

Phosphorus, P	mg	91	0	0
Potassium, K	mg	143	0	0
Sodium, Na	mg	40	0	0
Vitamins				
Vitamin C	mg	0	4	0
Vitamin D	IU	40.431	12	3.101
Lipids				
Fatty acids, total saturated	g	1.865	0	0
4:0	g	0.075	12	0
6:0	g	0.075	12	0
8:0	g	0.075	12	0
10:0	g	0.075	12	0
12:0	g	0.077	12	0.002
14:0	g	0.297	12	0.011
Fatty acids, total monounsaturated	g	0.812	0	0
16:1 undifferentiated	g	0.000	12	0
18:1 undifferentiated	g	0.812	12	0.032
20:1	g	0.000	12	0
22:1 undifferentiated	g	0.000	12	0
Fatty acids, total polyunsaturated	g	0.195	0	0
18:2 undifferentiated	g	0.120	12	0.005
18:3 undifferentiated	g	0.075	12	0
22:5 n-3	g	0.000	12	0
22:6 n-3	g	0.000	12	0
Cholesterol	mg	10	12	0.549
Amino acids				
Tryptophan	g	0.075	0	0
Threonine	g	0.143	0	0
Isoleucine	g	0.165	0	0
Leucine	g	0.265	0	0
Lysine	g	0.140	0	0
Methionine	g	0.075	0	0
Cystine	g	0.017	0	0
Phenylalanine	g	0.147	0	0
Tyrosine	g	0.152	0	0
Valine	g	0.192	0	0
Arginine	g	0.075	0	0

Histidine	g	0.075	0	0
Alanine	g	0.103	0	0
Aspartic acid	g	0.237	0	0
Glutamic acid	g	0.648	0	0
Glycine	g	0.075	0	0
Proline	g	0.342	0	0

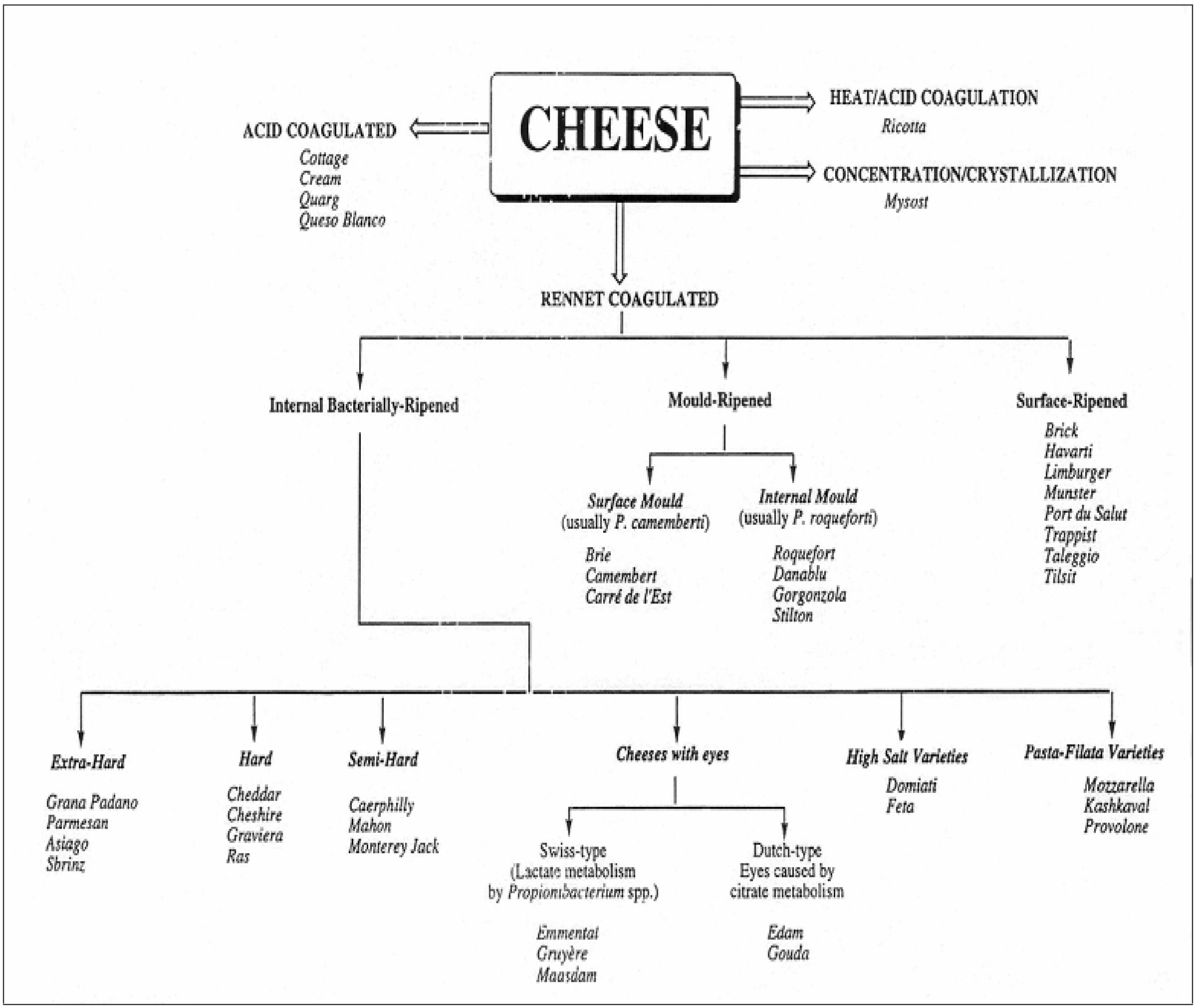


Figure 1: Classification of cheese families. Adapted from Fox *et al* 2000.

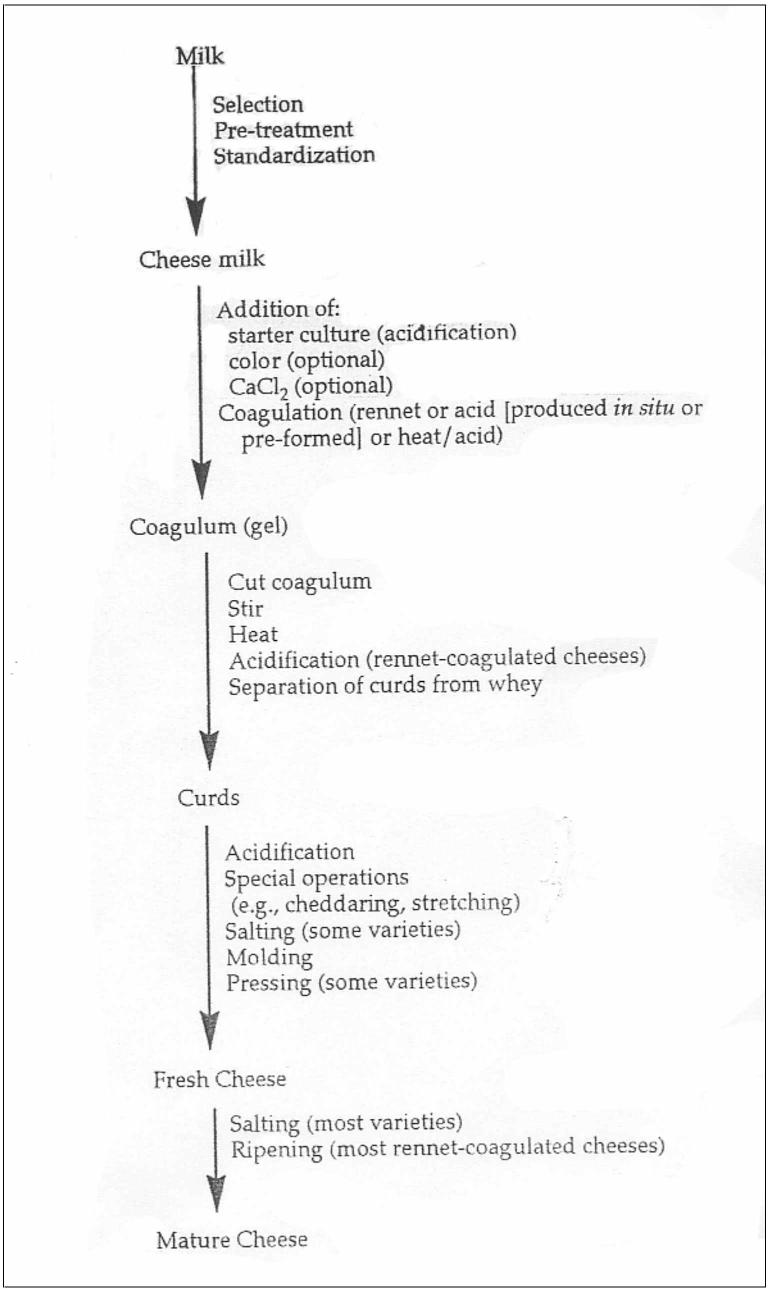


Figure 2: General protocol for cheese manufacture. Adapted form Fox *et al* 2000.

Yoghurt is defined as a food produced by culturing milk with bacterial culture that contains the lactic acid bacteria, *Lactobacillus delbrukii subsp. bulgaricus* and *Streptococcus thermophilus* (Hui 1993). There are various types for yoghurt classification, one is based on the microbial species which dominate. Based on this classification, yoghurt types are divided into three broad categories as follows:

- Lactic fermentation (*e.g.* yoghurt).
- Yeast –lactic fermentation (*e.g.* kefir).

Mould – lactic fermentation (*e.g.* Villi). Villi is a Finnish fermented milk, made with a ropy strain of *Lactococcus* sp.

Another classification of yoghurt types is based on the physical state of yoghurt production as illustrated in Table 2.

Table 2: Classification of all yoghurt products. Adapted from Tamime and Robinson 1999.

Category	**Physical state**	**Yoghurt products**
I	Liquid/viscous	Yoghurt
II	Semi-solid	Concentrated/strained
III	Solid	Frozen
IV	Powder	Dried

However, there is a general yoghurt production protocol which consist of a few main steps as follows:

- Heating the milk for a period of 5-30 min; the precise time depending on the temperature selected.
- Inoculating the milk with a bacterial culture in which *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* are the dominant organisms.
- Incubating the inoculated milk under conditions that promote the formation of a smooth viscous gel with the desired flavour/texture.
- Addition of fruits or other ingredients

➢ Cooling and packaging for distribution under chilled conditions.

Commercial yoghurts are divided into three main categories, plain/natural, fruit and flavoured and these types are manufactured in either the set or stirred/drinking form as in Figure 3.

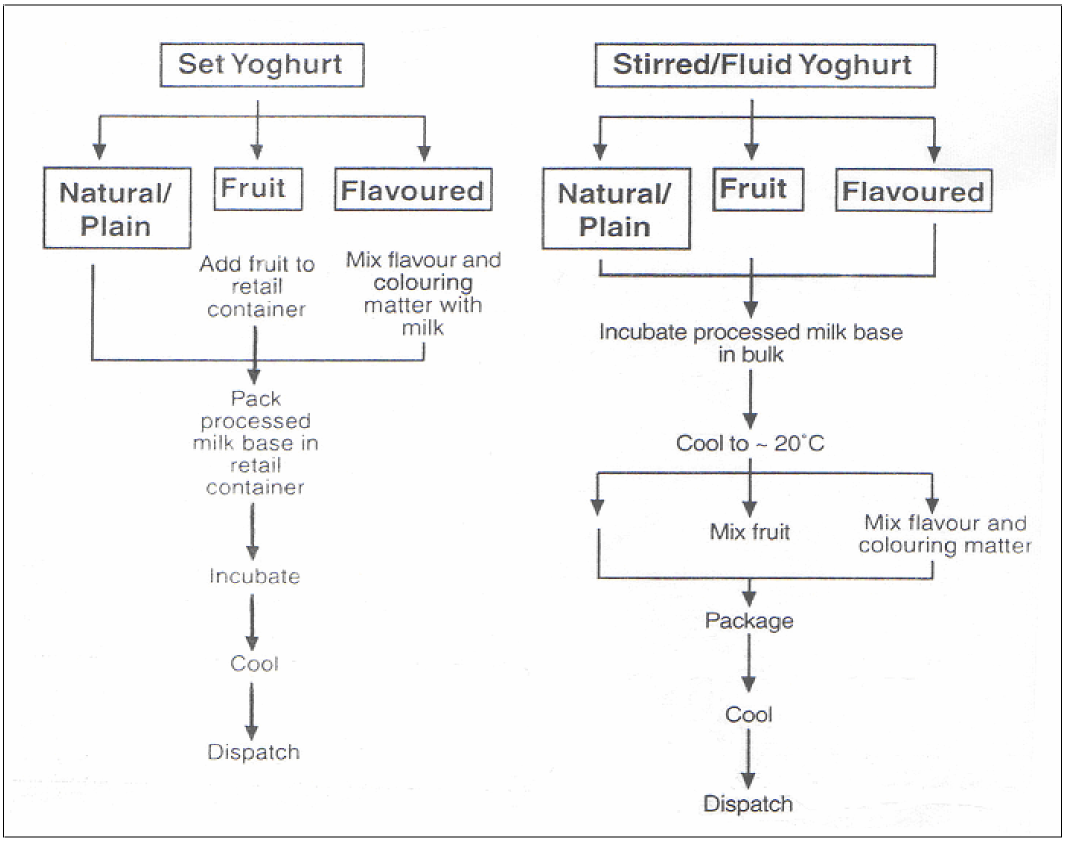


Figure 3: Manufacturing stages of yoghurt. Adapted from Tamime and Robinson 1999.

I . 2) Composition of Milk:

I . 2 . 1) Lactose:

Lactose is the main carbohydrate in the milk of all mammals. Milk contains trace amounts of other sugars, including glucose and fructose. Lactose is a disaccharide consisting of galactose and glucose linked by a β 1-4 glycosidic bond as shown in Figure 4. Lactose plays two important roles in milk and milk products; firstly, lactose contributes to the nutritive value of milk and its products. Secondly, lactose is essential in the production of fermented dairy products (cheese and yoghurt). In cheese and yoghurt, lactose is fermented to lactic acid by LAB, this process is vital since it causes reduction in pH of milk and the subsequent curd formation. This transformation is significant for people who are unable to digest lactose -(a problem known as lactose intolerance and this metabolic defect is the normal status of roughly half of the world's adult population (Wood 1998))- in which dairy fermented products (eg. cheese and yoghurt) help to solve this problem since the content of lactose is extremely low (Fox *et al* 2000, Hui 1993).

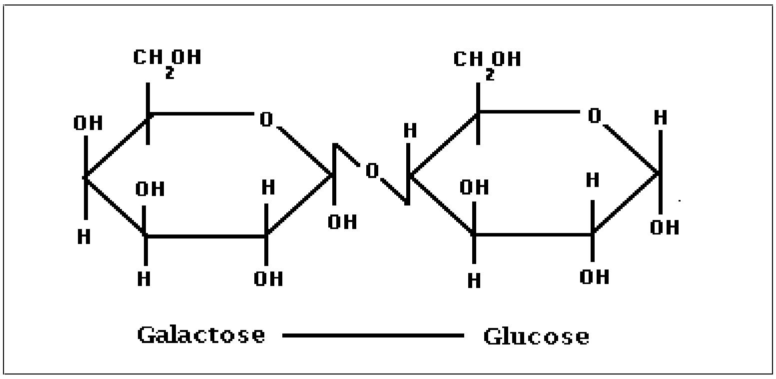


Figure 4: Structural formulae of lactose. Adapted from www.foodsci.uoguelph.ca/dairyedu/chem.html#overview

I . 2 . 2) Protein:

Milk proteins belong to two main categories that can be separated based on their solubility at pH 4.6 at 20°C, these are known as *caseins* while the proteins that remain soluble under these conditions are known as *whey* or *serum proteins*. Caseins are the main proteins involved in cheese and yoghurt production. Bovine caseins consists of four types of protein; αs_1-, αs_2-, β- and κ-casein, these make up 38%, 10%, 34% and 15% respectively. Minor components of the casein are the γ-caseins. Caseins are quite small molecules with molecular masses of 20-25 kDa as in Table 3.

Table 3: Principal proteins in dairy milk. Adapted from Fox *et al* (2000).

Protein	**Molecular wt (kDa)**
αs_1-casein	23.612
αs_2-casein	25.228
β-casein	19.005
κ-casein	23.980
γ_1-casein	20.520
γ_2-casein	11.822
γ_3-casein	11.557

All caseins are phosphorylated and relatively hydrophobic. κ-casein is glycosylated and contains galactose while (αs_1-, αs_2-, β-caseins) are not glycosylated. αs_1-, αs_2-, β-caseins constitute ~ 85% of whole casein and are calcium sensitive, they are precipitated by concentration of calcium greater than 6 mM. Since bovine milk contains about 30 mM calcium, it might be expected that these caseins would precipitate in milk. However, κ-casein is insensitive to calcium and it can stabilize up to 10 times its weight of the calcium sensitive caseins against precipitation by calcium. It does this via the formation of a type of quaternary structure known as *casein micelle* as illustrated in Figure 5. The micelles are composed of submicelles of mass around 5 x 10^6 kDa. The core of the submicelles is considered to consist of calcium sensitive αs_1-, αs_2-, β-caseins while κ-casein is concentrated at the surface. The submicelles are considered to be held together by microcrystals of calcium phosphate and perhaps hydrophobic and hydrogen bonds (Fox *et al* 2000), disulfide bonding, electrostatic interactions, van der Waals forces and colloidal calcium phosphate in the form of $Ca_9(PO_4)_6$ clusters acting to aggregate submiceles into micelles. The casein micelle is stable under conditions of processing (e.g. concentration, ultrafiltration, drying) but unstable under other conditions (eg. acidic conditions and presence of rennet enzyme) (Hui 1993). Manipulation of the casein micelles gives rise to many traditional dairy products.

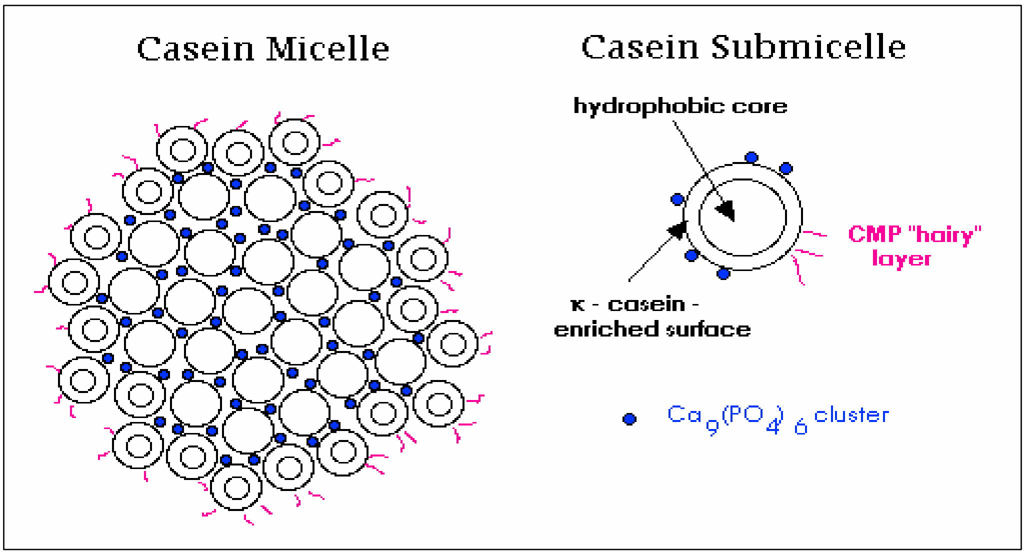


Figure 5: Schematic representation of the casein micelle and submicelle. Adapted from Hui 1993.

In cheese produced by rennet addition, κ-casein (molecular weight 19,000) is very resistant to calcium precipitation, stabilizing other caseins, the three dimensional structure of κ-casein is shown is Figure 6. Rennet cleavage at the Phe105-Met106 bond as in Figure 7, (Robinson and Wilbey 1998), eliminates the stabilizing ability leaving a hydrophobic portion, *para*-κ-casein, and a hydrophilic portion called κ-casein glycomacropeptide (GMP) or caseinomacropeptide (CMP). Once hydrolysed, the stability of the submicelles is reduced and the calcium sensitive caseins (αs_1-, αs_2-, β-) are exposed to the outside so they collide and build into a three-dimensional network, referred to as a coagulum or gel (Fox *et al* 2000).

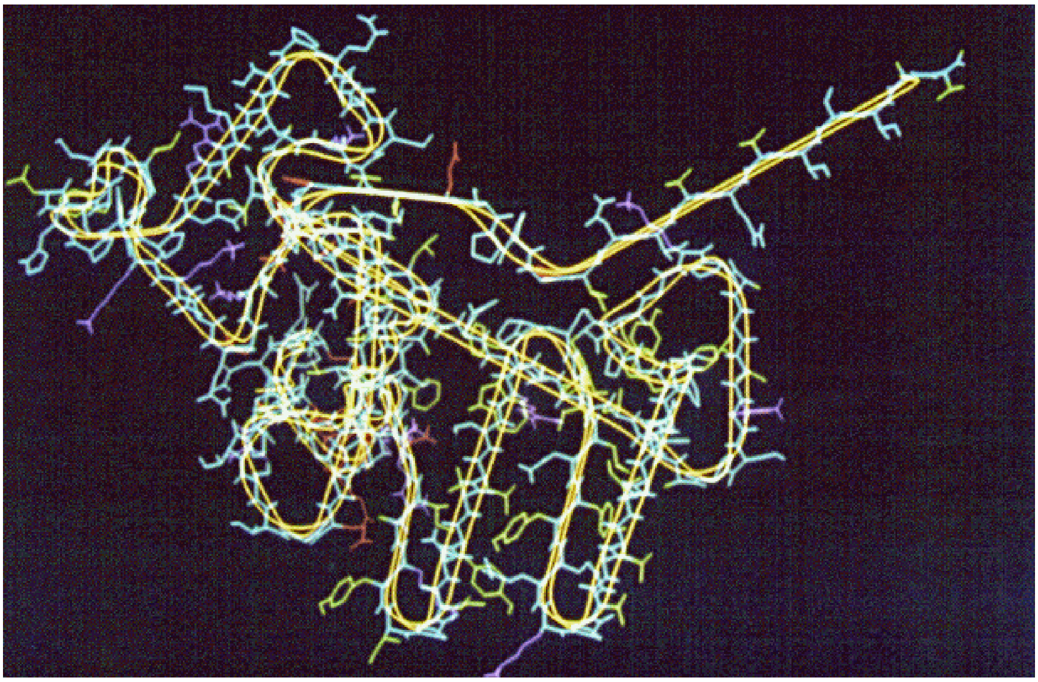


Figure 6: Three-dimensional model for κ-casein. Adapted from http://class.fst.ohio-state.edu/FST822/lectures/Cafunc.htm

1
Pyro- Glu-Glu-Gln-Asn-Gln-Glu-Gln-Pro-Ile-Arg-Cys-Glu-Lys-Asp-Glu-Arg-Phe-Phe-Ser-Asp-

21
Lys-Ile-Ala-Lys-Tyr-Ile-Pro-Ile-Gln-Tyr-Val-Leu-Ser-Arg-Tyr-Pro-Ser-Tyr-Gly-Leu-

41
Asn-Tyr-Tyr-Gln-Gln-Lys-Pro-Val-Ala-Leu-Ile-Asn-Asn-Gln-Phe-Leu-Pro-Tyr-Pro-Tyr-

61
Tyr-Ala-Lys-Pro-Ala-Ala-Val-Arg-Ser-Pro-Ala-Gln-Ile-Leu-Gln-Trp-Gln-Val-Leu-Ser-

81
Asn-Thr-Val-Pro-Ala-Lys-Ser-Cys-Gln-Ala-Gln-Pro-Thr-Thr-Met-Ala-Arg-His-Pro-His-

101 105 | 106
Pro-His-Leu-Ser-Phe↓Met-Ala-Ile-Pro-Pro-Lys-Lys-Asn-Gln-Asp-Lys-Thr-Glu-Ile-Pro-

121 Ile (Variant B)
Thr-Ile-Asn-Thr-Ile-Ala-Ser-Gly-Glu-Pro-*Thr*-Ser-*Thr*-Pro-*Thr*- -Glu-Ala-Val-Glu-
Thr (Variant A)

141 Ala (Variant B)
Ser-Thr-Val-Ala-Thr-Leu-Glu- - *SerP*-Pro-Glu-Val-Ile-Glu-Ser-Pro-Pro-Glu-Ile-Asn-
Asp (Variant A)

161 169
Thr-Val-Gln-Val-Thr-Ser-Thr-Ala-Val. OH

Figure 7: Amino acid sequence of κ-casein and the rennet enzyme cleavage site. Adapted from Fox *et al* 2000.

Amino acids are organic acids, which have $-NH_2$ (amino) group and a carboxylic end. Protein molecules have an electrical charge, which depends on the pH value of the solution. The charge can be positive or negative, depending on the availability of free amino groups or carboxylic groups. As long as the protein particles exist in the form of cations (in the acid range) or anions (in the basic range), they can move in an electric field or can repel each other if they are equally charged (Figure 8), therefore they remain in a soluble form (www.foodsci.uoguelph.ca). At pH 4.6 which is the isoelectric point of dairy milk, the solubility of casein is negligible, casein micelles disintegrate and casein precipitates as in Figure 9. In cheese prepared without rennet addition but with starter culture, as also in preparing yoghurt, the starter culture hydrolyses the sugar in the κ-casein causing a drop in pH and then the calcium sensitive casein (αs_1, αs_2, β) are exposed to the outside and they collide and form a gel. Aggregation occurs as a result of hydrophobic interaction (Hui 1993). Concentration of rennet and starter culture is a significant factor affecting κ-casein hydrolysis and the properties of the final product (Fox *et al* 2000).

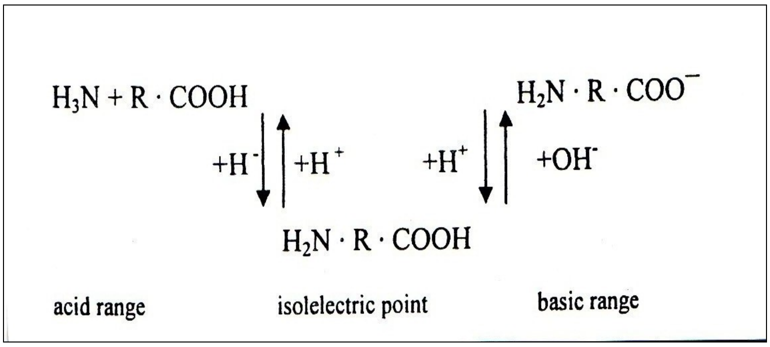


Figure 8: Isoelectric point of Dairy milk. Adapted from Spreer 1998.

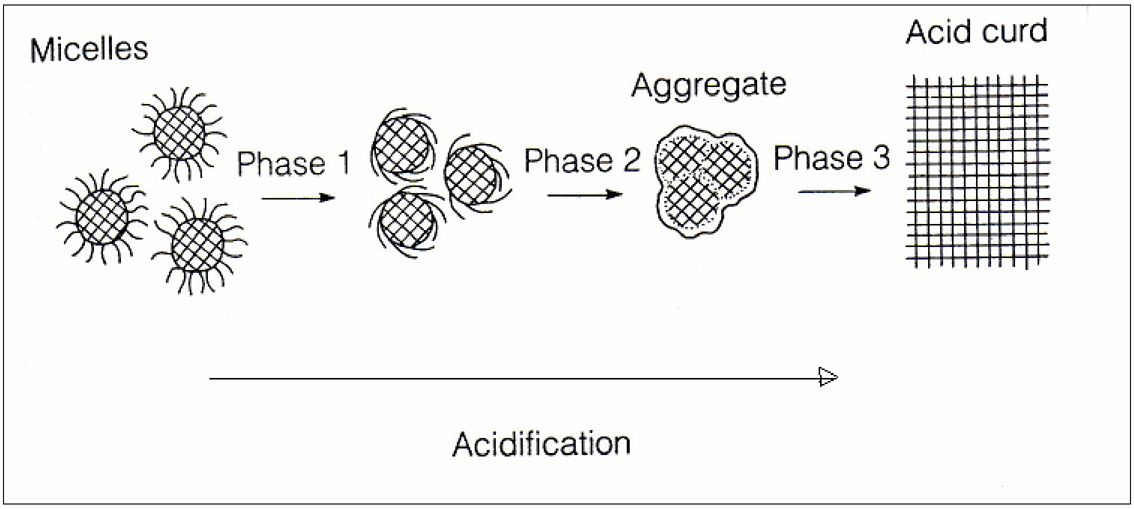


Figure 9: Curd formation by drop in pH as in yoghurt/cheese. Adapted from Varnam and Sutherland 2001.

I . 3) Soybeans

I . 3 . 1) Origin of Soybeans, Production and Marketing

Shu and *glycine max* are some of the terms referring to the same domesticate crop of soybean. Soybean first emerged around the 11th century in North China during the Zhou Dynasty and then it was known as *Shu*. *Shu* was found inscribed on tortoise shells from the Chang Dynasty (11th to the 16th century B.C.). In addition, soybean seeds have been discovered several times in relics unearthed in archaeological studies. For instance, in 1959, large amounts of soybean seeds dating back 2300 years were found in Shanxi Province. From China, soybean cultivation spread into Japan, Korea and throughout Southeast Asia. (Liu 1999, Hui *et al* 2001).

In Europe, soybean was first introduced by a German botanist as a curiosity. Later a Swedish botanist gave the genetic name '*Glycine max*' to soybeans. Glycine means 'sweet' and max means 'large' referring to the large nodules on the soybean plant as shown in Figure 10. Soybean introduction to the United States dates back to the mid-eighteenth century. It was reported in 1804 that soybeans grew in Pennsylvania. However, the large-scale official introduction did not occur until the early 1900s, resulting in a breakthrough in harvesting and processing of soybeans. In 1929, nine bushels (330.66 metric tons)* of soybean were produced. By 1939, the crop had increased to 91 million bushels (3343.34 metric tons). Until 1945 China led the world in soybean production and export, since then the United States has become the leader. The total estimated world production of soybean for the year 2000 was 6.299 million bushels, United States produce ~45% of it as illustrated in Figure 11.

*one bushel = eight gallons = 36.4 litre (USA).

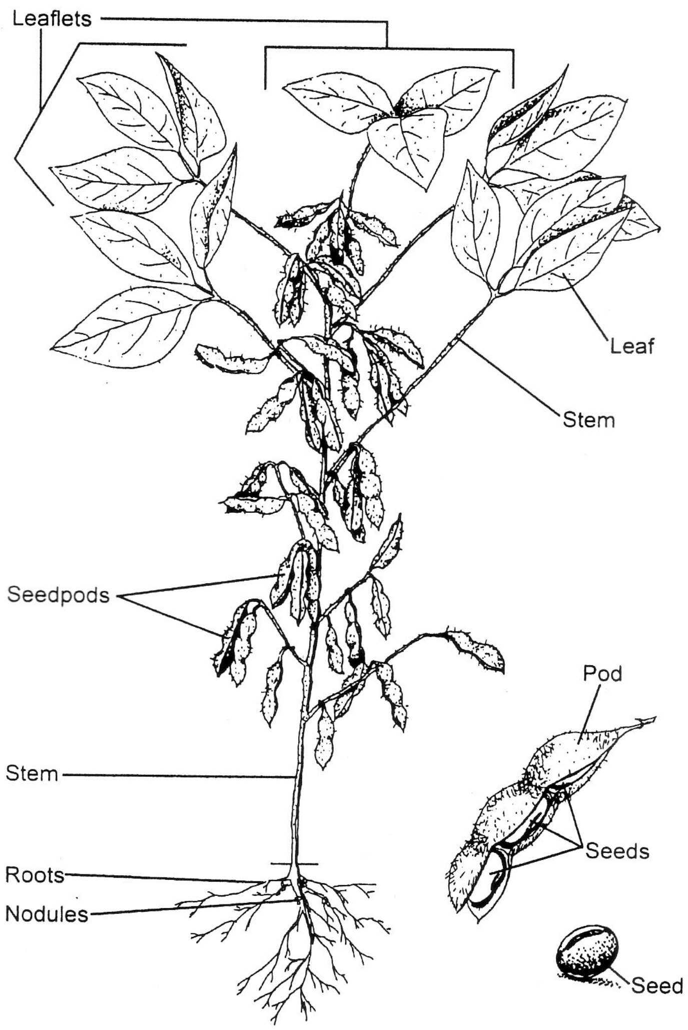
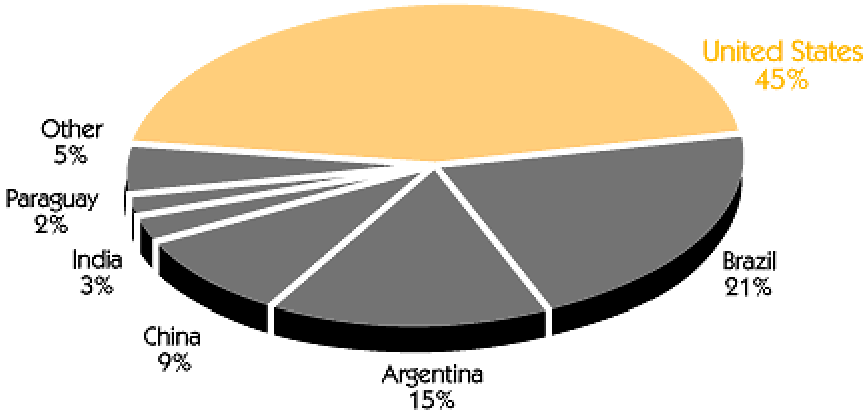


Figure 10: Soybean plant. Adapted from Ontario Soybean Growers.
(www.soybean.on.ca/teacher_files/picture-growth-history)

World Soybean Production 2000



	Million Bushels		Million Metric Tons
United States	**2,769**		**75.4**
Brazil	**1,341**		**36.5**
Argentina	**955**		**26.0**
China	**577**		**15.7**
India	**195**		**5.3**
Paraguay	**125**		**3.4**
Other	**337**		**9.2**
Total	**6,299**		**171.5**

Figure 11: World soybean production for the year 2000. Adapted from www.unitedsoybean.org

I . 3 . 2) Composition of Soybeans

Soybean is one of the most valuable agricultural commodities because of its unique chemical composition. Among legumes, it has the highest protein content (around 40%) and the second highest oil content (~20%). Other valuable components include phospholipids, vitamins and minerals. Furthermore, soybean includes minor substances such as oligosaccharides and isoflavones. Isoflavones are present in just a few botanical families and soybean is unique in that it contains the highest amount of isoflavones (~3mg/g) dry weight. (Liu 1999).

I . 3 . 2 . 1) Carbohydrate

Moisture free soybeans contain about 35% carbohydrates. Mature soybeans contain trace amounts of monosaccharide such as glucose and measurable amounts of di- and oligosaccharides, with sucrose in the range of 2.5-8.2%, raffinose 0.1-0.9% and stachyose 1.4-4.1%. Basically, the oligosaccharides in soybeans are non-reducing sugars, containing fructose, glucose and galactose linked by β-fructosidic and α-galactosidic linkages as shown in Figure 12. Raffinose and stachyose receive more attention, mainly because their presence has been linked to flatulence and abdominal discomfort associated with consumption of soybeans and soy products. The reason is humans are not endowed with the enzyme α-galactosidase necessary for hydrolysing the α-galactosidic linkage present in these oligosaccharides. When these oligosaccharides are consumed by humans, the oligosaccharides cannot be digested in the human duodenal and intestinal mucosa. The intact sugars go directly to the lower intestine where they are metabolised by micoorganisms that contain the enzyme. This results in production of gases as carbon dioxide, hydrogen, nitrogen. An effective way of eliminating these oligosaccharides is fermenting soymilk with cultures of lactic acid bacteria which posses the enzyme and cause hydrolysis of the α-galactosidase linkage. (Liu 1999). And as flatulence is one of the major factors limiting soybean utilization as food (Liu 1999, Hou *et al* 2000), fermented soy products such as cheese and yoghurt may help to overcome this problem (Wang *et al* 2002).

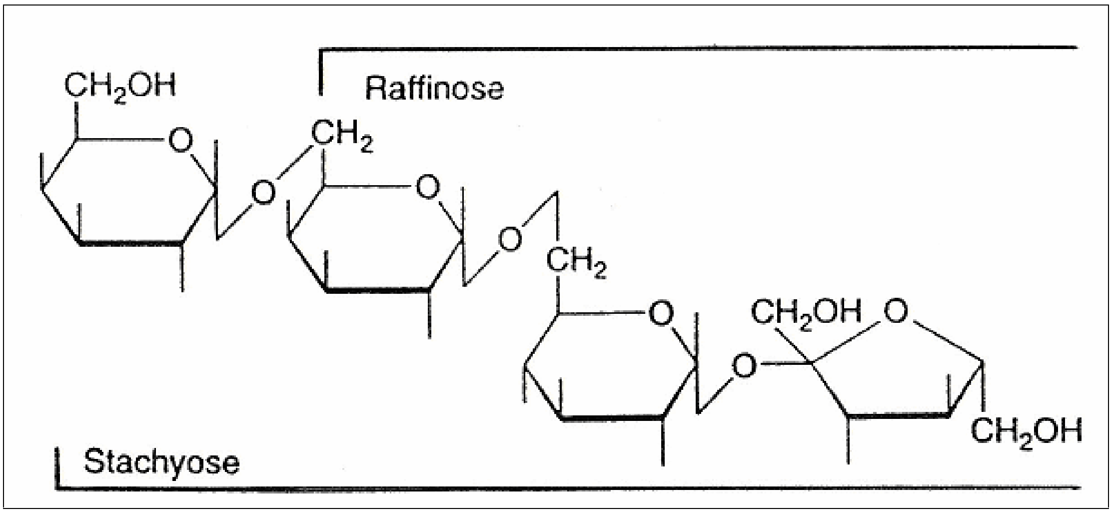


Figure 12: Structure of oligosaccharides found in soybeans. Adapted from Liu 1999.

I . 3 . 2 . 2) Protein

Proteins comprise 40% of soybean on a dry weight basis. Proteins can be categorized based on their solubility pattern into two types; *albumins* and *globulins***.** Albumins are water soluble whereas globulins are only saline soluble. Under this classification system, most soy proteins are globulins. Globulins are further divided into legumin and vicilin which are also known as glycinin and conglycinin respectively. A more precise means of identifying proteins has been based on approximate sedimentation coefficient using ultracentrifugation to separate seed proteins. Soy proteins exhibit four fractions after centrifugation, these fractions are designated as 2S, 7S, 11S and 15S. 2S and 7S fractions are heterogeneous. 7S fraction consists of conglycinin, α- amylase and lipoxygenase. 11S fraction is the glycinin, 15S is a polymer of glycinin. However, the two major protein fractions frequently studied are 7S globulin (β-conglycinin) and 11S (glycinin) (Liu 1999).

11S Glycinin is the largest fraction of total seed protein (25–35%) and accounts for over 40% of total seed globulin. 11S is a hexamer with molecular weight of about 360 kDa (Figure 13) . Its monomeric subunits have the generalized structure A-S-S-B, where:

- A represents an acidic polypeptide of 34-44 kDa.
- B is a basic polypeptide of 20 kDa.
- S-S is a single disulphide bond that links the two polypeptides.

Glycinin molecule (11S) which has a complex quaternary structure consisting of two layers of trimers. Each trimer has three acidic and basic polypeptides paired and held together by disulfide and hydrogen bonds. The secondary structure of glycinin consist of 25% α-helices, 25% β-sheet, 42% turns, and 8% unordered forms. (Liu 1999, Renkema 2001).

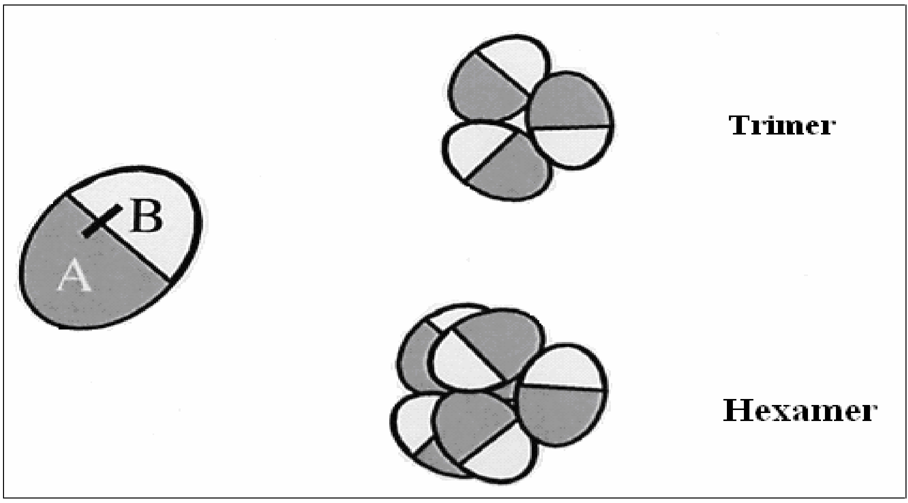


Figure 13: Schematic presentation of a glycinin molecule and its trimeric and hexameric complexes. A and B are the acidic and basic polypeptides, respectively. The small bar connecting A and B represents the disulfide bond. Adapted from Rekema 2001.

7S (Beta-conglycinin) (Figure 14) is a trimer which consists of ά (MW ~ 72,000), α (MW ~ 68,000) and β (MW ~ 52,000) subunits. (Mujoo *et al* 2003).

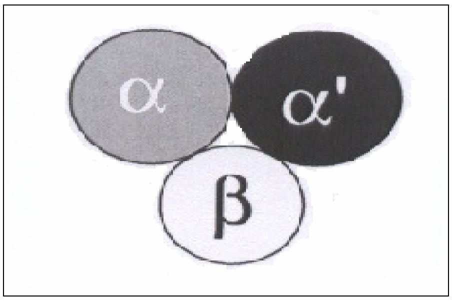


Figure 14: Schematic presentation of a β-conglycinin molecule (type βαά). Adapted from Rekema 2001.

7S is glycosylated. In contrast to 7S (conglycicnin), only a small portion of 11S is glycosylated. 11S (glycinin) has a better gel formation ability than 7S (conglycinin) while 7S has a greater emulsifying capacity and emulsion stability. Both 7S and 11S form gels when induced by heat and/or coagulant. However, the denaturation temperature of 7S is lower than 11S.

Just as in dairy milk, soy milk can be formed into an edible coagulum or gel like tofu. Liu (1999) stated that gelation of soymilk is a two steps process as illustrated in Figure 15:

- 1^{st} step: Protein denaturation by heat.
- 2^{nd} step: Hydrophobic coagulation promoted by protons from glucono-δ-lactone (GDL) or calcium ions from coagulant.

In soymilk, the hydrophobic regions of the protein molecules in the native state are located inside. Upon heating, these regions are exposed to the outside. Since the heat-denatured soy protein is negatively charged, either protons induced by GDL or calcium ions neutralize the net charge of the protein in the second step. As a result, the hydrophobic interaction of the neutralized protein molecules become more predominant and this leads to the aggregation. The gels are formed by random aggregation and become turbid. It is also assumed that besides the hydrophobic interaction, hydrogen bonds and disulfide interaction may also be involved in forming the network (Fiora *et al* 1990).

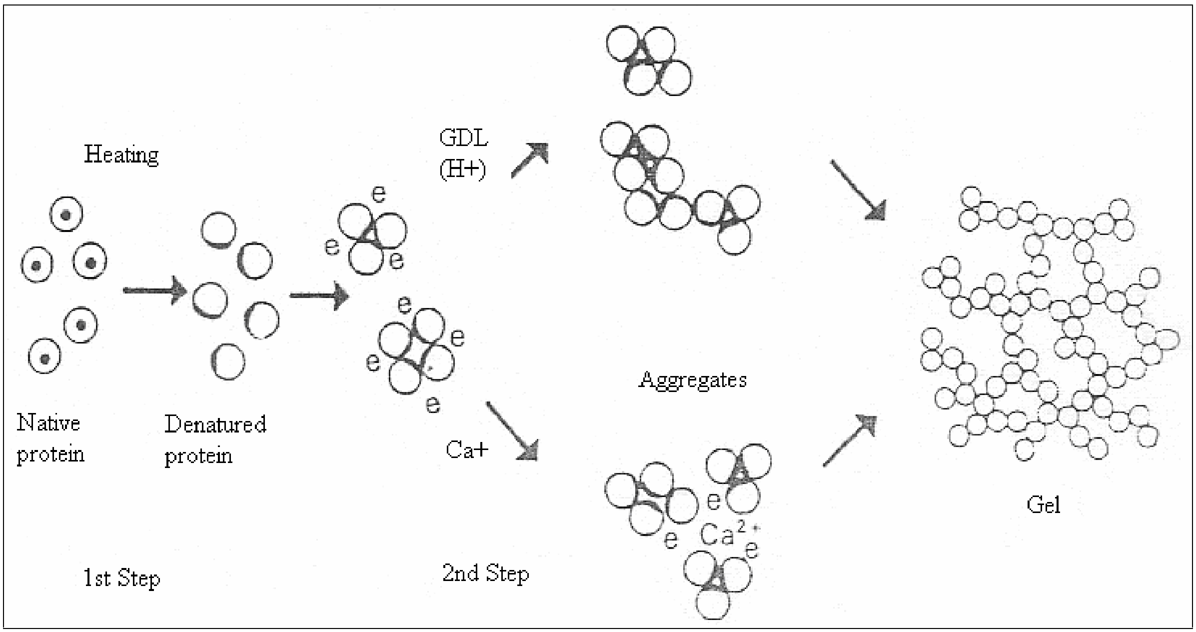


Figure 15: Gelation mechanism of soybean proteins in the presence of GDL or $CaSO_4$. circle=protein molecules, dot=hydrophobic regions. Adapted from Liu 1999.

I . 3 . 2 . 2) Lipid

Soybeans store lipids mainly in the form of triglycerides, in organelles known as oil bodies. Those oil bodies are relatively homogenous in size ranging from 0.2-0.5μm in diameter. The major component of the soy oil is triglycerides which constitute 99% of the oil, the rest are minor components of phospholipids, unsaponifiable material (*e.g.* tocopherols), free fatty acids and trace metals (eg. iron, copper), (Liu 1999). Most of the free fatty acids in soy oil are unsaturated as shown in Table 4.

Table 4: Soy lipid composition.USDA National Nutrient Database for Standard Reference 2002. Adapted from www.nal.usda.gov (NDB NO: 16120)

Lipids	Unit	Per 100g (edible portion)
Fatty acids, total monounsaturated	g	0.326
16:1 undifferentiated	g	0.004
18:1 undifferentiated	g	0.322
Fatty acids, total polyunsaturated	g	0.833
18:2 undifferentiated	g	0.735
18:3 undifferentiated	g	0.098
Cholesterol	mg	0

I . 3 . 2 . 4) Isoflavones

Isoflavones are secondary vegetable substances (www.isoflavonesinfo.com) which belong to a group of compounds (flavonoids) that share a basic structure consisting of two phenyl rings joined by a three-carbon bridge which may or may not be closed in a pyran ring as in Figure 16. Isoflavones differ from flavonoids in that benzyl ring B is joined at position 3 instead of position 2. Flavonoids are water soluble and they often undergo changes during processing (Ward 2002). Isoflavones have an extremely limited distribution in nature. Isoflavones are present in just few botanical families, including soybeans. The isoflavones in soybeans and soy products are of three types, with each type being present in four chemical forms as shown in Figure 17. Thus there are twelve isomers of isoflavones. The terms, daidzein, genistein and glycitein refer to the aglycones of soybean isoflavones. In the β-glucoside form, they become daidzin, genistin and glycitin. In the acetylglucoside form, soybean isoflavones are named as 6”-*O*-acetyldaidzin, 6”-*O*-acetylgenistin and 6”-*O*-acetylglycitin. In the malonylglucoside form, the corresponding names are 6”-*O*-malonyldaidzin, 6”-*O*-malonylgenistin and 6”-*O*-malonylglycitin. (Griffith and Collison 2001).

Unfortunately isoflavones are thought to be mainly responsible for the objectionable aftertaste of soymilk and soy products which is usually described as sour, bitter and astringent (Shi *et al* 2002). This is due to the phenolic/tannin like chemical form of these compounds. The isoflavones concentration in soy proteins varies due to the effect of soy protein processing conditions. Soy products that have been subject to minimal processing generally have an isoflavones level similar to unprocessed soybeans, for example; raw soybeans have a total isoflavones of 1789mg/kg while firm tofu contains 355mg/kg on a wet weight basis (Franke *et al* 1999). However, the isoflavones level in raw soybeans themselves varies substantially with cultivars as well as environmental conditions during the plant growth (Liu 1999).

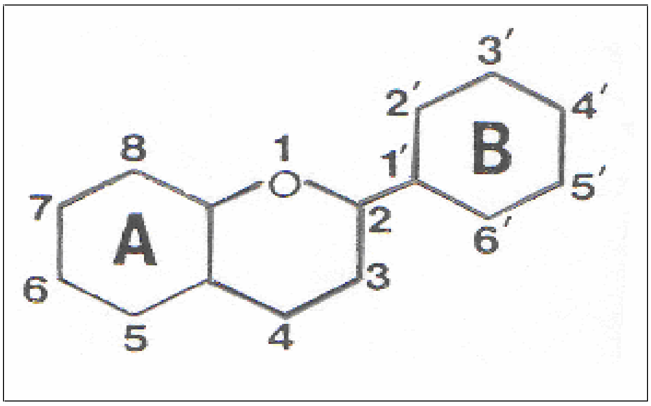
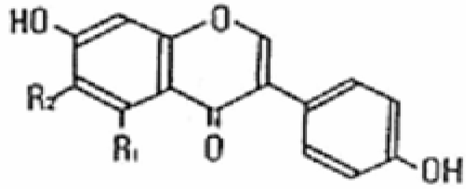
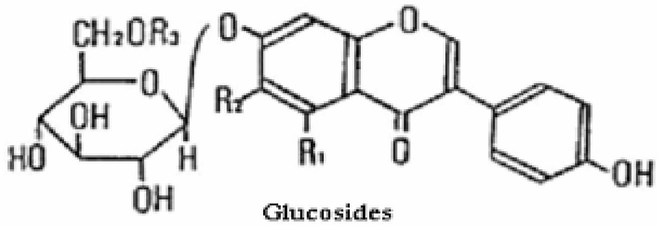


Figure 16: General structure of flavonoids. Adapted from Liu 1999.



Aglycones

R1	R2	Compounds
H	H	Daidzein
OH	H	Genistein
H	OCH3	Glycitin



Glucosides

R1	R2	R3	Compounds
H	H	H	Daidzin
OH	H	H	Genistin
H	OCH3	H	Glycitin
H	H	COCH3	6"-O-malonyldaidzin
OH	H	COCH3	6"-O-malonyldaidzin
H	OCH3	COCH3	6"-O-malonyldaidzin
H	H	COCH2COOH	6"-O-malonyldaidzin
OH	H	COCH2COOH	6"-O-malonyldaidzin
H	OCH3	COCH2COOH	6"-O-malonyldaidzin

Figure 17: Chemical structures of 12 isoflavones isomers found in soybeans. Adapted from Liu 1999.

I . 3 . 2 . 5) Lipoxygenase

Lipoxygenase (LOX), is an iron-containing dioxygenase that catalyses the oxidation of certain polyunsaturated fatty acids, producing conjugated unsaturated fatty acid hydroperoxides. LOXs have been found in plants, animals and fungi. Soybeans are the richest known source of LOXs and it is of particular interest because they have been implicated as the principal cause of the undesirable flavours, commonly known as “greeny” or “beany” flavour associated with soy bean products. (Liu 1999). LOX catalyzes hydroperoxidation of linoleic acid and other polyunsaturated lipids that contain a cis,cis-1,4-pentadiene moiety, in the presence of molecular oxygen as in figure 18. The primary products are hydroperoxides. Three steps are assumed for the reaction: (1) activation of the native anzyme, (2) removal of a proton from the activated methylene group, and (3) insertion of the oxygen into the substrate molecule with formation of the hydroperoxide. The initial products of lipoxygenase activity are into a variety of C-6 and C-9 products through the action of hydroperoxide lyases and/or isomerases as in figure19. These volatile carbonyl compounds are aldehydes, ketones, and alcohols. Many of them have an objectionable odor or undesirable flavour, which is responsible for off flavours associated with various soy products. Among the volatile compounds, hexanal is primarily responsible for the “greeny” flavour of soy products due to its extremely low flavour threshold (less than 1 ppm). Liu 1999.

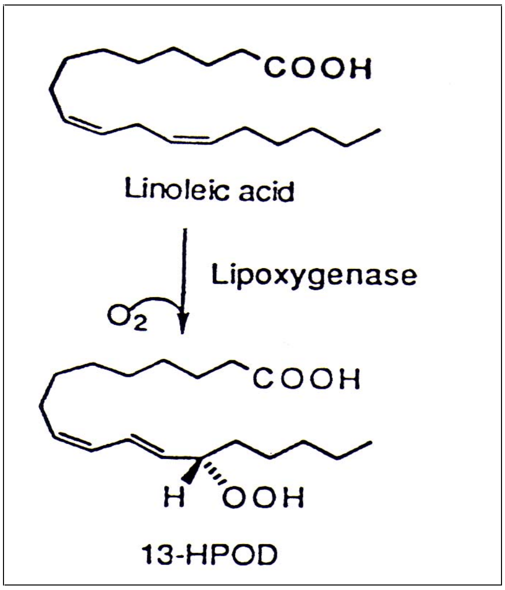


Figure 18: Lipoxygenase catalised oxygenation of linoleic acid to produce13-hydroperoxy-cis-9,trans-11-octadecadienoic acid (13-HPOD). Adapted from Liu 1999.

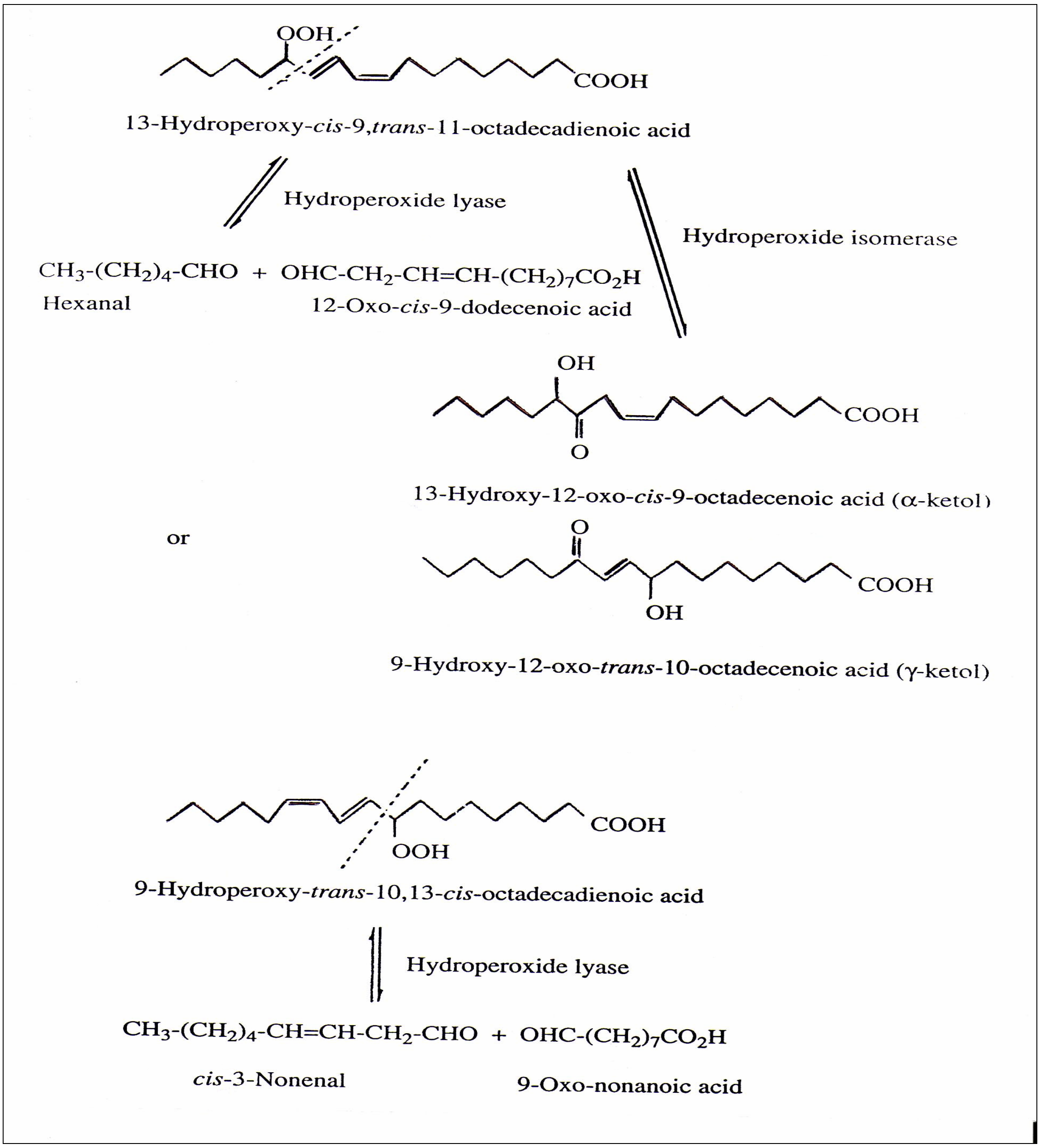


Figure 19:Possible degradation products arising from the action of hydroperoxide lyases and isomerases. Adapted from Liu 1999.

I . 3 . 3) Nutritional Value of Soybeans

Soy based foods are considered to be "Functional Foods". There are many definitions of functional foods, however generally it can be defined as foods that provide an additional physiological benefit that may prevent disease or promote health (Hasler 1996).

Numerous scientific studies report that soybeans have certain beneficial activities against osteoporosis (Choi *et al* 2002, Ohr 2003, Xie *et al* 2003), obesity (Lee and Kim 2000), cardiovascular disease (Choi *et al* 2002, Ohr 2003) and cancer cells (Mejia *et al* 2004, Head *et al* 1996) particularly prostate, breast, (Ohr 2003, Franke *et al* 1999) colon, rectum and stomach cancer (Storm *et al* 2001).

I . 3 . 3 . 1) Cancer

Overall soy isoflavones consumption has been associated with a reduced risk of most hormone-associated health disorders (Tsangalis *et al* 2002) particularly breast cancer (Ohr 2003) due to the fact that isoflavones are considered to be weak oestrogens that can prolong the oestrus cycle in women who consume soy products (Gibson and Williams 2000). Wu *et al* 2002 suggested that soy food intake, particularly during early life (adolescence) may have a lasting protective effect on breast cancer risk during adulthood. Cancer protective effect of isoflavones may be based on their antioxidant activity (Franke *et al* 1999).

I . 3 . 3 . 2) Cardiovascular Disease/Coronary Heart Disease

Cardiovascular disease includes arteriosclerosis and atherosclerosis. The primary cause and the major risk factor is hypercholesterolemia. Hypercholesterolemia is a condition where the circulating total cholesterol, LDL-cholesterol and triglycerides are high. Hypercholesterolemia predisposes individuals to cardiovascular disease (Bidlack *et al* 2000). Studies have shown that inclusion of soy protein in the diet accompanied by low levels of saturated fat and cholesterol may reduce the risk of coronary heart disease (CHD) (Anthony *et al* 1996). This prompted the FDA in 1999 to approve a health claim that can be used on labels of soy based foods as follows:

"Diets low in saturated fat and cholesterol that include 25g of soy protein a day may reduce the risk of heart disease. One serving of (name of food) provides__ grams of soy protein". Provided that foods must contain per serving:

- 6.25 g of soy protein.
- Low fat (less than 3 g).
- Low cholesterol (less than 20 mg).
- Sodium value of less than 480 mg for individual foods, less than 720 mg if considered a main dish and less than 960mg if considered a meal." (FDA 1999).

Cholesterol lowering effect of soy protein on is believed to be caused by the specific amino acids content of soy protein which is different from animal protein and appears to alter the synthesis and metabolism of cholesterol in the liver. (Gibson and Williams 2000). In a study conducted on amino acid to test their effect on raising serum total and LDL cholesterol concentration; the essential amino acids lysine and methionine were found to be the most hypercholesterolemic, whereas arginine appeared to counteract the hypercholesterolemic effects of other essential amino acids (Kenneth and Kurowska 1995).

Lysine, methionine, arginine and glycine contents of soymilk and dairy milk and structure are shown in table 5 and figure 20 respectively. Dairy milk contains higher methionine content than soymilk but a lower concentration of lysine (0.039%) difference, whilst soymilk contains more than twice the amount of arginine than dairy milk. Belleville (2002) stated that glycine is present at almost twice the concentration in soy protein. The higher methionine: glycine ratio in casein is suggested to be responsible for elevation of serum cholesterol.

Table 5: Dairy milk and soymilk content of lysine, methionine, arginine and glycine.

Amino acid	**Dairy Milk %**	**Soymilk %**
Lysine	0.140	0.179
Methionine	0.074	0.040
Arginine	0.075	0.214
Glycine	0.075	0.120

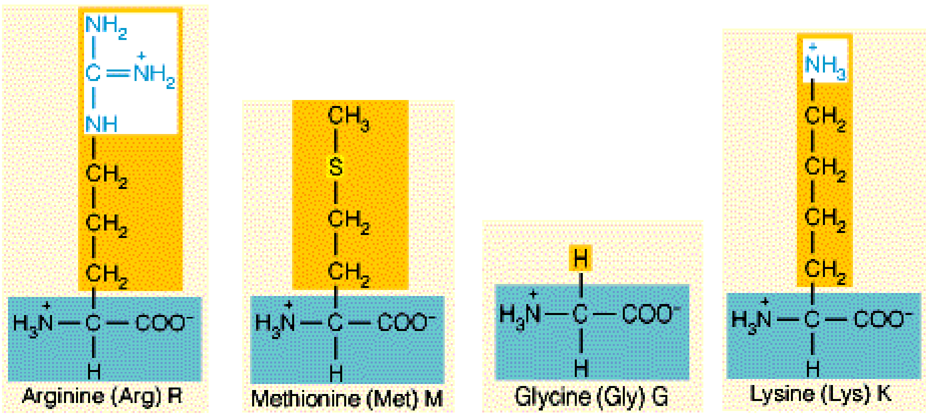


Figure 20: Arginine, methionine, glycine and lysine structure. Adapted from Mathews *et al* 2000.

I . 4) Soy Dairy Analogues

Soybeans have become an increasingly important agricultural commodity with a steady increase in the world wide annual production. Soybeans are one of the world's leading crops because of their wide adaptability and their health benefits. However, there are distinct differences in how the East and West use soybeans. In the East, soybeans are mainly processed for human consumption while in the West soybeans are crushed into oil and defatted meal; most of the oil is used for human consumption while the defatted meal is mainly used as animal feed. Only a small portion is processed into soy protein ingredients (soy flour, isolate and concentrate). These ingredients may be used in the new generation soy foods such as soy cheese, yoghurt, frozen dessert. Figure 21 outlines soybean food utilization.

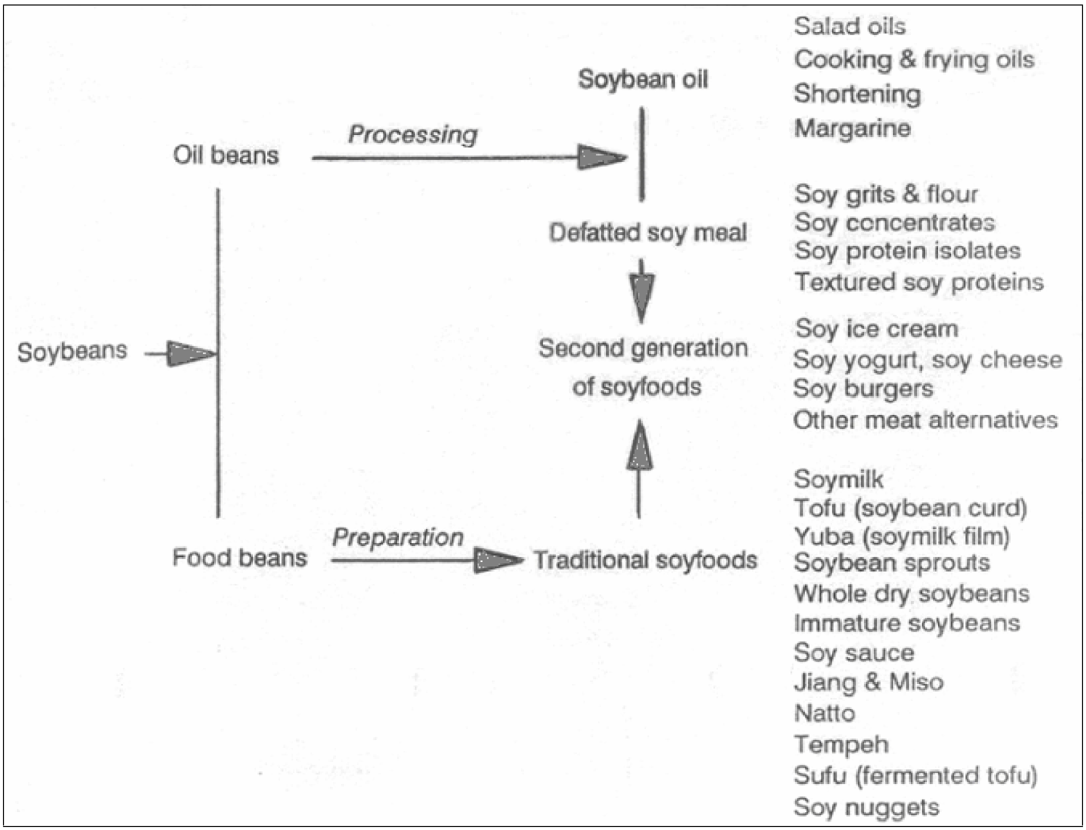


Figure 21: A general outline of soybean food use based on classification of oil and food beans. Adapted from (Liu 1999).

Soymilk is defined as the aqueous extract of whole soybeans (Hou *et al* 2000) closely resembling dairy milk in appearance and composition. Soymilk is rich in protein, calcium, phosphorous and other nutrients as shown in Table 6. Soymilk has been used traditionally as a drink and used in the preparation of other products like tofu (soybean curd) and yuba (soymilk film). Soymilk had also been used to prepare a second generation of soy foods such as soy ice cream, soy yoghurt, soy cheese and other products as in Figure 21. In this study, the focus will be on fermented dairy analogues such as soy cheese and soy yoghurt.

Table 6: Soymilk composition.USDA National Nutrient Database for Standard Reference 2002. Adapted from www.nal.usda.gov (NDB NO: 16120)

Nutrient	**Units**	**Value per 100 grams of edible portion**	**Std. Error**	**1 cup ------- 245 g**
Proximates				
Water	g	93.27	0.225	228.511
Energy	kcal	33	-	80.850
Protein	g	2.75	0.104	6.737
Total lipid (fat)	g	1.91	0.440	4.679
Ash	g	0.27	-	0.662
Carbohydrate, by difference	g	1.81	-	4.434
Fiber, total dietary	g	1.3	-	3.185
Minerals				
Calcium, Ca	mg	4	-	9.800
Magnesium, Mg	mg	19	-	46.550
Phosphorus, P	mg	49	-	120.050
Sodium, Na	mg	12	4.830	29.400
Lipids				
Fatty acids, total saturated	g	0.214	-	0.524
4:0	g	0.000	-	0.000
6:0	g	0.000	-	0.000
8:0	g	0.000	-	0.000
10:0	g	0.000	-	0.000
12:0	g	0.000	-	0.000
14:0	g	0.004	-	0.010
16:0	g	0.157	-	0.385
18:0	g	0.053	-	0.130
Fatty acids, total monounsaturated	g	0.326	-	0.799
16:1 undifferentiated	g	0.004	-	0.010
18:1 undifferentiated	g	0.322	-	0.789
20:1	g	0.000	-	0.000
22:1 undifferentiated	g	0.000	-	0.000
Fatty acids, total polyunsaturated	g	0.833	-	2.041
18:2 undifferentiated	g	0.735	-	1.801
18:3 undifferentiated	g	0.098	-	0.240

20:4 undifferentiated	g	0.000	-	0.000
20:5 n-3	g	0.000	-	0.000
22:5 n-3	g	0.000	-	0.000
22:6 n-3	g	0.000	-	0.000
Cholesterol	mg	0	-	0.000
Amino acids				
Tryptophan	g	0.043	-	0.105
Threonine	g	0.113	-	0.277
Isoleucine	g	0.144	-	0.353
Leucine	g	0.241	-	0.590
Lysine	g	0.179	-	0.439
Methionine	g	0.040	-	0.098
Cystine	g	0.047	-	0.115
Phenylalanine	g	0.151	-	0.370
Tyrosine	g	0.112	-	0.274
Valine	g	0.141	-	0.345
Arginine	g	0.214	-	0.524
Histidine	g	0.071	-	0.174
Alanine	g	0.122	-	0.299
Glutamic acid	g	0.550	-	1.347
Glycine	g	0.120	-	0.294
Proline	g	0.162	-	0.397

I . 4 . 1) Soy Cheese

One of the most promising soybean foods is soybean cheese which is also known as cheese substitute or imitation cheese. An imitation cheese is defined as a product which is a substitute for and resembles another cheese but is nutritionally

inferior, where nutritional inferiority implies a reduction in the content of an essential nutrient(s) present in a measurable amount but does not include a reduction in the calorie or fat content" (FDA regulation 101.3). A substitute cheese is defined as a product that is a substitute for and resembles another cheese and is not nutritionally inferior. (Fox *et al* 2000). Cheese analogues are categorized as dairy, partial dairy or non dairy depending on whether the fat and/or protein components are from dairy or vegetable sources as indicated in Figure 22. Analogue cheeses constitute by far the largest group of imitation or substitute cheese products. The annual production of analogue cheese in the USA , the major producing region, is around 300,000 tonnes. The European production is around 20,000 tonnes/annum. The major products are substitute or imitations for mozzarella and cheddar. These products find application mainly as cheese topping for pizza and as slices in beef burgers, spaghetti sprinkling, cheese sauces, cheese dips and ready – made meals (Fox *et al* 2000).

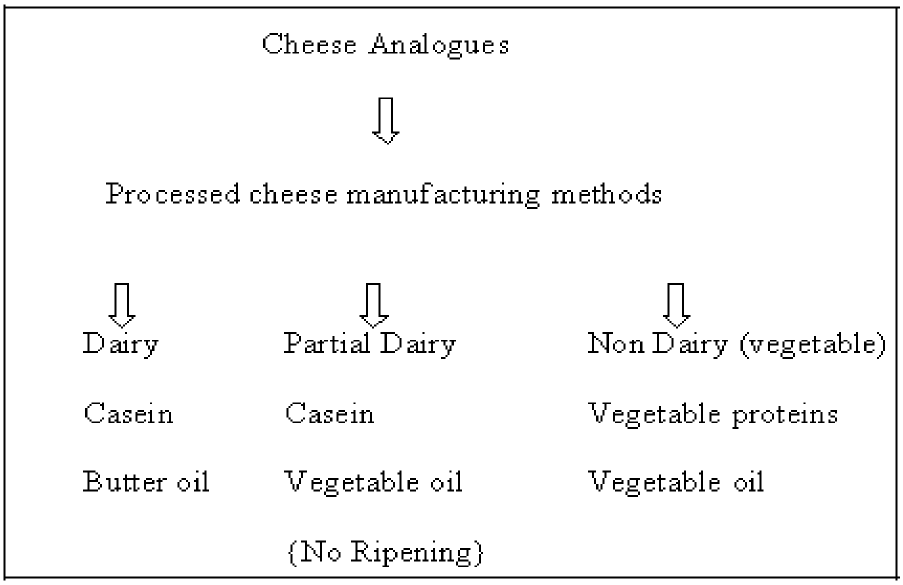


Figure 22: Classification of analogue cheeses based on the sources of proteins and oils used in product formulations. Adapted from Fox *et al* 2000.

Although Fox *et al* (2000) has stated that generally cheese analogues are not ripened, some studies indicated that soy cheese analogues have been prepared and ripened according to the basic dairy cheese making procedures. For instance, Abou El-ella (1980) prepared a hard cheese substitute from soy milk and ripened the cheese for three months.

I . 4 . 2) Soy Yoghurt

Yoghurt can be defined as a cultured milk. The word "cultured" is used to describe the process of innoculation of milk with a combination of microorganisms which ultimately convert lactose in dairy milk to lactic acid (Early 1998). Yoghurt is regarded to have some health benefits as yoghurt starter culture may produce antibiotic-like substance preventing the growth of pathogens in the intestine (Tamime and Robinson 1999).

Soybeans provide an inexpensive and rich source of protein and it can be used for the manufacture of a yoghurt like product. Although the contents of soymilk are different from dairy milk, for example, the main sugar in dairy milk is lactose, while the main sugar in soymilk is sucrose and stachyose. However, some studies investigated the ability of yoghurt starter growth in soymilk.

I . 5) LITERATURE REVIEW AND BACKGROUND TO THE STUDY

I . 5 . 1) Soy Cheese

1) Abou El-Ella, (1980) investigated the use of soymilk in making a hard cheese substitute. He prepared five cheese samples, the first one using only soymilk as a base, the second sample was prepared by using a mix soymilk:dairy milk (1:1), the third sample is a mix of 1:2 soymilk:dairy milk, fourth sample was 1:3 soymilk: dairy milk and the fifth sample is 100% dairy milk (control). *Streptococcus lactis* was used as a starter culture and calcium lactate was added to improve coagulation. All cheese samples were ripened for three months. Colour, flavour, appearance, body and texture of cheese samples were evaluated after 1, 2, 3 months. After the experiment, Abou El-Ella concluded that a hard cheese substitute can be made successfully using

soymilk. However, flavour, colour, body and texture were improved by increasing the percentage of dairy milk.

2) Yang and Taranto (1982) studied the textural properties of Mozzarella cheese analogues prepared from soy base which includes (soy protein concentrate, soy protein isolate, soy flour, defatted soy flour, gelatin, gum Arabic and solid coconut oil). These authors used different formulations of soy base (mentioned above), gelatin and gum Arabic to get a gel with desired characteristics. They also studied the effect of different coconut oil concentration on the gel. After that cheese samples were incorporated with 10% soy protein isolate or 10% soy protein concentrate or 10% defatted soy flour or 10% full fat flour. Since the final product was Mozzarella cheese intended for pizza toppings, the authors found that fortification of cheese with 10% soy protein isolate yielded the best stretchability, so they studied the effect of addition of different concentration of soy protein isolate (10 – 40 %), as soy protein isolate concentration increased up to 30% hardness, adhesiveness and stretchability increased. After the experiment, Yang and Taranto concluded that a Mozzarella cheese analogue prepared from soy protein, gelatin and gum Arabic can be made to exhibit both textural and stretching properties that are similar to natural Mozzarella cheese. However, further research concerned with the possible reduction in the concentration of gelatin and gum was required in the formulations and use of other gums beside gum Arabic (eg. Xanthan, guar gum).

3) Choi et al 2002 conducted a study to determine the effect of five different strains of LAB growth in Man, Rogosa and Sharpe MRS broth or soymilk media. Soymilk medium was prepared with commercial soymilk and glucose. Ninety

ml of commercial soymilk was added into sterilized separately and was supplemented aseptically. Lactic acid bacteria strains were grown in MRS medium and had been inoculated at 2% (v/v) and incubated for 24 h at 37˚C. Culture broth, 0.2 ml, was added to 1.8 ml 0.1 M phosphate/citrate buffer, pH 6, containing 100 µg genistin or daidzin. The mixture was held at 45˚C for 30 minutes and then boiled for 10 minutes. The composition of isoflavones was analysed by HPLC. After the experiment, Choi et al found out that *Lactobacillus delbrueckii* subsp. *delbrueckii*, grown in MRS broth or soymilk media, completely hydrolysed the isoflavone glucosides, genistin and daidzin at 50µg ml^{-1}, into their respective aglycones, genistein and daidzein within 30 minutes. The strains of lactic acid bacteria studied did not produce β-gucosidase, the enzyme responsible for the hydrolysis of isoflavone glucosides, when cultured in MRS medium. Glucoside-hydrolysing activity was induced in some lactic acid bacteria when cultured in soymilk medium. These strains hydrolysed 70-80% of genistin into genistein and 25-40% of daidzin into daidzein.

I . 5 . 2) Soy Yoghurt

1) Cheng *et al* 1990, prepared a yoghurt like soybean product (soghurt) using *Streptococcus thermophilus* and *Lactobacillus casei* as starter culture. Soymilk - which was prepared by mixing defatted soy flour and water-was used as a base, gelatin (0.5%) calcium acetate (0.15%) and lactose (0 or 2%) were also added to the soymilk. Two soghurt samples (with 0 or 2% lactose) prepared by the authors and one yoghurt sample bought from the market as external control had been studied and compared for texture, aroma, taste, colour, pH and acidity. After the experiment, Cheng *et al* concluded that soghurt, yoghurt like product

can be prepared. Although, soghurt (with 2% lactose) was similar to yoghurt in acidity, sour taste and intensity of butter aroma, soghurt can be differentiated from yoghurt by a beany, bitter and astringent taste. Cheng *et al* suggested that bleaching of soy flour and using a stabilizer at a lower concentration may produce a soghurt more similar to yoghurt.

2) Darke and Gerard (2003) conducted a study to determine the consumer acceptance of yoghurt fortified with 2.5% soy protein concentrate and to determine the consumer knowledge of soy and dairy foods. They prepared four yoghurt samples with dairy milk as a base, sucrose (10% w/w), stabilizer (0.375%) and non-fat dried milk (5%w/w). Soy-based yoghurt was prepared by replacing part of non-fat dried milk by 2.5% soy protein concentrate. Two flavours (lemon and strawberry) were chosen. Thus, the following four yoghurts were prepared (strawberry no soy, strawberry +2.5% soy, lemon no soy, and lemon +2.5% soy). Two sites were selected for sensory evaluation; (university campus and local grocery store). Consumers were asked to assess texture, flavour and overall acceptance on a 9-point hedonic scale with (number 1 being dislike extremely to number 9 being like extremely). Authors found that soy fortified yoghurt were not as well liked as regular yoghurt but acceptability scores were greater than 5 (neither like nor dislike). However, fortification of dairy yoghurt with low amounts of soy protein may provide a way to introduce soy protein into the diet.

3) Buono et al 1990 prepared soymilk yoghurt using LAB as starter culture. Three yoghurt samples were prepared with fortifiers, 25% fructose, evaporated milk, non-fat dry milk, those samples were compared for total sugar and

stachyose content, pH and sensory evaluation. Four attributes of sensory tests were discussed (sweet, sour, beany flavour and viscosity). External yoghurt (control) samples were included in the sensory evaluation test. After the experiment, Buono et al found that the three soymilk yoghurt samples prepared showed decrease in stachyose content. Soymilk yoghurt fortified with non-fat dry milk was most viscous, while soymilk yoghurt fortified with 25% fructose was least viscous and it had also the lowest beany flavour and the highest sweetness. However, beany flavour was reduced significantly in all soymilk yoghurt samples prepared.

I . 6) Objectives of This Study

The objective of this study is to test the applicability of basic cheese-making and yoghurt making processes to soymilk as a mean of producing a cheese substitute and yoghurt substitute acceptable to traditional dairy product consumers. The following experiments were carried out:

1) Preparation of soy cheese analogues in the following forms:

   1.1) Soy Cheddar analogue by coagulation of soymilk with mesophilic bacteria.

   1.2) Soy Mozzarella analogue by coagulation of soymilk with thermophilic bacteria.

   1.3) Soy Brie analogue by coagulation of soymilk with mesophilic bacteria and *Penicillium candidum* mould.

   1.4)To study the effect of oil and /or okara supplementation of cheese on the chemical and textural properties of soy cheese analogues.

   1.5)To quantify the total isoflavones recovery in the proposed cheese analogues.

   1.6) To compare chemical composition and texture of the proposed cheese analogues to their dairy-based cheese.

2) Preparation of soy yoghurt

   2.1) To study the ability of yoghurt bacteria culture to grow in soymilk.

   2.2) To study the effect of fortification by the tropical fruit papaya "*Carica papaya*" of soymilk on the growth of yoghurt bacteria culture and subsequent effect as a yoghurt product.

   2.3) To study the effect of $CaCl_2$ fortification of soymilk and the subsequent effect as a yoghurt product.

2.4) To compare using sensory analysis, chemical and physical attributes soymilk based yoghurt with dairy milk based yoghurt.

II. MATERIALS AND METHODS

II . 1) Soy Cheese Analogues:

1. Cheese Manufacturing: Unsweetened pasteurized soymilk Brix 12, okara (soymilk residue) was supplied by Unicurd Pte Ltd-Singapore. Three types of cheese analogue were prepared, hard cheese (Cheddar), semi hard (Mozzarella) and soft (Brie) with some modification to the protocols of traditional cheese manufacturing described by Fox *et al* 2000.

2.1) Soy Cheddar Analogue

2.1.1) Starter culture: starter cultures of mesophilic starter (product code: C101 Mesophilic Direct Set Culture, composed of *Lactococcus lactis subsp lactis*) were purchased from New England Cheese Making Supply Company, Ashfield-MA, USA.

2.1.2) Manufacturing protocol: Cheddar analogue was prepared by heating the soy milk to 35°C then 0.5% mesophilic starter was inoculated, after 7-8 hours curd is obtained then it was cut to allow whey drainage and cheddared until whey acidity reached 0.5%. The curds were then milled and dry salted at 2% level, moulded and pressed at 5°C for 14 days.

2.2) Soy Mozzarella Analogue

2.2.1) Starter culture: starter cultures of thermophilic starter (product code: C202: Mozzarella Direct Set Culture, composed of *Streptococcus thermophilus, Lactobacillus delbrueckii subsp. Lactis,* *Lactobacillus helveticus*) were purchased from New England Cheese Making Supply Company, Ashfield-MA, USA.

2.2.2) Mozzarella cheese analogue was prepared by adding 0.5% thermophilic starter, then cheese was cooked at 40°C for 6-7 hours after that coagulum was cut to allow whey drainage. The curds were then heated and stretched at 65°C for 30 minutes after that curds were dry salted at 2% level and transferred into moulds and aged for 14 days at 5°C.

2.3) Soy Brie Analogue

2.3.1) Starter culture: starter cultures of mesophilic (product code: C101 Mesophilic Direct Set Culture) and *Penicillium candidum* (product code: C8 *Penicillium candidum* (white)) mould were purchased from New England Cheese Making Supply Company, Ashfield-MA, USA.

2.3.2) Brie cheese analogue was prepared by adding 0.1% mesophilic starter and 0.1 % *Penicillium candidum* to soy milk, all were mixed together and heated at 30°C for 6-7 hours, once curd was formed it was cut into large cubes and left to drain whey overnight then curds were dry salted and stored in the fridge at 5°C for 14 days. (in an aerated condition).

Soy Cheese samples were supplemented with 6 and 30% oil or okara or both (Figures chosen to represent high and low concentration). Soy cheese (control) and dairy cheese (control) had also been prepared for comparison. Each cheese prepared was coded as in Table 7.

Table 7: Formulations and code of all cheese samples prepared.

Sample Number	Cheese Type	Cheese Code	Ingredients
1	Cheddar –soy control	C1	Soymilk + 0.5 % mesophilic starter
2	Cheddar	C2	Soymilk + 0.5 % mesophilic starter + 6 % soyoil
3	Cheddar	C3	Soymilk + 0.5 % mesophilic starter + 6 % okara
4	Cheddar	C4	Soymilk + 0.5 % mesophilic starter + 6 % soyoil + 6 % okara
5	Cheddar	C5	Soymilk + 0.5 % mesophilic starter + 30 % soyoil
6	Cheddar	C6	Soymilk + 0.5 % mesophilic starter + 30 % okara
7	Cheddar	C7	Soymilk + 0.5 % mesophilic starter + 30 % soyoil + 30 % okara
8	Cheddar	dairy control	Low fat dairy milk + 0.5 % mesophilic starter
9	Mozzarella-soy control	M1	Soymilk + 0.5 % thermophilic starter
10	Mozzarella	M2	Soymilk + 0.5 % thermophilic starter + 6 % soyoil
11	Mozzarella	M3	Soymilk + 0.5% thermophilic starter + 6%okara
12	Mozzarella	M4	Soymilk + 0.5% thermophilic starter + 6%soyoil + 6%okara
13	Mozzarella	M5	Soymilk + 0.5% thermophilic starter + 30%soyoil
14	Mozzarella	M6	Soymilk + 0.5% thermophilic starter + 30%okara
15	Mozzarella	M7	Soymilk + 0.5% thermophilic starter + 30%soyoil + 30%okara
16	Mozzarella	dairy control	Low fat dairy milk + 0.5% thermophilic starter
17	Brie-soy control	B1	Soymilk + 0.1 % mesophilic starter + 0.1 % *Penicillium candidum*
18	Brie	B2	Soymilk + 0.1 % mesophilic starter + 0.1 % *Penicillium candidum* + 6 % soyoil
19	Brie	B3	Soymilk + 0.1 % mesophilic starter + 0.1 % *Penicillium candidum* + 6 % okara
20	Brie	B4	Soymilk + 0.1 % mesophilic starter + 0.1 % *Penicillium candidum* + 6 % soyoil + 6 % okara
21	Brie	B5	Soymilk + 0.1 % mesophilic starter + 0.1 % *Penicillium candidum* + 30 % soyoil
22	Brie	B6	Soymilk + 0.1 % mesophilic starter + 0.1 % *Penicillium candidum* + 30 % okara
23	Brie	B7	Soymilk + 0.1 % mesophilic starter + 0.1 % *Penicillium candidum* + 30 % soyoil + 30 % okara
24	Brie	dairy control	Low fat dairy milk + 0.1 % mesophilic starter + 0.1 % *Penicilluim candidum*

II . 2) Soy Yoghurt Analogues:

II . 2 . 1) Starter culture: commercial yoghurt starter culture (Product code: Y4 Yoghurt Direct Set with *Bifidus* and *Acidophilus*) was purchased from New England Cheese Making Supply Co. Ashfield, MA. USA.

II . 2 . 2) Preparation of Yoghurt: pasteurized sweetened soymilk was heated in the oven at 30-35˚C for 30 minutes, then yoghurt starter culture was added at 0.5% and samples incubated at 30- 35˚C for 16 hours. Soy yoghurt samples were supplemented with 5 or 10 % papaya fruit "*Carica papaya*" and or $CaCl_2$ as in Table 8. However, any other fruit can be chosen, for example pineapple, durian, etc. $CaCl_2$ is added to some dairy products (eg. cheese, yoghurt) to enhance curd firmness. The rate at which $CaCl_2$ is added range from 0.01-2.0% and it depends on the final chosen characteristics of the product, (Fox *et al* 2000, Early 1998). The value chosen for this study (0.5%) represent an intermediate value within the range.

Table 8: Formulations of all yoghurt samples prepared.

Sample #	**Ingredients**
1	Soymilk + 0.5% starter culture (control)
2	Soymilk + 0.5% starter culture + 5% papaya
3	Soymilk + 0.5% starter culture + 10% papaya
4	Dairy milk + 0.5% starter culture (control)
5	Soymilk + 0.5% starter culture + 0.5% $CaCl_2$ (control)
6	Soymilk + 0.5% starter culture + 5% papaya + 0.5% $CaCl_2$
7	Soymilk + 0.5% starter culture + 10 % papaya + 0.5% $CaCl_2$

II . 3) Chemical Analysis:

Cheese samples were analysed on day 1, 7, and 14 as follows:

- Moisture: samples were dried in an oven at 100° C for 6-7 hrs (to constant weight).
- pH: pH meter was immersed in the cheese curd and the yoghurt curd.
- Acidity: AOAC official method 920.124. Mix 10g cheese/yoghurt sample with 105ml 40° C H_2O and shake vigorously, filter, titrate 25ml portion filtrate representing 2.5g sample with 0.1M NaOH using phenolphthalein as indicator. Results expressed as % lactic acid.
- Ash: samples were ashed in Muffle Furnace at ~500° C for 16 hrs.
- Isoflavones: Isoflavones concentration of samples were analysed using HPLC according to the method published by Institute for Nutraceutical Advancement method 118:000 with some modification, isoflavones were extracted for two hours in 80% methanol and saponified at ambient temperature in 2M sodium hydroxide for 10 minutes. This saponification step converts the isoflavones glycoside esters (acetyl and malonyl ester) to the corresponding isoflavones glycoside conjugates, leaving the three parent isoflavones (aglycones) intact. The reaction is stopped with 10% glacial acetic acid then the extract is centrifuged at 5000rpm for 15 min. The supernatants were filtered and injected into the HPLC. High performance liquid chromatography (HPLC) apparatus with auto sampler consisted of two Waters 515 pumps, a Waters in-line degasser AF, a Waters 2996 photodiode array detector (PDA) monitoring 200-350nm (Waters

corporation), and a Compaq computer with Waters PDA data processing software. A Shim-pack VP-ODS C_{18} reverse phase column (4.6µm,250mmX4.6 mm id, Shimadzu Corporation, Japan) was used for quantitative HPLC analyses. All HPLC analyses were performed at ambient temperature.

A linear HPLC gradient was used in the analysis. It consisted of solvent A (0.1 % Acetic acid in water),and B (0.1 % Acetic acid in acetonitrile). Solvent B increased from 15 % to 35 % over 50 minutes and held at 35% for 3 min, then reduced back to 15 % over the next 2 min as in Table 9. The total run time was 55 min. the sample injection volume was 20µl, the solvent flow rate was controlled at 1ml/min.

Table 9: A linear HPLC gradient was used for isoflavones analysis.

Time/min	Flow Rate ml/min	Percent Mobile A%	Percent Mobile B %
0.00	1.00	85	15
50.00	1.00	65	35
53.00	1.00	65	35
55.00	1.00	85	15

Determination of Isoflavones Concentration and Distribution:

The identification of peaks of interest in the samples during HPLC analysis was based on the retention time of standards. The concentrations of isoflavones in samples were calculated according to the following equation:

Isoflavones (aglycones or glucosides), µg/g=[(A-c)/m] x V/W, where,

A = Peak area

c = y-intercept of the calibration curve

m = Gradient of the calibration curve

V = Volume of extract (ml)

W = Weight of sample (g)

- Sugar analysis: glucose, galactose, raffinose, stachyose and lactose content were analysed using HPLC according to the method of Wang et al 2003. HPLC apparatus with auto sampler consisted of two Waters 515 pumps, a Waters in-line degasser AF, a Waters 2996 photodiode array detector (PDA) monitoring 200-350nm (Waters corporation), and a Compaq computer with Waters PDA data processing software. Yoghurt samples (1g) were mixed with 10 ml of distilled water and centrifuged for 30 minutes at 5000 rpm. The supernatants were filtered and injected into the HPLC. The mobile phase consisted of 65% acetonitrile in distilled deionised water. A Hypersil (APS-2) column (250X4.6mm) was purchased from Phenomenex-USA. Sugar standards stachyose, galactose and lactose were purchased from Sigma chemical company, USA. Glucose was purchased from Anala R, and raffinose was purchased from Aldrich, USA.
- Electrophoresis of protein constituents: Analysis was carried out according to the method of Cavalier-salou and Cheftel, 1991 with some modifications. The separating gel (15% acrylamide) contained 4.3M urea and 1.5M Tris-HCl, pH 8.8. The stacking gel (4% acrylamide) contained 4.3M urea and 0.5%M Tris-HCl, pH 6.8. The running buffer was Tris glycine, pH 8.6. Electrophoresis was performed at 170V for one hour. Gels were stained in 0.025% commasie blue in acetic/methanol/water (7/40/53 v/v) for one hour at room temperature. Then gels were washed by destaining solution one (acetic acid/methanol/water 7/40/53 v/v) for one hour at room temperature. Then gels were destained in destaining

solution two (acetic acid/methanol/water 7/5/88 v/v) for one hour at room temperature.

- Texture Evaluation: Texture evaluation was performed using a Stable micro system analyser. Two consecutive bites were taken and six textural parameters were evaluated as listed in Table 10. Probe 6 mm Diameter cylinder of stainless steel was used. The pre test, test speed and post test speed were all set at 1mm/s. Distance of penetration through the samples was set to 3 mm and the force used was 4g. Dairy Cheddar, Mozzarella and Brie cheese had been bought from the local market and included as external controls for comparison purposes.

Table 10: Definitions of rheological terminology, adapted from Yang and Taranto 1982.

1. Hardness	The force necessary to attain a given deformation, is the peak force during the first compression cycle (first bite). (soft---firm--- hard).
2. Adhesiveness	The work necessary to overcome the attractive forces between the surfaces of the food and the other materials with which the food comes in contact (eg.Tongue,teeth,etc), is the negative force area for the first bite. (sticky---tacky--- gooey).
3. Cohesiveness	The strength of the internal bonds making up the body of the product, is the ratio of the positive area during the second compression to that during the first compression (A_2/A_1).
4. Springiness	The height that the food recovers during the time that elapses between the end of the first bite and the start of the second bite.(plastic---elastic).
5. Gumminess	The energy required to disintegrate a semi-solid food product to a state ready for swallowing, it is related to primary parameters of hardness x cohesiveness.(short--- meaty--- pasty--- gummy).
6. Chewiness	The energy required to masticate a solid food product to a state ready for swallowing. It is related to primary parameters of hardness, cohesiveness, and springiness, or a product of gumminess x springiness. (tender---chewy--- tough).

III . RESULTS AND DISSCUSSION

III . 1) Soy Cheese Analogues

III . 1 . 1) Soy Cheddar Analogues

Cheddar cheese is classified as a bacterial ripened cheese with moisture content <42% so it is classified as hard cheese (Wood 1998). In all soy Cheddar analogues prepared, a curd was obtained. Results of compositional analysis (% moisture, % ash, pH, % acidity) of prepared cheese analogues are presented in Figures 23 - 26.

Moisture Content:

All cheese samples showed reduction in moisture content after 7 and 14 days of pressing. During 1st-7th days of ripening, the greatest moisture content loss was observed in dairy control Cheddar with 6.78% loss followed by C1 (soy Cheddar control) and C7 (cheese supplemented with 30% oil and 30% okara) in which both lost 5.80% moisture. The lowest moisture loss was observed in C6 (cheese supplemented with 30% okara) and C4 (cheese supplemented with 6% oil and 6% okara) with 1.15 and 1.90% loss respectively. During 7th -14th day of ripening, the greatest moisture loss was in control soy cheese C1 and C5 (cheese supplemented with 30 % oil) with 19.35 and 13.55% respectively.

After 14 days, C5 (cheese supplemented with 30% oil) and C7 (cheese supplemented with 30% oil and 30 % okara) had the lowest moisture content of 34.8 and 54.4 % respectively. These results suggest that addition of soy oil w/wo okara at high concentration ~30% promoted syneresis during ripening. Syneresis is defined as fluid droplets that appeared on the gel/coagulum surface. When the cheese coagulum is formed, the molecules aggregate together from "solution" phase to "gel" phase through junction zone regions to form three-dimensional gel network structure. If these junction zone regions grow larger after formation of gel to become more compact, the structure contracts and syneresis results (Fennema 1996). Addition of oil without okara at a relatively lower concentration~6% as in C2, also lowered the moisture content up to (58.6%) which is also lower than cheese sample supplemented with 6% oil and 6% okara as in C4 which contains (65.2%).

On the other hand, the greatest moisture content was in C6 (cheese supplemented with 30% okara) and C3 (cheese supplemented with 6% okara) which contained 81.1 and 74.5% moisture. This observation suggest that addition of okara at both high ~30% and low~6% promoted water retention or increased the water holding capacity of the cheese gel. Water holding capacity is the ability of a matrix of molecules to entrap large amounts of water in a manner that inhibits exudation (Fennema 1996). This can be explained by the high water content of okara which ranges from 76 – 80% (Liu 1999).

Ash Content:

Ash content represents the total mineral content in foods. Ash refers to the inorganic residue remaining after either ignition or complete oxidation of organic matter in a food. All cheese analogues showed increase in the ash content during ripening. Values ranged from (0- 0.05%) after the first production day and increased up to (0 -2.00%) after 7 days and increased again from 0.77 to 2.99% after 14 days. C4 (cheese supplemented with 6% oil and 6% okara) showed the greatest ash content while C7 (cheese supplemented with 30% oil and 30% okara) showed the lowest ash content. Ash values were not directly related to moisture content values, however, during ripening all samples showed reduction in their moisture content and increased their ash content. However, since the mineral content of cheese samples is constant, increase in ash content upon ripening does not mean increase in mineral content of cheese samples, but the mineral content increased per cheese unit (portion) analysed mainly due to total water loss of cheese upon ripening.

All soy cheddar samples showed reduction in moisture content after 7 and 14 days of ripening as in Figure 23 and all samples showed increase in ash content after 7 and 14 days of ripening as in Figure 24. However, theses values were not directly related, for example C1 (control) showed the greatest moisture loss (19.4%) after 14 days, while C4 showed the greatest ash content increase (2.28%) after 14 days.

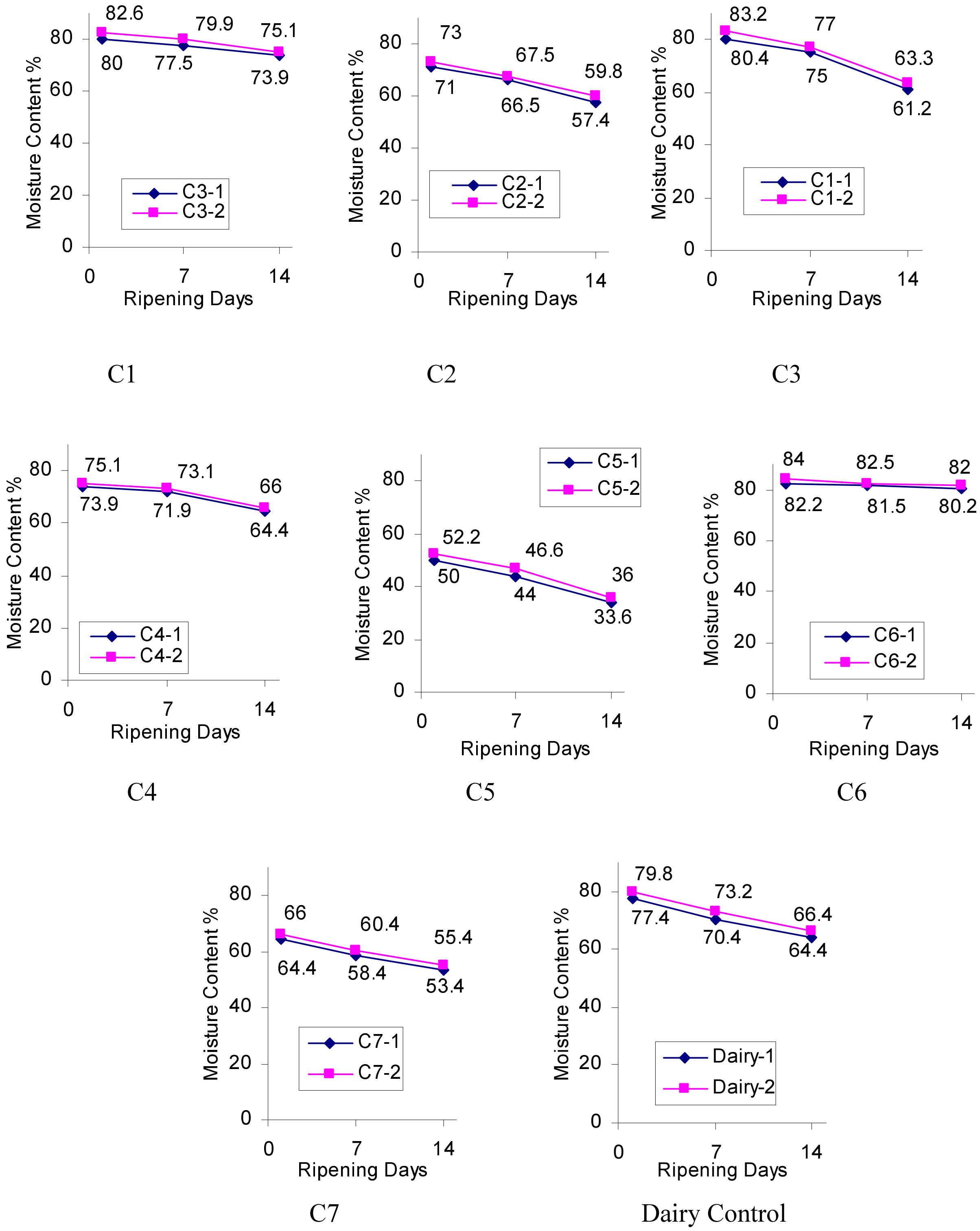


Figure 23: Moisture content of soy Cheddar analogues, day 1, 7 ,14. Each scatter plot represent duplicate measurements (numbers 1 & 2) for every sample studied.

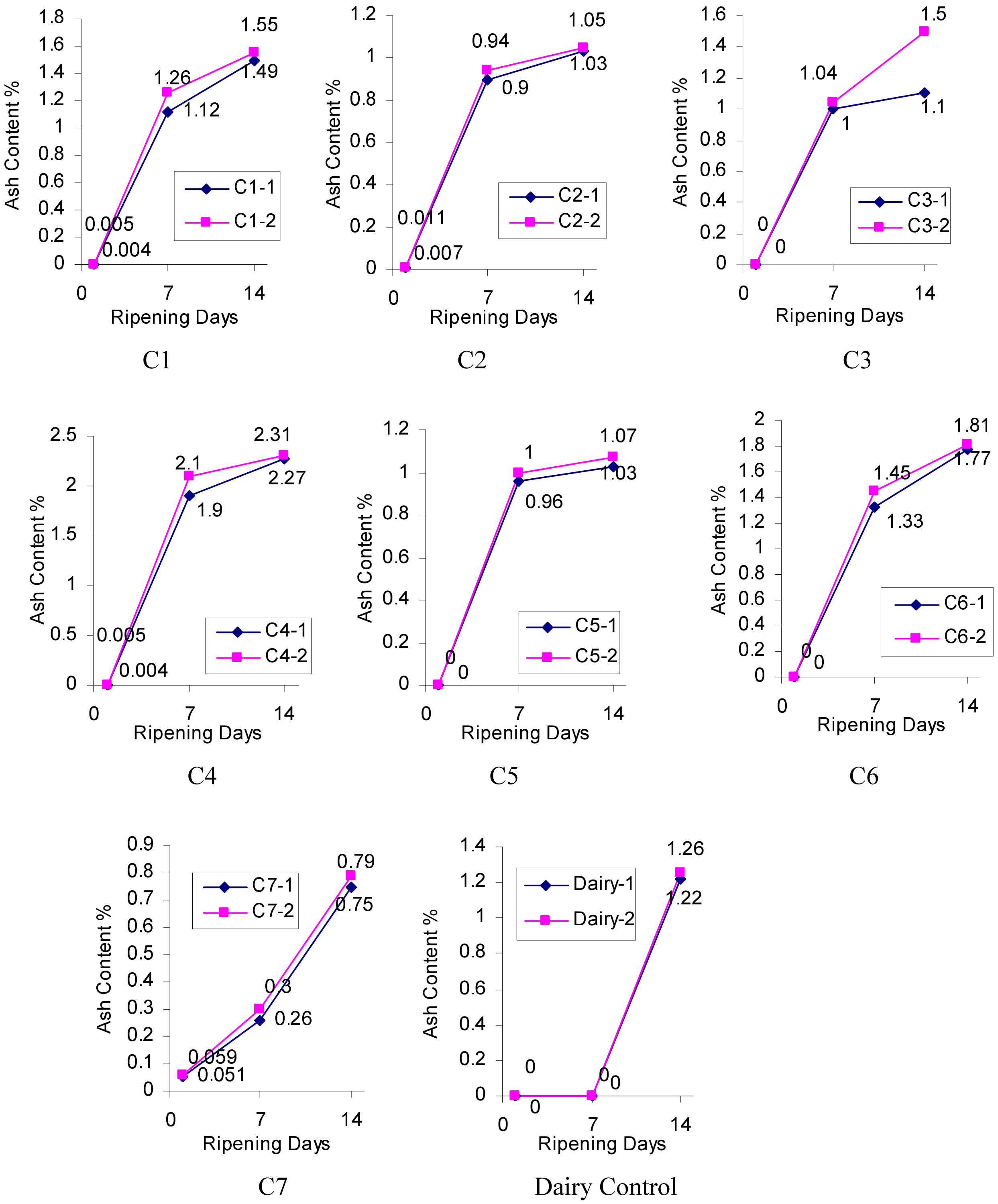


Figure 24: Ash content of soy Cheddar analogues, day 1, 7 ,14. Each scatter plot represent duplicate measurements (numbers 1 & 2) for every sample studied.

pH and Acidity:

Acid production in the medium depends upon the growth of the organisms and their ability to ferment available carbohydrates. pH and acidity values are presented in Figures 25, 26. pH values for all cheese analogues increased during ripening while acidity values showed some variations. During 1^{st}-7^{th} day of ripening pH values increased and their values ranged from 5.03 to 6.13. The lowest pH value after 7 days was observed in dairy control and C5 - cheese supplemented with 30% oil- with their pH values 4.68 and 5.03 respectively. C6 -cheese supplemented with 30% okara- and C3 - cheese supplemented with 6% okara- showed the highest pH values 6.13 and 5.61. During 7^{th}-14^{th} day, all samples showed 0.05-0.74 increase in pH values, with the greatest value observed for C6 -30% okara- and C3 -6%okara- with pH values of 6.61 and 6.35 respectively. The lowest pH values were in dairy control and C4 -6%oil and 6% okara- with their pH values of 5.03 and 5.10 respectively. This may suggest that addition of okara increased pH values compared with control sample C1 or samples in which oil was added.

The increase in pH was related to decrease in % acidity values as represented in Figure 26. During 1^{st} -7^{th} day of ripening % acidity values increased 0.04-0.06%. the lowest value was in C1 –control- and the highest value was in dairy control. During 7^{th}-14^{th} day of ripening, % acidity values decreased 0.08-0.78%, the lowest acidity content was in C6 -30% okara- and C3 -6% okara- with 0.08 and 0.09% respectively. This can be explained by the high water content of okara which may have a buffering effect. The highest acidity content was in dairy control and C7 -30% oil and okara- with 0.66 and 0.17% respectively.

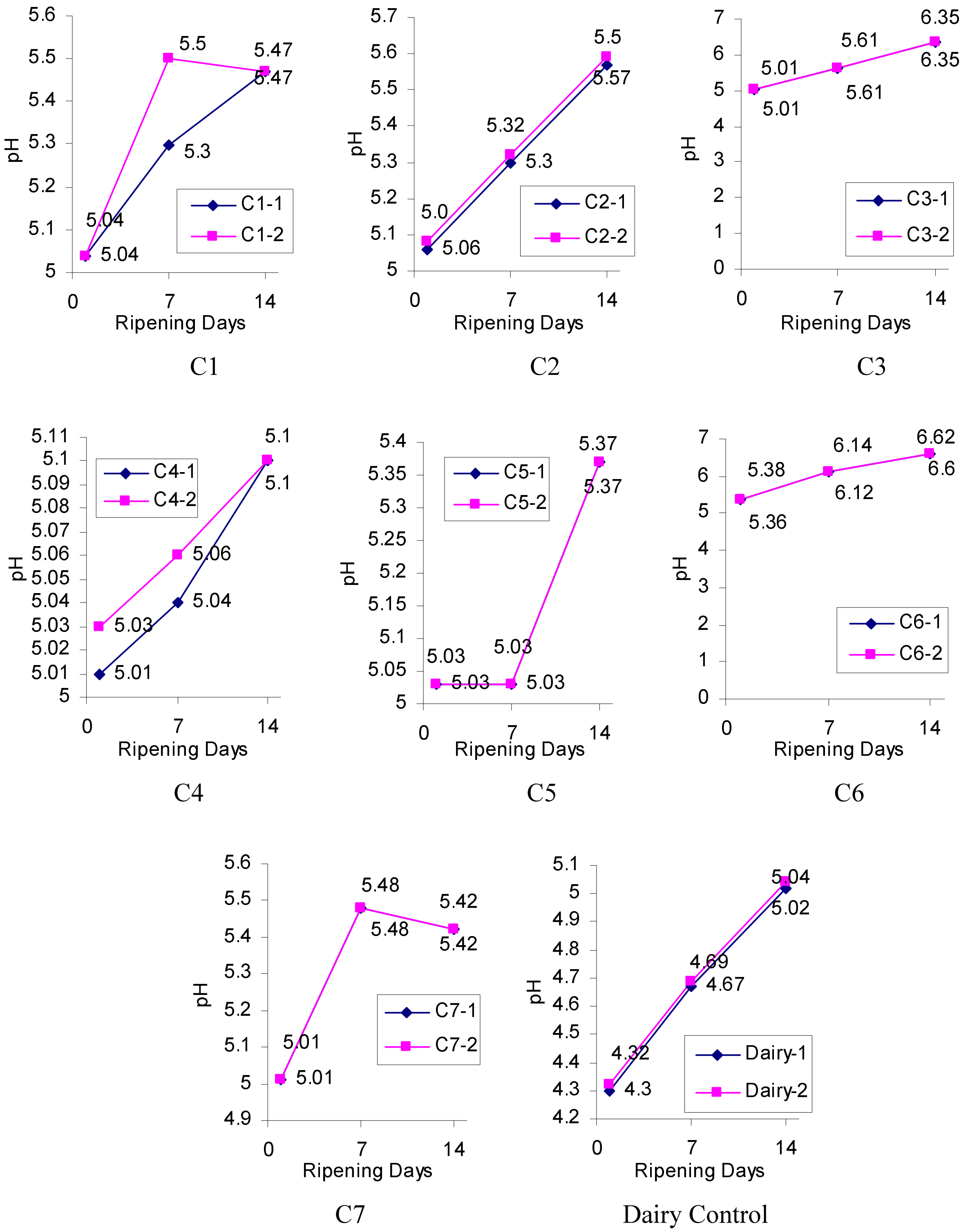


Figure 25: pH content of soy Cheddar analogues, day 1, 7 ,14. Each scatter plot represent duplicate measurements (numbers 1 & 2) for every sample studied.

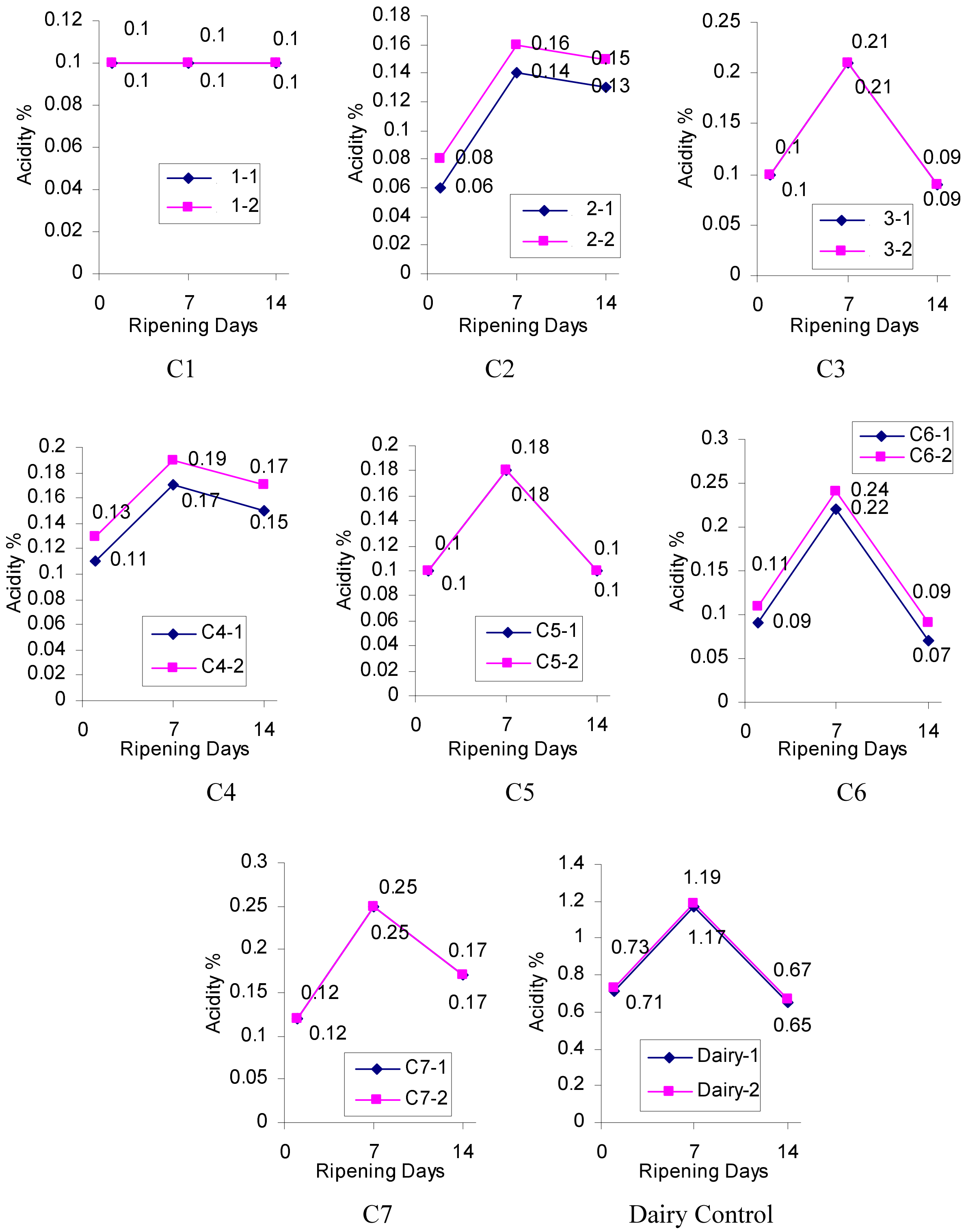


Figure 26: Acidity content of soy Cheddar analogues, day 1, 7 ,14. Each scatter plot represent duplicate measurements (numbers 1 & 2) for every sample studied.

Isoflavones Content:

The change in the concentration of isoflavones aglycones, isoflavones glucosides and total isoflavones are presented in Tables 11, 12, 13. The fermentation of soymilk with mesophilic bacteria after 14 days significantly increased the concentration of isoflavones aglycones due predominantly to the hydrolysis of the isoflavones glucosides. Supplemented cheese with 6 (C3) and 30% okara (C6) showed the greatest increase in aglycones concentrations from 26.7µg/g cheese to 99.2µg/g and 35.7 to 95.4µg/g respectively, while the lowest increase in aglycones concentration was in supplemented cheese with 6% oil (C2) and 30 % oil (C5). Additionally, control soy cheese C1 showed greater increase in aglycones compared with C7 (30 % okara and 30 % oil).

Between 7th-14th day of ripening, fermentation of C1 cheese increased the aglycones concentration from 37.7 to 58.9µg/g. However, the increase in aglycones concentration occurring in control cheese was greater than C7 (cheese samples supplemented with 30% oil and okara) and C5 (30% oil) but lower than C4 and C6 as presented in Table 11. Within 7th-14th day of ripening, control cheese C1 showed 27.9µg/g increase in aglycones concentration higher than C3, C5 and C7 but lower than C4 and C6. C2 (cheese supplemented with 6% oil) is the only sample which showed decrease in aglycones concentration.

However, all cheese samples prepared showed an increase in aglycones concentration between 1st-14th day of ripening. With the increase in aglycones concentration, cheese samples showed reduction in the concentration of isoflavones glucosides as presented in Table 12. The decrease in glucosides concentration was greatest in C1, concentration decreased from 398.8 to

48.7μg/g. C1, C2, C3 and C4 showed a greater decrease in glucosides concentration during the ripening period than cheese samples supplemented with higher concentration of oil and/or okara as in C5, C6 and C7, but this observation was not correlated to the increase in aglycones concentration where the greatest increase was in C6, C3, C4 and C1 while the lowest increase in aglycones was in C2, C5.

Recent research had shown that isoflavones aglycones (daidzein, genistein, glycitein) are absorbed faster and in higher amounts in humans than their glucosides (daidzin, genistin, glycitin). Intestinal micro flora are able to hydrolyse ingested isoflavones glucosides into bioactive aglycones (Franke *et al* 1999). This is similar to fermented cheese in which starter culture enhance hydrolysis (conversion) of glucosides to aglycones. Mean total isoflavones levels in soy cheese investigated varied between 117.6-191.3μg/g cheese (Table 13), with lowest values found in C5 and C7 and highest levels found in C4 and C6. This large variability in isoflavones content may be related to cheese moisture content, in which the cheese with the lowest total isoflavones also showed the lowest moisture content and cheese samples with 6 or 30 % okara seemed to increase total isoflavones concentration. These results are in agreement with Franke *et al* 1999 who evaluated isoflavones content in different soy products and suggested isoflavones concentration present in soy product is related to their moisture content. pH and temperature also affect total isoflavones content and the rate of glucosides conversion to aglycones (Xie *et al* 2003). The fluctuation in the content of isoflavones, aglycones and glucosides among samples from C1 to C7 can be explained by the hydrophobic nature of isoflavones (Griffith and Collison 2001), therefore, isofalvones is affected by pH, temperature and moisture, and since there is a change in moisture and pH during ripening, consequently isoflavones will be affected.

Table 11: concentration of isoflavones aglycones in soy Cheddar analogues fermented with mesophilic species. Results are performed twice, both values are presented in the table. Results are expressed on wet weight basis.

Age of cheese	Cheese code	Isoflavones Aglycones (µg/g- ppm)			Total Aglycones (µg/g)
		Daidzein	Genistein	Glycitein	
Day 1	C1	26.0 / 30.0	7.5 / 9.9	0.0 / 2.0	33.5 / 41.9
	C2	26.4 / 28.0	7.2 / 9.0	0.6 / 0.8	34.2 / 37.8
	C3	24.6 / 27.0	6.4 / 9.2	1.2 / 3.0	39.4 / 39.2
	C4	27.2 / 29.0	12.0 / 16.0	0.2 / 0.4	39.4 / 45.4
	C5	14.4 / 16.0	3.4 / 6.2	-	17.8 / 22.2
	C6	17.2 / 20.2	6.9 / 8.9	-	24.1 / 29.1
	C7	9.2 / 12.2	1.2 / 3.0	0.7 / 0.9	11.1 / 16.1
Day 7	C1	34.9 / 36.9	24.0 / 25.0	-	58.9 / 61.9
	C2	32.9 / 34.9	18.4 / 21.2	19.9 / 21.1	71.2 / 77.2
	C3	51.5 / 53.9	19.2 / 21.6	2.2 / 4.6	72.9 / 80.1
	C4	44.2 / 46.0	14.2 / 16.6	4.2 / 6.0	62.6 / 68.6
	C5	29.4 / 31.0	8.0 / 10.0	0.2 / 2.0	37.6 / 43
	C6	29.0 / 31.6	11.0 / 13.6	15.2 / 17.0	55.2 / 62.2
	C7	12.1 / 14.0	0.3 / 0.5	1.0 / 3.6	13.4 / 18.1
Day 14	C1	50.9 / 52.1	26.2 / 28.6	6.5 / 8.9	83.6 / 89.6
	C2	26.0 / 28.6	16.4 / 18.8	14.2 / 16.6	56.6 / 64
	C3	26.0 / 30.0	42.2 / 44.6	15.0 / 17.0	83.2 / 91.6
	C4	67.5 / 69.9	28.0 / 30.0	2.9 / 4.1	98.4 / 104
	C5	34.5 / 36.9	10.2 / 12.6	0.4 / 2.8	45.1 / 52.3
	C6	56.4 / 58.8	32.2 / 34.6	7.0 / 9.0	95.6 / 102.4
	C7	26.9 / 28.9	17.9 / 19.1	16.4 / 18.2	61.2 / 66.2

Table 12: Concentration of isoflavones glucosides in soy Cheddar analogues fermented with mesophilic species. Results are performed twice, both values are presented in the table. Results are expressed on wet weight basis.

Age of cheese	Cheese code	Isoflavones Glucosides (µg/g)			Total Glucosides (µg/g)
		Daidzin	Genistin	Glycitin	
Day 1	C1	306.7 / 307.7	40.1 / 41.1	50.6 / 51.4	397.4 / 400.2
	C2	61.0 / 61.5	96.0 / 96.5	55.4 / 56.0	212.4 / 214.0
	C3	111.3 / 112.3	25.4 / 26.0	85.3 / 86.3	222.0 / 224.6
	C4	95.2 / 60.2	84.3 / 85.3	55.9 / 56.7	199.4 / 202.2
	C5	43.8 / 44.8	58.2 / 60.2	37.0 / 38.0	139.0 / 143.0
	C6	41.9 / 42.4	65.8 / 66.6	41.5 / 42.0	149.2 / 151.0
	C7	30.7 / 31.7	59.1 / 60.1	28.6 / 29.4	118.4 / 121.2
Day 7	C1	3.7 / 4.3	9.8 / 10.3	40.9 / 41.4	54.4 / 56.0
	C2	28.0 / 28.0	35.9 / 36.9	55.0 / 56.0	118.9 / 120.9
	C3	38.0 / 38.4	36.8 / 37.8	42.4 / 43.3	117.2 / 119.6
	C4	117.6 / 119.6	38.8 / 39.8	31.6 / 32.6	188.0 / 192.0
	C5	29.0 / 29.0	5.3 / 5.3	54.9 / 57.3	89.2 / 91.6
	C6	22.8 / 23.8	67.6 / 68.6	23.0 / 23.8	113.4 / 116.2
	C7	28.7 / 29.7	34.2 / 34.6	23.3 / 24.3	86.2 / 88.6
Day 14	C1	3.2 / 3.2	26.3 / 26.7	18.0 / 20.0	47.5 / 49.9
	C2	8.9 / 8.9	25.1 / 25.9	39.2 / 40.2	73.2 / 75.0
	C3	8.0 / 9.0	36.5 / 37.5	29.5 / 29.5	74.0 / 76.0
	C4	50.0 / 55.0	4.4 / 7.4	30.6 / 32.6	85.0 / 95.0
	C5	7.0 / 7.7	28.1 / 29.2	33.3 / 33.3	67.4 / 70.2
	C6	21.3 / 22.7	25.3 / 26.3	20.6 / 20.6	67.2 / 69.6
	C7	24.1 / 24.1	29.8 / 30.8	17.1 / 17.1	71.0 / 72.0

Table 13: Concentration of total isoflavones (Aglycones and Glucosides) in soy Cheddar analogue fermented with mesophilic species for 1, 7 and 14 days of ripening. Results presented in the table is the average of total glucosides, total aglycones.

Age of cheese	Cheese code	Total Glucosides (µg/g)	Total Aglycones (µg/g)	Total Isoflavones (µg/g)
Day 1	C1	398.8	37.7	436.6
	C2	213.2	36.0	249.3
	C3	223.3	35.7	259.0
	C4	200.8	42.5	243.3
	C5	141.0	20.0	161.1
	C6	150.1	26.7	176.8
	C7	119.8	15.2	135.1
Day 7	C1	55.2	58.9	114.1
	C2	119.9	74.3	194.3
	C3	118.4	76.7	195.1
	C4	190.0	65.6	255.7
	C5	90.4	40.3	130.8
	C6	114.8	58.8	173.6
	C7	87.4	21.4	96.3
Day 14	C1	48.7	86.8	135.5
	C2	74.1	60.4	134.5
	C3	75.0	87.4	162.4
	C4	90.0	101.3	191.3
	C5	68.8	48.8	117.6
	C6	68.4	99.2	167.6
	C7	74.0	47.3	121.3

Texture analysis :

Cheese samples were prepared with different formulations with the addition of 6 % and 30 % oil or okara or both. It is of interest to evaluate the effect of this form of incorporation into the cheese analogue on the product textural properties. Dairy cheese samples (external control) of light Cheddar cheese were used as reference samples and results are presented in Table 14. Samples were evaluated after 1, 7 and 14 days of production and results are presented in Table 15.

- Hardness: Data indicated that the analogue required less force to break through the surface of the cheese as compared with the dairy cheese (external control) but required more force compared to dairy Cheddar prepared from low fat dairy milk. Between 1^{st} – 7^{th} and 7^{th} - 14^{th} day, hardness values increased, however, fat addition and/or okara at low concentration and high concentration was not found to enhance the hardness of the analogue up to the hardness level of dairy external control.
- Adhesiveness and cohesiveness: Results for adhesiveness for all cheese analogues showed a range of 0.92-14.47 g/mm with the lowest value for control C1 and the highest value for C7. Dairy light Cheddar (external control) had intermediate value around 7.68 g/mm, almost close to C4 with a value of 7.42g/mm. Control cheese prepared from dairy milk showed a value around 4.82g/mm less than dairy cheese (external control). For cohesiveness, results indicated that there were differences between dairy cheese (external control), dairy control cheese and cheese analogues. Values range from 0.32-0.69 with the

lowest value for C7 and the highest value was in C2, dairy Cheddar (external control) had a valve ~0.46 similar to C1 which showed a value of 0.47 and C4 which showed a value of 0.44.

- Springiness, gumminess and chewiness: There were differences between dairy cheese (external control), dairy control and analogue. Gumminess is related to hardness and cohesiveness while chewiness is related to gumminess and springiness. Since results indicated variation in cohesiveness, springiness and hardness as in Table 15, thus the determining factor for gumminess and chewiness can not be determined. C4 and C1 showed the closest texture characteristics to the dairy cheese (external control).

Table 14: Texture evaluation of dairy cheese (external control). Results are performed twice, both values are presented in the table.

Dairy external control	Hardness (g)	Adhesiveness (g/mm)	Cohesiveness	Springiness (mm)	Gumminess	Chewiness (g/mm)
Cheddar	119.7 /121.7	6.68 / 8.68	0.45 / 0.47	1.56 / 1.58	55.51/55.53	87.16/87.16
Mozzarella	208.45/210.45	5.64 / 7.64	0.22 / 0.24	1.10 / 1.30	48.16/48.18	57.80/57.80
Brie	84.65 / 86.65	11.38 / 13.38	0.28 / 0.30	2.96 / 2.98	24.82/24.84	73.77/73.77

In conclusion, addition of other ingredients (oil or okara) did not help to improve the overall texture of the soy Cheddar analogues prepared. For example, addition of 6% okara as in C3 improved the hardness and cohesiveness but it did not improve the other textural parameters evaluated in this study. Another example is C6 in which 30% okara had been added, this okara addition improved the springiness but it did not improve the overall quality of the soy cheese samples prepared.

Table 15: Texture evaluation of soy Cheddar analogues. Results are performed twice, both values are presented in the table.

Age of Cheese	Cheese Code	Hardness (g)	Adhesiveness (g/mm)	Cohesiveness	Springiness (mm)	Gumminess (mm)	Chewiness (g/mm)
Day 1	C1	43.8/43.8	0.15 / 0.17	0.50 / 0.60	0.52 / 0.54	16.32 /16.40	8.67/8.67
	C2	31.7/31.7	0.17 / 0.19	0.53 / 0.55	0.51 / 0.51	14.79/ 14.79	7.54/7.54
	C3	48.6/48.6	0.60 / 0.80	0.41 / 0.43	0.95 / 0.97	15.15 /15.17	14.6/14.6
	C4	38.8/38.8	0.99 / 0.99	0.43 / 0.43	0.87 / 0.87	17.96 /17.98	15.6/15.6
	C5	17.4/17.4	-	-	4.46 / 4.48	-	-
	C6	33.7/33.7	2.12 / 2.14	0.32 / 0.34	1.68 / 1.70	12.76/ 12.78	21.0 /22.0
	C7	19.9/19.9	2.68 / 2.68	0.41 / 0.43	3.39 / 3.41	6.72 / 6.72	22.8/ 22.8
	Dairy control	10.0/12.0	-	0.35 / 0.35	3.18 / 3.20	3.80 / 3.90	12.26/12.3
Day 7	C1	54.2/54.4	0.43 / 0.45	0.45 / 0.47	0.65 / 0.65	25.50 /25.50	16.0 /16.0
	C2	58.7/58.9	0.60 / 0.60	0.48 / 0.48	0.84 / 0.86	25.97 /25.97	19.78/19.7
	C3	57.3/57.5	0.62 / 0.64	0.39 / 0.41	1.12 / 1.14	23.92/ 23.92	25.48/25.4
	C4	56.1/56.3	1.73 / 1.73	0.38 / 0.38	1.30 / 1.30	21.83 /21.83	26.98/26.9
	C5	28.5/28.7	0.80 / 0.80	0.63 / 0.65	2.28 / 2.30	18.17 /18.17	43.07/43.0
	C6	44.7/44.9	3.38 / 3.38	0.32 / 0.34	1.82 / 1.82	15.39 /15.39	27.52/27.5
	C7	47.1/47.3	8.31 / 8.33	0.33 / 0.33	1.82 / 1.84	15.91 /15.91	27.62/27.6
	Dairy control	37.0/39	8.28 / 8.30	0.38 / 0.40	0.17 / 0.17	14.84 /14.84	10.53/10.5
Day 14	C1	115/117	0.91 / 0.93	0.47 / 0.47	0.84 / 0.86	55.87 /55.87	43.11/43.1
	C2	73 / 75	3.40 / 3.40	0.68 / 0.70	2.97 / 2.97	52.0 / 52.0	194.7/196
	C3	91.5/91.7	1.23 / 1.25	0.38 / 0.38	1.34 / 1.36	36.69 /36.69	46.08/46.0
	C4	81.1/81.3	7.41 / 7.43	0.43 / 0.45	1.98 / 1.98	38.67 /38.67	83.39/83.3
	C5	40.4/40.4	1.18 / 1.18	0.55 / 0.57	1.80 / 1.82	29.30 /29.30	58.71/58.7
	C6	67.5/67.7	4.92 / 4.94	0.33 / 0.33	1.82 / 1.82	22.58 /22.58	40.17/40.1
	C7	69.0/69.0	14.47 / 14.47	0.31 / 0.33	1.95 / 1.99	22.06 /22.06	42.34/42.3
	Dairy control	60.0/60.0	4.81 / 4.83	0.36 / 0.36	1.18 / 1.22	21.60 /21.60	68.47/68.4

III . 1 . 2) Soy Mozzarella Analogues

Mozzarella cheese is classified as a bacterial ripened cheese with moisture content 43 – 55 % thus it is also classified as a hard cheese (Wood 1998). Mozzarella produced for pizza toppings has a moisture content 45 – 52%, while Mozzarella produced as table cheese usually has a moisture content between 45 – 50% (Mead and Roupas 2001). Results of compositional analysis (% moisture, % ash, pH, % acidity) after 1, 7 and 14 days of ripening of prepared Mozzarella cheese analogues are presented in Figures 27 - 30.

<u>Moisture content</u>:

All cheese samples showed reduction in the moisture content after 7 and 14 days of ripening. During 1^{st}-7^{th} day of ripening, the greatest moisture content loss was observed in M7 and dairy control with 4.50 and 4.20% loss respectively. This is similar to soy Cheddar samples discussed earlier where dairy Cheddar control and C7 also showed the greatest moisture loss. The lowest moisture loss observed in M2 and M5 with 1.81, 2.38% loss respectively. During 7^{th}-14^{th} days of ripening, the greatest moisture loss was observed in M2 and M7 with 6.96 and 6.87% loss respectively.

However, after 14 days, M5 and M7 showed the lowest moisture content with 41.8 and 50.0% respectively. On the other hand, the greatest moisture content was in M6, M1 and M3 with 77.0, 76.1 and 74.2% moisture content. These results are similar to soy Cheddar analogues in which high 30% and low 6% addition of okara observed to promote water retention and addition of oil at 30% promoted syneresis.

Ash Content:

For ash values, all cheese samples increase in ash content during ripening. After 1 day ripening, all samples showed 0% ash content, while after 7 days, values increased and ranged from 0.61-2.38 % with the greatest value in M4, M2 as shown in Figure 27. The lowest value was in M5, M7.

All moisture values decreased upon ripening and all ash values increased upon ripening, this is similar to soy Cheddar analogues. In soy Cheddar analogues and soy Mozzarella analogues, the lowest moisture content was in C5, M5 with 34.8 and 41.8% moisture content. The greatest moisture content was in C6 and M6 with 81.1 and 77.0% moisture respectively. For ash values, (C4, M4) with their values (2.29, 2.97%) showed the greatest ash content. While (C7, M7) and their values were (0.77, 1.22%) showed the lowest values.

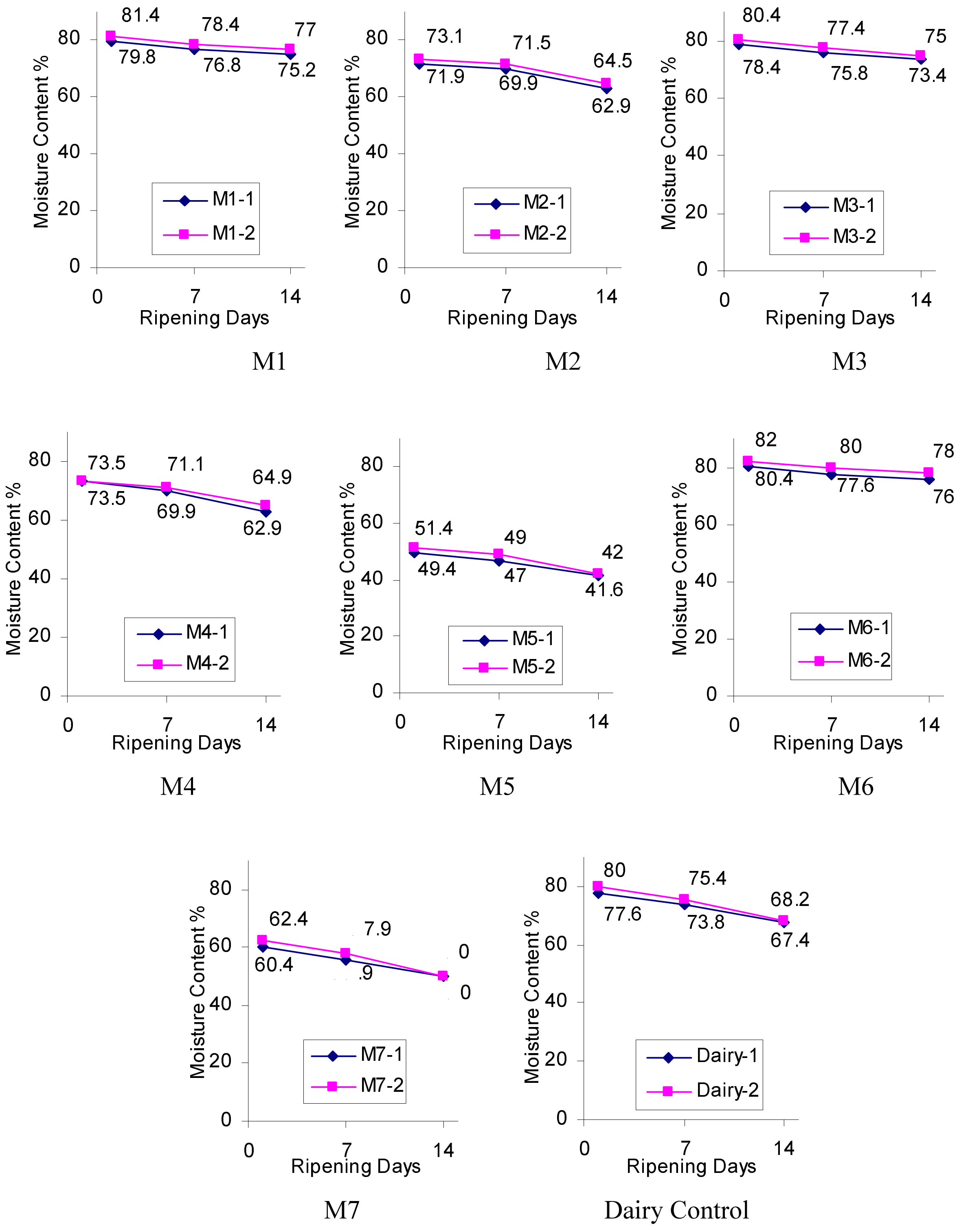


Figure 27: Moisture content of soy Mozzarella analogues, day 1, 7 ,14. Each scatter plot represent duplicate measurements (numbers 1 & 2) for every sample studied.

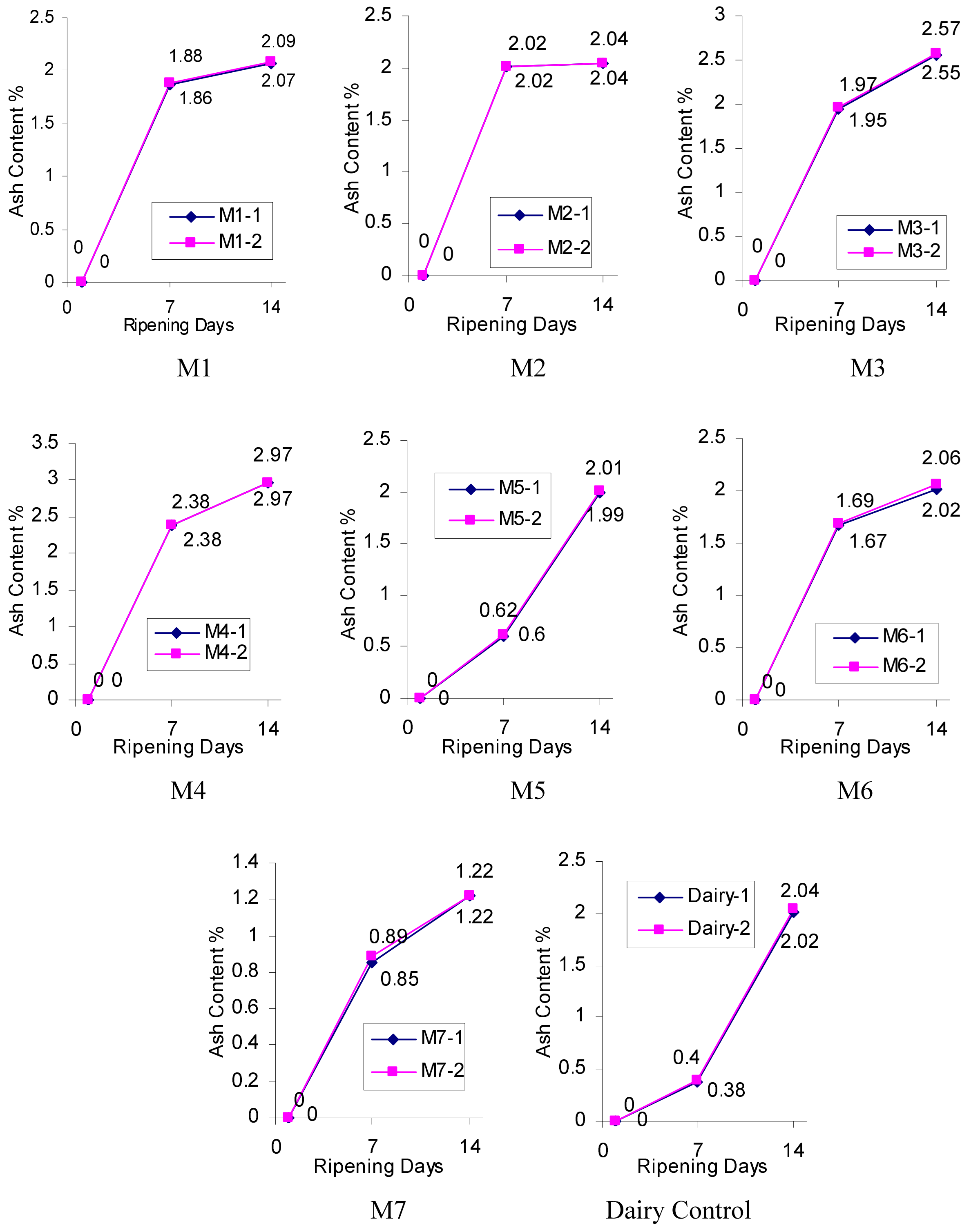


Figure 28: Ash content of soy Mozzarella analogues, day 1, 7 ,14. Each scatter plot represent duplicate measurements (numbers 1 & 2) for every sample studied.

pH and Acidity:

pH and acidity values are presented in Figures 29, 30. During 1^{st}-7^{th} day of ripening pH values increased for all cheese during ripening while acidity values showed some variations. The lowest pH values was in dairy Mozzarella control and M5 with pH value of 4.04 and 4.63. The greatest pH value was in M4 and M6 with 5.60 and 5.54 respectively. After 14 days of ripening, pH values increased and ranged from (5.06-6.22). The lowest pH value was in M5 and dairy Mozzarella-control with pH value of 5.06 and 5.14 respectively. The greatest pH value was in M6 and M1 with 6.22 and 5.76.

Just like in soy Cheddar analogue increase in pH was accompanied by decrease in acidity as presented in Figure 30. During 1^{st}-7^{th} day of ripening some cheese samples showed increase in % acidity as M3, M6, M7 with 0.14, 0.21, 0.13% increase respectively. While the rest M1, M2, M4, M5 and dairy Mozzarella control showed decrease in % acidity. But during 7^{th}-14^{th} day of ripening all cheese samples showed decrease in % acidity. Thus pH and acidity values observed during 7^{th} and 14^{th} day were similar to soy Cheddar analogue in that all samples increased their pH values and decreased acidity values. The greatest pH value after 14 days was in C6, M6 with values of 6.61, 6.22 respectively while the lowest pH values was in dairy Cheddar and M5 with values of 5.03, 5.06 respectively. Acidity values were greatest in dairy Cheddar and dairy Mozzarella with values of 0.66 and 0.84% and the lowest acidity values were in C6, M1 with values of 0.08, 0.07% respectively.

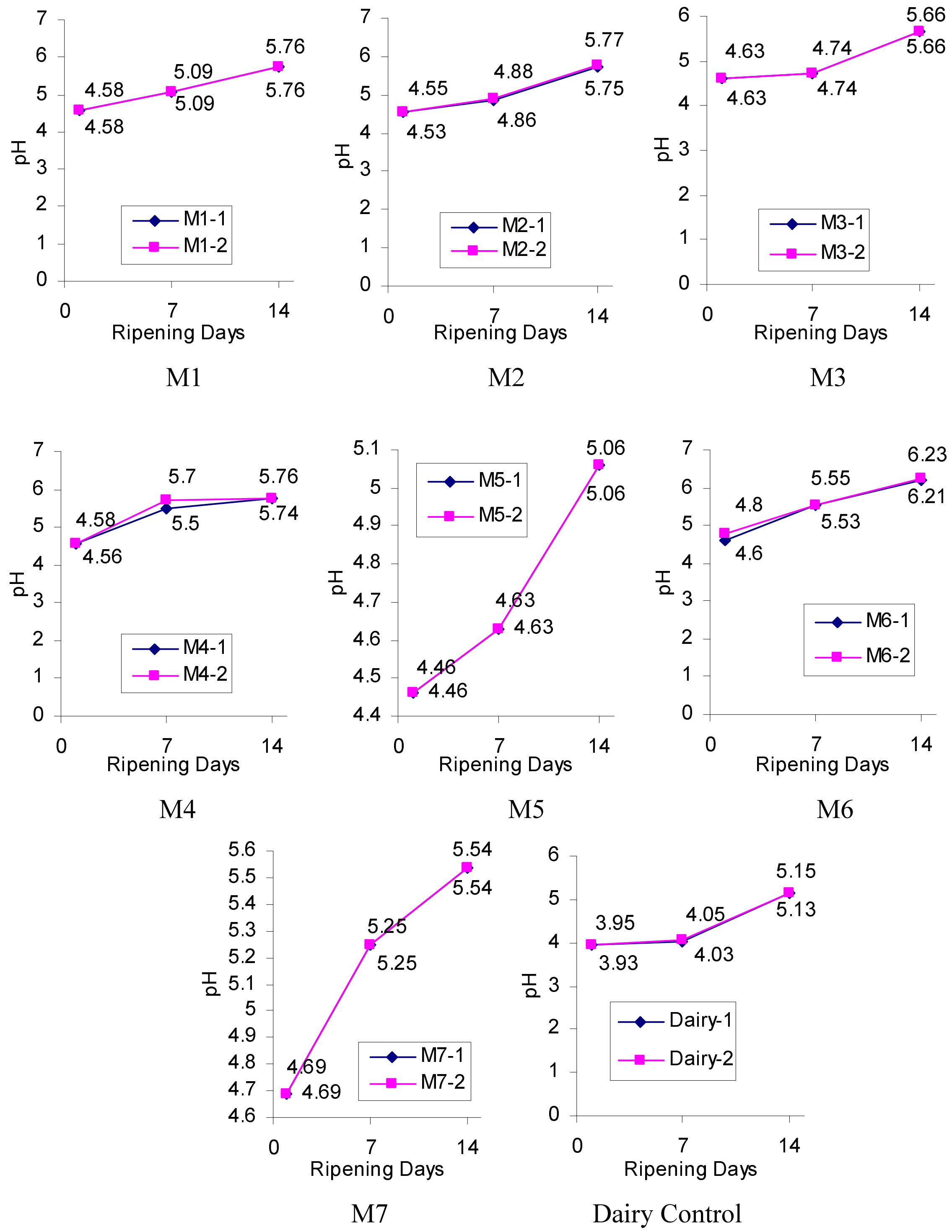


Figure 29: pH values of soy Mozzarella analogues, day 1, 7 ,14. Each scatter plot represent duplicate measurements (numbers 1 & 2) for every sample studied.

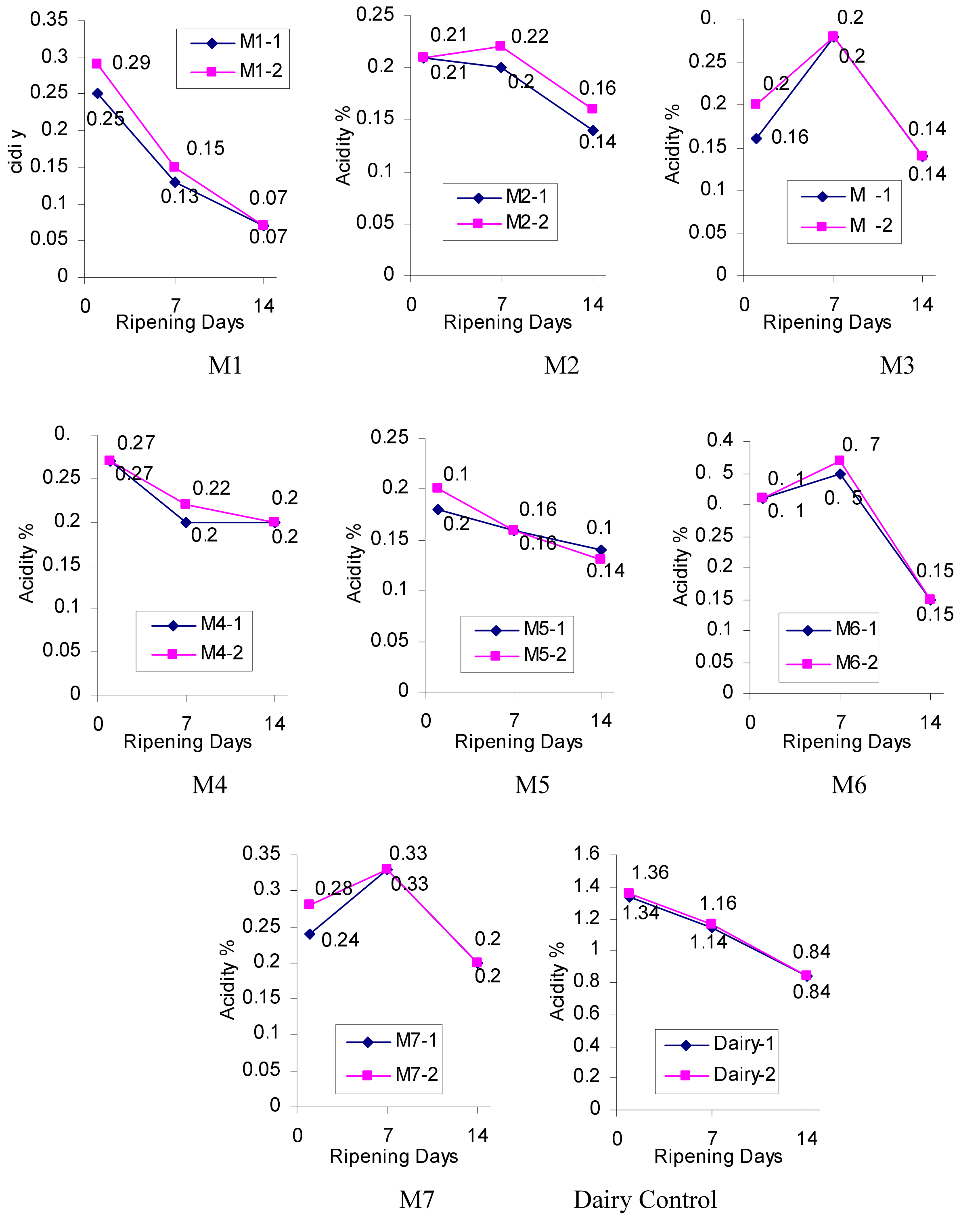


Figure 30: Acidity content of soy Mozzarella analogues, day 1, 7 ,14. Each scatter plot represent duplicate measurements (numbers 1 & 2) for every sample studied.

Isoflavones content:

The change in concentration of isoflavones aglycones, glucosides and total isoflavones of soy Mozzarella analogue are shown in Tables 16, 17, 18. The fermentation of soymilk with thermophilic bacteria and their effect on the isoflavones content was evaluated after 1, 7 and 14 days of ripening. During 1^{st}-7^{th} day of ripening, all cheese analogues showed increase in aglycones concentration with the greatest increase observed in M1 and M6. The lowest isoflavones aglycones observed in M3 and M2. During 7^{th}-14^{th} day of ripening, all cheese analogues increase their aglycones concentration. The greatest isoflavones aglycones concentration was in M1 and M7. While the lowest aglycones concentration was in M5. After 14 days, the highest aglycones concentration was in M1 and M7 with 207.7 and 136.8µg/g and the lowest aglycones concentration was in M5 and M3 with 33.0 and 56.0 µg/g respectively.

All cheese samples showed increase in aglycones concentration after 14 days of ripening, this increase was accompanied by decrease in glucosides concentration. Soy Mozzarella analogues fermented with thermophilic starter decreased glucosides concentration after 14 days of ripening as in Table 17. The lowest glucosides concentration was in M4 while the greatest glucosides concentration after 14 days was in M1. After 14 days, the highest glucosides concentration was in M3 and M2 with 120.6 and 114.7 µg/g. While the lowest glucosides concentration was in M4 and M1 with 33.5 and 44.7 µg/g respectively.

However, mean total isoflavones levels investigated varied between 122.5-252.5µ/g with lowest values found in M4 and M5 while the highest levels found in M1, M2 as presented in Table 18. Some of these results are similar to soy Cheddar analogue for instance both C5, M5 showed a lower total isoflavones content than other formulations while values showing the highest total isoflavones were different, for example M1, M2 showed the highest total isoflavones among soy Mozzarella. While C4, C6 showed the highest total isoflavones among soy Cheddar analogues.

Table 16: Concentration of Isoflavones aglycones in soy Mozzarella analogues fermented with thermophilic species. Results are performed twice, both values are presented in the table. Results are expressed on wet weight basis.

Age of cheese	**Cheese code**	**Isoflavones Aglycones (µg/g)**			**Total Aglycones (µg/g)**
		Daidzein	**Genistein**	**Glycitein**	
Day1	M1	0.3 / 0.3	9.2 / 9.4	6.9 / 7.1	16.4 / 16.8
	M2	0.7 / 0.7	2.3 / 2.4	1.2 / 1.4	4.3 / 4.5
	M3	1.0 / 1.2	-	0.6 / 0.6	1.6 / 1.8
	M4	0.6 / 0.6	-	1.8 / 1.8	2.4 / 2.4
	M5	3.0 / 3.0	-	0.4 / 0.4	3.4 / 3.4
	M6	3.4 / 3.4	9.8 / 9.8	1.1 / 1.1	14.3 / 14.3
	M7	1.2 / 1.2	16.9 / 16.9	0.1 / 0.1	18.2 / 18.2
Day 7	M1	4.3 / 4.3	28.6 / 28.6	32.0 / 32.0	64.9 / 64.9
	M2	11.8 / 11.8	6.9 / 6.9	4.6 / 4.6	23.3 / 23.3
	M3	12.9 / 12.9	4.5 / 4.5	1.3 / 1.3	18.7 / 18.7
	M4	25.7 / 25.7	22.0 / 22.0	2.2 / 2.2	49.9 / 49.9
	M5	17.1 / 17.1	6.7 / 6.7	3.4 / 3.6	27.2 / 27.4
	M6	35.3 / 35.3	17.2 / 17.2	9.9 / 9.9	62.4 / 62.4
	M7	34.7 / 34.7	17.8 / 18.0	2.9 / 2.9	55.4 / 55.6
Day 14	M1	102.6/102.6	66.4 / 66.4	38.7 / 38.7	207.7 / 207.7
	M2	60.9 / 60.9	32.9 / 32.9	7.8 / 8.0	101.6 / 101.8
	M3	26.7 / 26.7	22.9 / 22.9	6.3 / 6.5	55.9 / 56.1
	M4	51.3 / 51.3	26.8 / 26.8	10.9 / 10.9	89.0 / 89.0
	M5	18.4 / 18.4	7.5 / 7.9	6.9 / 6.9	32.8 / 33.2
	M6	45.7 / 45.7	26.6 / 26.6	10.1 / 10.3	82.4 / 82.6
	M7	37.2 / 37.2	35.8 / 35.8	63.6 / 64.0	136.6 / 137.0

Table 17: Concentration of Isoflavones glucosides in soy Mozzarella analogues fermented with thermophilic species. Results are performed twice, both values are presented in the table. Results are expressed on wet weight basis.

Age of cheese	**Cheese code**	**Isoflavones Glucosides (µg/g)**			**Total Glucosides (µg/g)**
		Daidzin	**Genistin**	**Glycitin**	
Day 1	M1	143.3 / 143.5	105.0 / 105.2	96.3 / 96.3	344.6 / 345.0
	M2	66.8 / 66.8	83.9 / 83.9	63.5 / 63.9	214.2 / 214.6
	M3	133.6 / 133.6	134.4 / 134.4	83.0 / 84.0	351 / 352
	M4	57.1 / 57.1	40.5 / 40.7	63.0 / 63.0	160.6 / 160.8
	M5	44.2 / 44.6	65.5 / 65.5	52.3 / 52.3	162.0 / 162.4
	M6	73.4 / 73.4	98.6 / 98.6	69.5 / 69.5	228.0 / 228.3
	M7	100.5 / 102.5	59.2 / 59.2	38.7 / 38.7	200.2 / 200.4
Day 7	M1	19.8 / 19.8	46.9 / 48.9	31.3 / 31.3	98.0 / 100.0
	M2	56.6 / 56.6	65.3 / 65.3	28.0 / 28.0	148.8 / 150.0
	M3	48.5 / 48.5	65.9 / 65.9	56.6 / 56.6	170.6 / 171.0
	M4	138.1 / 138.1	78.3 / 78.3	31.0 / 31.0	147.2 / 147.4
	M5	41.1 / 41.1	57.1 / 57.1	20.7 / 20.7	118.7 / 118.9
	M6	25.0 / 25.0	68.3 / 69.3	16.7 / 17.7	110.0 / 112.0
	M7	25.3 / 25.7	54.4 / 54.4	13.9 / 13.9	93.6 / 94.0
Day 14	M1	12.3 / 12.3	18.5 /18.6	14.0 / 14.1	44.8 / 45.0
	M2	29.6 / 29.8	49.1 / 49.1	35.1 / 35.1	114.6 / 114.8
	M3	29.1 / 30.1	50.7 / 50.7	40.8 / 40.8	119.6 / 121.6
	M4	8.7 / 8.9	4.6 / 4.6	20.1 / 20.1	33.4 / 33.6
	M5	22.4 / 22.6	44.6 / 44.6	28.0 / 28.0	95.0 / 95.2
	M6	22.1 / 22.1	46.7 / 46.9	17.5 / 17.5	86.3 / 86.5
	M7	121.1 / 121.3	37.1 / 37.1	15.9 / 15.9	74.1 / 74.3

Table 18: Concentration of total isoflavones (Aglycones and Glucosides) in soy Mozzarella analogues fermented with thermophilic species. Results presented in the table is the average of total glucosides, total aglycones.

Age of cheese	Cheese code	Total Aglycones (µg/g)	Total Glucosides (µg/g)	Total Isoflavones (µg/g)
Day 1	M1	16.6	344.8	361.5
	M2	4.4	214.4	218.9
	M3	1.7	351.5	353.2
	M4	2.4	160.7	163.2
	M5	3.4	162.2	165.6
	M6	14.3	228.1	242.4
	M7	18.2	200.3	218.5
Day 7	M1	64.9	99.0	164.0
	M2	23.3	148.9	172.3
	M3	18.7	170.8	189.6
	M4	49.9	147.3	197.3
	M5	27.3	118.8	146.1
	M6	62.4	111.0	173.5
	M7	55.5	93.8	149.3
Day 14	M1	207.7	44.9	252.5
	M2	101.7	114.7	216.5
	M3	56.0	120.6	176.6
	M4	89.0	33.5	122.5
	M5	33.0	95.1	128.2
	M6	82.5	86.4	168.9
	M7	136.8	74.2	211.0

Texture Analysis:

Cheese samples were prepared with formulations similar to the soy Cheddar analogue with the addition of 6%, 30% oil or okara or both. Texture characteristics after 1, 7 and 14 days of ripening were evaluated and are summarized in Table 19. Dairy Mozzarella (external control) was included and evaluated in the same way as soy Mozzarella analogues were tested and results are presented in Table 14, [Page 64].

Table 19: Texture evaluation of soy Mozzarella analogues. Results are performed twice, both values are presented in the table.

Age of Cheese	Cheese Code	Hardness (g)	Adhesiveness (g/mm)	Cohesiveness	Springiness (mm)	Gumminess (mm)	Chewiness (g/mm)
Day 1	M1	44.0/44.0	1.50/1.70	0.40 / 0.42	1.05 / 1.07	18.76/18.78	19.8/20.0
	M2	36.2/36.2	2.15 / 2.15	0.36 / 0.38	1.40 / 1.42	9.50 / 9.70	13.53/15.5
	M3	35.2/35.2	1.36 / 1.38	0.38 / 0.40	1.29 / 1.31	16.25/16.27	21.14/21.1
	M4	30.0/30.0	0.43 / 0.45	0.40 / 0.42	1.17 / 1.19	15.88/15.88	18.74/18.7
	M5	22.6/22.6	0.57 / 0.59	0.50 / 0.52	0.9 / 1.1	12.06/12.06	12.06/12.0
	M6	51.8/51.8	2.01 / 2.03	0.37 / 0.39	1.66 / 1.68	28.89/28.89	48.26/48.2
	M7	32.0/32.0	2.60 / 2.64	0.32 / 0.34	1.62 / 1.64	13.11/13.11	21.38/21.3
	Dairy Mozzarella	21.8/21.8	1.49 / 1.51	0.23 / 0.25	1.77 / 1.79	5.23 / 5.25	9.31 /9.33
Day 7	M1	72.6/72.6	0.59 / 0.61	0.41 / 0.43	0.77 / 0.79	30.81/30.81	24.26/24.3
	M2	59.2/59.2	1.14 / 1.16	0.37 / 0.39	1.18 / 1.20	22.39/22.39	26.67/26.6
	M3	68.6/68.6	0.97 / 0.99	0.39 / 0.41	1.52 / 1.54	27.76/27.78	42.47/42.4
	M4	62.2/62.2	2.01 / 2.01	0.36 / 0.38	1.39 / 1.41	24.06/24.08	31.31/31.3
	M5	31.2/31.2	0.49 / 0.51	0.72 / 0.74	2.05 / 2.07	24.09/24.09	58.9/60.9
	M6	91.4/91.4	2.72 / 2.74	0.33 / 0.35	1.88 / 1.90	33.67/33.67	58.86/58.8
	M7	56.3/56.3	5.69 / 5.71	0.29 / 0.31	1.92 / 1.94	17.32/17.34	32.84/32.8
	Dairy Mozzarella	26.5/26.7	2.59 / 2.61	0.35 / 0.37	2.13 / 2.15	9.52 / 9.54	20.39/20.3
Day 14	M1	71.97/72	2.47 / 2.49	0.48 / 0.52	1.16 / 1.16	34.17/34.17	38.69/38.6
	M2	60.0/60.2	4.20 / 4.22	0.53 / 0.53	1.89 / 1.89	32.49/32.49	67.13/67.1
	M3	80.4/80.4	2.85 / 2.87	0.35 / 0.39	1.44 / 1.44	30.09/30.09	43.28/43.2
	M4	93.1/93.1	5.06 / 5.08	0.43 / 0.43	2.25 / 2.25	38.6 / 38.6	65.82/65.8
	M5	43.6/43.6	3.30 / 3.30	0.35 / 0.37	1.30 / 1.30	15.94/15.94	19.41/19.4
	M6	109/110	4.20 / 4.24	0.30 / 0.32	1.86 / 1.86	35.76/35.76	63.11/63.1
	M7	95.4/95.4	7.00 / 7.04	0.28 / 0.30	1.92 / 1.94	28.77/28.77	52.68/52.6
	Dairy Mozzarella	45.2/45.2	4.00 / 4.20	0.41 / 0.43	1.74 / 1.76	18.98/18.98	33.21/33.2

- Hardness**:** Data indicated that dairy Mozzarella (external control) required more force to break through the surface than soy Mozzarella analogue as presented in Tables 14 and 19. Among soy Mozzarella analogues, M6 required the highest force and exhibited a greater hardness than the rest of the analogues, followed by M7 and M4 (both samples had been supplemented with oil and okara at different concentration). However, lowest hardness values were in M5, M2. This may suggest that addition of oil result in reduction in the force needed to break cheese surface. This is not in agreement with Yang and Taranto 1982 who prepared Mozzarella cheese from soy and found that fat addition enhance the hardness of the curd. This difference in results may be due to the type and amount of lipid added. In this study, soybean oil was added at 6 and 30% oil while Yang and Taranto 1982 used a solid coconut fat. Besides they used texture enhancers such as gelatin and gum Arabic which were not used in soy cheese preparation in this study.

- <u>Adhesiveness and Cohesiveness</u>: Results for adhesiveness of all cheese analogues showed a range from 2.48-7.02g/mm with the lowest value observed for M1 control (just as C1 in soy Cheddar which also showed the lowest adhesiveness). The highest value was in M7 with a value of 7.02g/mm. However, dairy Mozzarella (external control) showed a value of (6.64). The closest value to Mozzarella external control was 7.02g//mm and was observed in M7 and M4 and their values were 7.02 and 5.07 respectively. For cohesiveness, dairy Mozzarella (external control) showed again a close value to M4 and it was also the lowest cohesiveness value. M2 showed the highest cohesiveness value followed by M1. Adhesiveness and cohesiveness values for soy Mozzarella analogue are similar to soy cheddar analogue. In both trials, C7 and M7 showed the lowest cohesiveness while C1 and M1 showed the lowest adhesiveness.

- Springiness, Gumminess and Chewiness: Springiness values range from 1.16 to 2.25mm with the highest value for M4 and the lowest value for M1. Dairy Mozzarella (external control) value was 1.20 and was closest to M1 (control) which had a value of 1.16mm followed by M5 with 1.30mm springiness value. The rest of soy cheese analogues had lower gumminess values than external control, among these the greatest values were in M4 and M6 and the lowest value was in M5 followed by M7 as presented in Table 19. Chewiness is a function of gumminess and springiness, since dairy Mozzarella showed the greatest gumminess and springiness values so it will also get the highest chewiness values. The lowest chewiness value was in M4 and it also showed the lowest gumminess value. Yang and Taranto 1982 stated that a good Mozzarella cheese should possess a moderate hardness and an adequate stringiness. Compared with dairy Mozzarella (external control), M6 showed the closest textural characteristics.

The manufacture of reduced-fat and low fat Mozzarella cheeses with sensory properties similar to full fat cheeses poses a challenge to cheese manufacturers. The main texture defects ascribed to low-fat cheese are crumbly and chewy mouth feel. The presence or absence of fat in cheese impacts the texture and the sensory properties of cheese (Bhaskaracharya and Shah 2001).

III . 1 . 3) Soy Brie Analogues

Brie cheese is classified as a mould ripened cheese with moisture content > 55 % so it is classified as soft cheese. Results of compositional analysis (% moisture, % ash, pH, % Acidity) after 1,7 and 14 days of ripening of prepared soy Brie analogues are presented in Figures 31 - 34.

Moisture and Ash:

Similar to soy Cheddar and soy Mozzarella analogues, all soy Brie analogues showed reduction in moisture content after 7, 14 days of ripening. During 1st-7th day of ripening, the greatest loss was in B5 and B7. The lowest moisture loss was observed in B6 and B1. During 7th-14th days of ripening, the greatest moisture loss was observed in B2 and B5 with 15.37 and 10.17% loss respectively. While the lowest moisture loss was in B7 and B6 with 1.40 and 1.81% loss respectively. After 14 days of ripening all cheese samples showed reduction in moisture content as presented in Figure 31. B5 and B7 contained the lowest moisture content with values of 36.9 and 53.8% respectively. While the greatest moisture content was observed in B6 and B3 with 79.2 and 73.5% moisture content respectively. B1 (control) showed 70.5% moisture content.

Ash Content:

For ash values, all cheese samples showed increase in ash content during ripening as presented in figure 32. After the first day of the ripening process ash was detected as trace amounts 0% in all samples, while after 7 days, values increased with a range from 0.82-2.59% with the greatest value observed in B1 (2.59% ash) and the lowest content was in B7 (0.82%). After 14 days, ash content continued to increase and ranged from 1.02-2.90%. The greatest ash content was in B3 and B1 with 2.90and 2.63% and the lowest content was in B7 and B2 with 1.02 and 1.07% ash respectively. Among all cheese analogues prepared, (C6, C3), (M6, M1), (B6, B3) showed the greatest moisture content and their values were (81.1, 74.5), (77.0, 76.1), (79.2, 73.5) respectively. Addition of 6 and 30% okara seemed to enhance water retention and increase moisture content, control soy Cheddar, Mozzarella and Brie contained 62.4, 76.1 and 70.5% respectively. Dairy control Cheddar, Mozzarella and Brie contained 65.4, 67.8 and 65.7% moisture respectively. This suggested that syneresis in dairy curd is enhanced more than in soy milk based curd. The lowest moisture content values were in (C5, C7), (M5, M7), (B5, B7) and their values were (34.8, 54.4), (41.8, 50.0), (36.9, 53.8%) respectively. These values suggested that addition of 30% oil w/wo okara promoted syneresis while addition of 6 and 30% okara promoted water retention.

For ash values (C4, C6), (M4, M3), (B3, B4) and their values were (2.29, 1.79), (2.97, 2.56), (2.90, 2.63%) respectively. Cheese supplemented with 6% oil and okara showed the greatest ash content among all cheese analogues, this is due to the fact that okara contains 25.4-28.40% protein (Liu 1999) so its mineral content is high. Soy control Cheddar, Mozzarella and Brie contained 1.52, 2.08 and 2.44% ash while dairy control contained 1.24, 2.03 and 1.05% ash respectively. All soy cheese analogues and dairy control cheeses prepared showed reduction in moisture content and increase in ash content upon ripening.

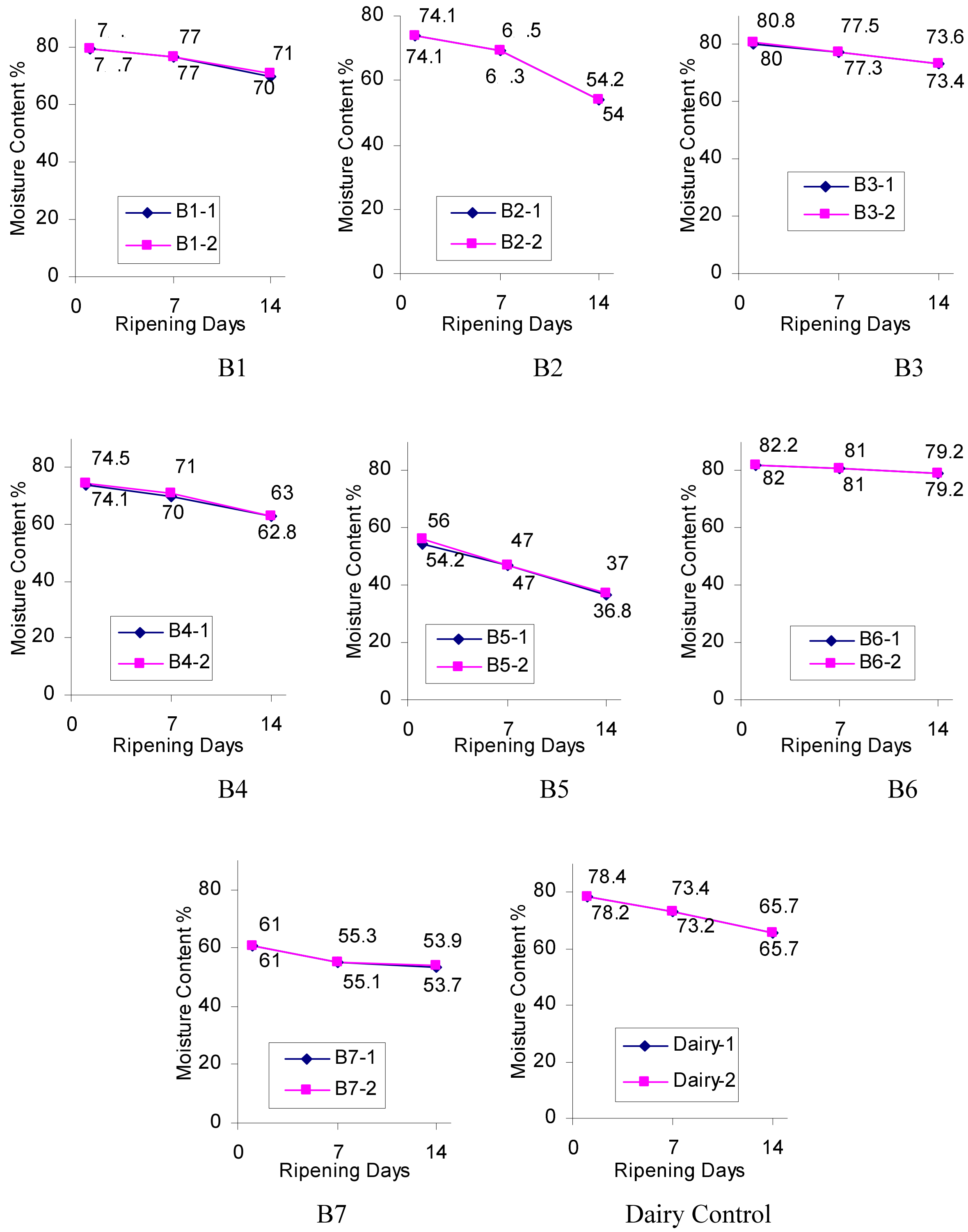


Figure 31: Moisture content of soy Brie analogues, day 1, 7 ,14. Each scatter plot represent duplicate measurements (numbers 1 & 2) for every sample studied.

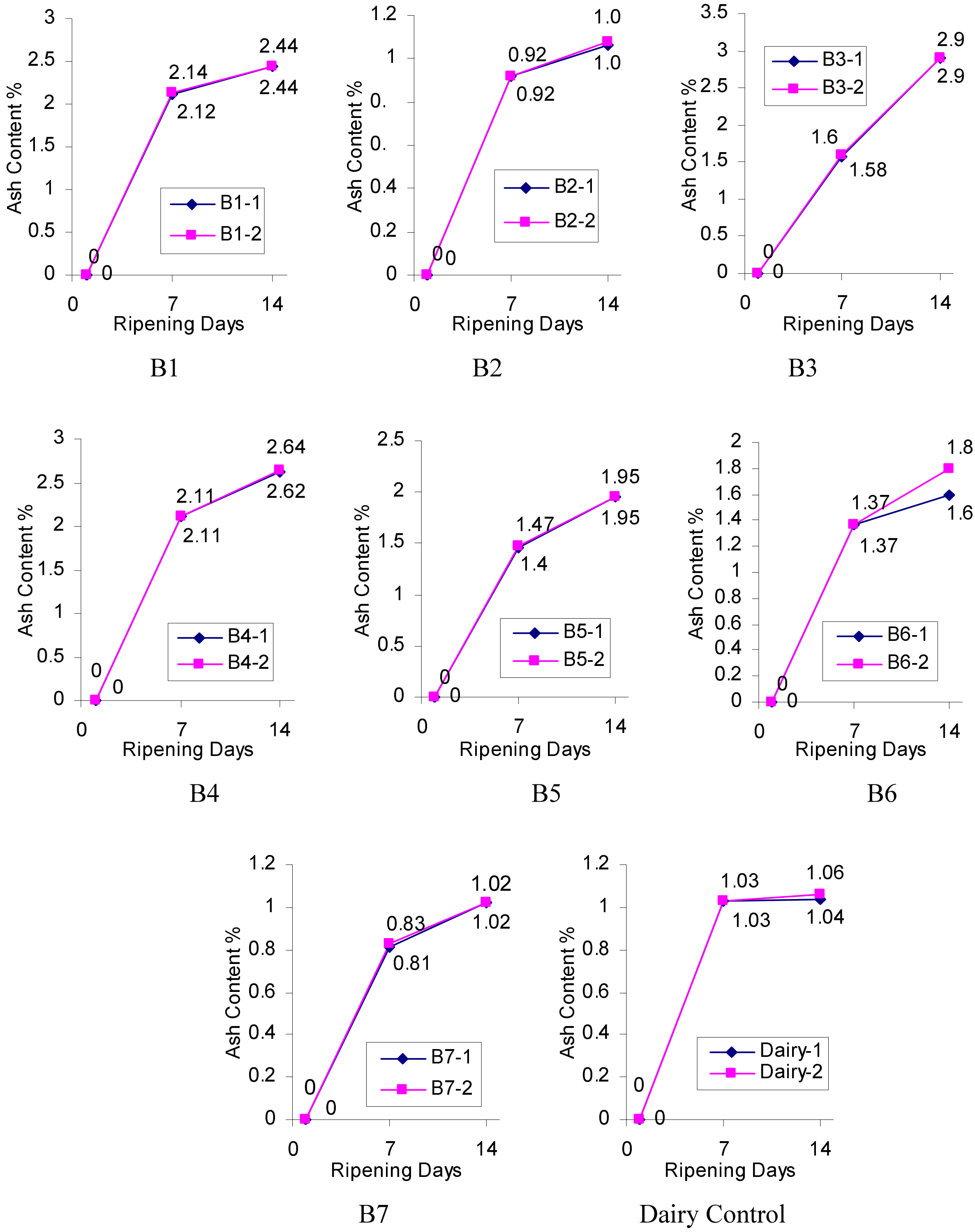


Figure 32: Ash content of soy Brie analogues, day 1, 7 ,14. Each scatter plot represent duplicate measurements (numbers 1 & 2) for every sample studied.

pH and Acidity:

pH and acidity values are presented in Figures 33, 34. During 1^{st}-7^{th} day of ripening pH values increase for all cheese samples during ripening while acidity values showed some variations. After 1st day of ripening, pH values increase for all cheese analogues, values range from 4.40-5.11. Dairy Brie showed the lowest values while B4 showed the highest one. During 1^{st}-7^{th} day of ripening, pH values range from 4.52 and 5.79 with the lowest value observed in dairy Brie and the highest value observed in B2. After fourteen days, pH values continued to increase and range from 5.18-7.26, the lowest values observed in B5 and dairy Brie. While the highest values observed in B3 and B6 as in Figures 33 and 34.

Acidity values showed some variations, for instance B2, B4, B5 and B6 showed increase in %acidity after 7 days while B1, B3, B7, dairy control showed decrease. After 14 days, all samples showed decrease except for B2 which showed slight increase of 0.01%. Among the three cheese analogues prepared, (C6, C3), (M6, M2), (B3, B6) showed the highest pH values. These results suggest that 30% okara addition seemed to increase pH values regardless of the microorganisms inoculated in the milk. The lowest pH values were observed in (dairy Cheddar, C4), (M5, dairy Mozzarella-control), (B5, dairy Brie-control). Thus, dairy control seemed to possess the lowest pH values among samples. From the soy analogues, 30% oil addition as in B5, M5 seemed to be associated with lower pH values, this may suggest that high fat content affected microorganisms optimum growth and enhanced acid production while high okara content did not enhance acid production as presented in results obtained in this study. For acidity values, (dairy Cheddar, C7), (dairy Mozzarella, M7) and (dairy Brie, B7) showed the greatest % acidity their values were (0.66, 0.17), (0.84, 0.20), (0.50, 0.38) respectively. Dairy cheese showed the lowest pH values and the highest % acidity compared with soy cheeses. However, the highest % acidity among soy cheese analogues was in C7, M7, B7 with values (0.17, 0.20, 0.38) respectively. These samples contained 30% oil and okara and as mentioned earlier B7 displayed the greatest acidity level, this may be due to presence of two types of microorganisms (the mesophilic starter - which is already known to grow and produce acid in soymilk [as in soy Cheddar]- and *Penicillium candidum* which appear to have a similar effect of enhancing acid production).

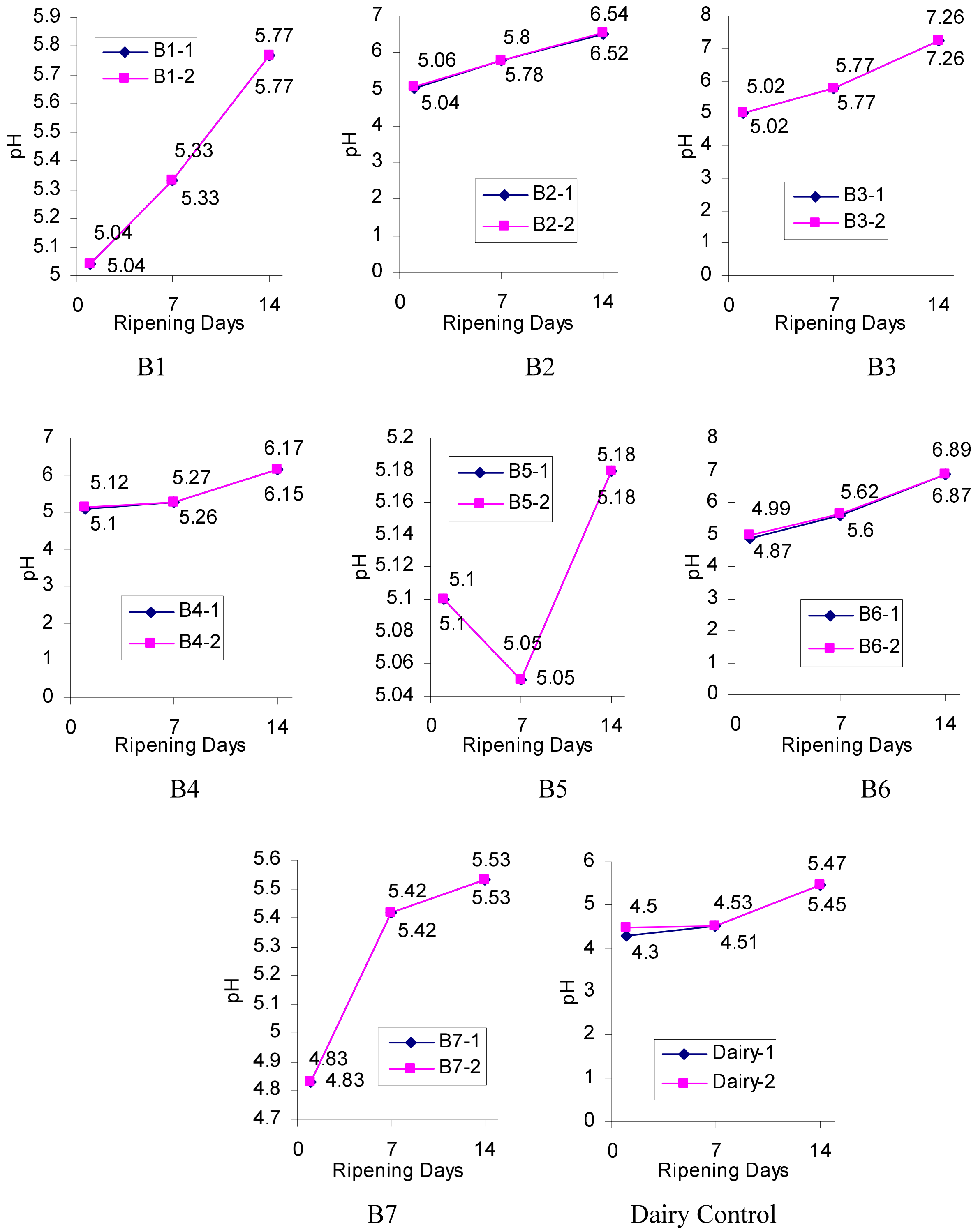


Figure 33: pH values of soy Brie analogues, day 1, 7 ,14. Each scatter plot represent duplicate measurements (numbers 1 & 2) for every sample studied.

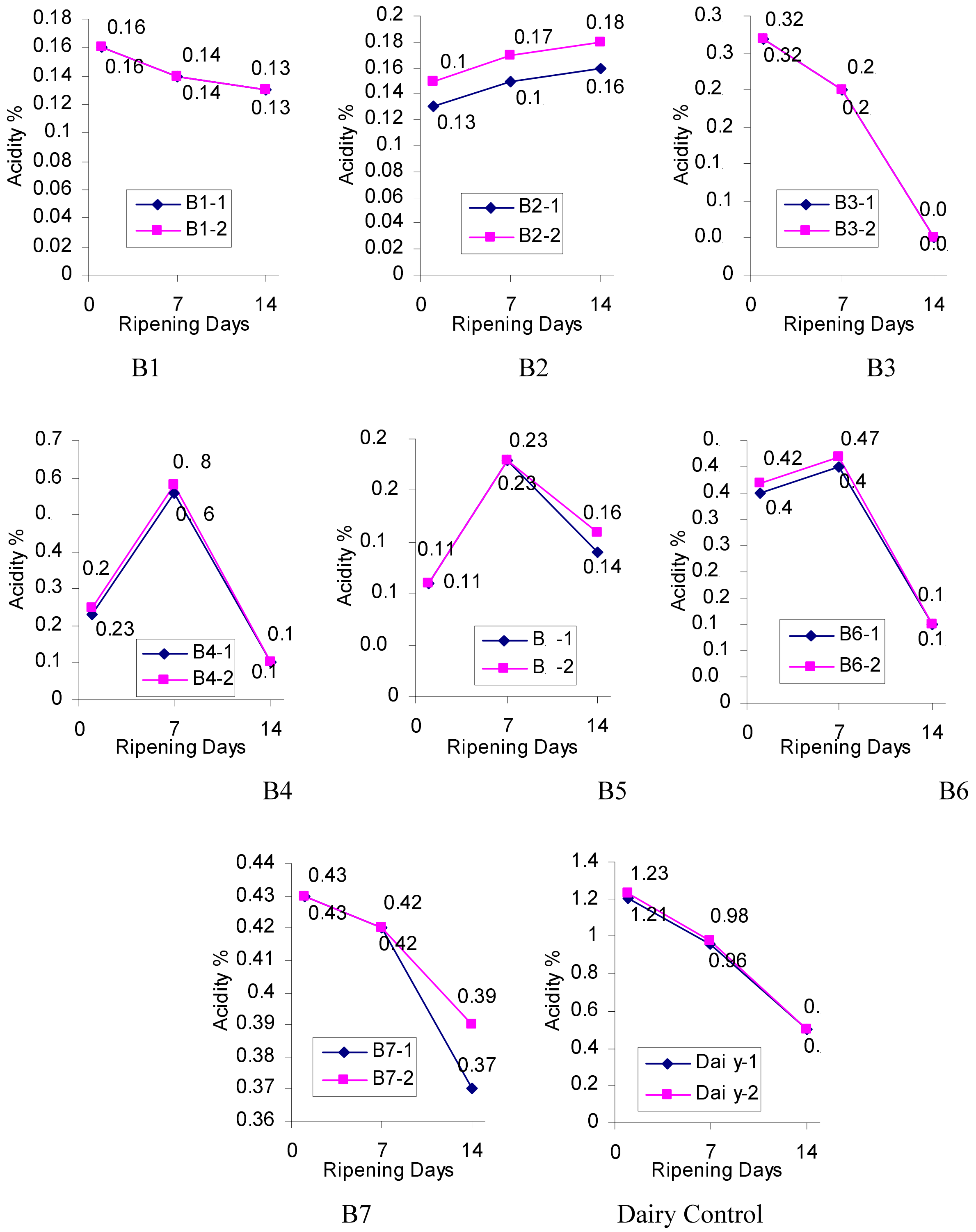


Figure 34: Acidity content of soy Brie analogues, day 1, 7 ,14. Each scatter plot represent duplicate measurements (numbers 1 & 2) for every sample studied.

Isoflavones Content:

The change in concentration of isoflavones aglycones, isoflavones glucosides and total isoflavones of soy Brie analogues are shown in Tables 20, 21, 22. The fermentation of soy milk with mesophilic bacteria and *Penicillim candidum* and their effect on isoflavones content was evaluated after 1, 7 and 14 days of ripening. During 1^{st}-7^{th} day of ripening, all cheese analogues showed increase in aglycones concentration with the greatest increase observed in B5 and B6 with 94.3 and 65.5 µg/g respectively. The lowest increase observed in B2 and B1 with 17.5 and 37.1µg/g respectively.

During 7^{th}-14^{th} day B2 and B6 showed the greatest increase with 127.7 and 98.8µg/g respectively and the lowest increase was in B5 and B7 with 58.5 and 65.4µg/g respectively. After 14 days, B2, B6 contained the greatest aglycones concentration with 266.4 and 217.7µg/g respectively and the lowest concentration observed in B7 and B1 with 163.3 and 193.4µg/g respectively.

Increase in aglycones concentration was accompanied by glucosides loss. There was a steady decrease in glucosides concentration in all samples as presented in Table 21. During 1^{st}-7^{th} day, B7 and B2 showed the greatest glucosides loss. While B5 and B1 showed the lowest loss. During 7^{th}-14^{th} day, B3 and B7 showed the greatest decrease while B2 and B4 showed the lowest one. After 14 days of ripening, B2 and B1 showed the greatest glucosides concentration and their values were 110.1 and 85.0µg/g while the lowest concentration was in B7 and B5 with their values of 14.5 and 39.3µg/g. However, the total isoflavones concentration presented in Table 22, showed that B2 and B4 contained the greatest content of isoflavones and their values were 376.5 and 286.1µg/g respectively while B7 and B5 which contained 177.9 and

242.8μg/g showed the lowest values of total isoflavones concentration. Results among all cheese analogues suggested that addition of 30% oil as in C5, M5 and B5 cause isoflavones loss. On the other hand cheese formulations with 6% okara may help in isoflavones recovery upon ripening. This is partly in agreement with Franke *et al* 1999 who found out that total isoflavones content in soybean based products is related to the product moisture content mainly due to the hydrophobic nature of isoflavones.

Table 20: Concentration of isoflavones aglycones in soy Brie analogues fermented with thermophilic and *Penicillium candidum*. Results are performed twice, both values are presented in the table. Results are expressed on wet weight basis.

Age of cheese	**Cheese code**	**Isoflavones Aglycones (µg/g)**			**Total Aglycones (µg/g)**
		Daidzein	**Genistein**	**Glycitein**	
Day 1	B1	47.4 / 47.4	29.7 / 29.7	8.0 / 8.1	78.0 / 79.6
	B2	49.3 / 49.3	62.6 / 62.8	9.1 / 9.1	121.0 / 121.2
	B3	51.7 / 51.6	48.5 / 48.4	4.4 / 4.6	104.6 / 104.8
	B4	43.3 / 43.3	43.5 / 43.5	4.3 / 4.4	81.2 / 81.2
	B5	33.5 / 33.5	19.9 / 19.9	6.8 / 7.2	50.4 / 50.8
	B6	44.5 / 44.5	13.3 / 13.3	5.4 / 5.4	53.3 / 53.3
	B7	35.7 / 35.7	21.0 / 21.4	8.0 / 10.0	56.1 / 56.1
Day 7	B1	61.1 / 61.1	48.8 / 48.8	15.9 / 15.9	115.8 / 116.0
	B2	58.2 / 58.4	74.9 / 74.9	15.2 / 15.4	138.4 / 138.8
	B3	63.8 / 64.0	78.4 / 78.4	11.6 / 11.6	144.0 / 144.2
	B4	54.5 / 54.5	69.5 / 69.5	16.2 / 16.2	130.4 / 130.4
	B5	70.6 / 71.0	52.6 / 52.6	31.2 / 31.4	144.8 /145.0
	B6	73.3 / 73.5	35.8 / 35.8	19.6 / 19.6	118.8 / 119.0
	B7	58.2 / 58.2	31.6 / 31.8	17.8 / 18.0	97.8 / 98.0
Day 14	B1	114.0 / 115.0	67.5 / 67.7	21.0 / 21.2	193.4 / 193.4
	B2	109.7 / 109.9	146.1 / 146.3	20.1 / 20.3	266.3 / 266.5
	B3	101.0 / 101.2	104.9 / 104.9	21.5 / 21.5	217.5 / 217.7
	B4	54.6 / 54.6	114.9 / 114.9	56.1 / 56.1	215.7 / 215.7
	B5	93.0 / 95.0	74.0 / 75.0	44.8 / 45.0	203.3 / 203.5
	B6	100.7 / 100.9	69.8 / 70.0	56.9 / 56.9	217.6 / 217.8
	B7	89.6 / 89.8	62.8 / 63.0	20.7 / 20.7	163.2 / 163.4

Table 21: Concentration of isoflavones glucosides in soy Brie analogues fermented with mesophilic and *Penicillium candidum*. Results are performed twice, both values are presented in the table. Results are expressed on wet weight basis.

Age of cheese	Cheese code	Isoflavones Glucosides (µg/g)			Total Glucosides (µg/g)
		Daidzin	Genistin	Glycitin	
Day 1	B1	11.3 / 12.3	69.1 / 69.1	51.6 / 51.6	132 / 133
	B2	81.3 / 81.9	68.8 / 68.8	45.6 / 45.6	196.0 / 196.6
	B3	58.7 / 58.7	66.0 / 66.8	38.3 / 38.3	163.0 / 163.8
	B4	13.9 / 13.9	65.8 / 66.8	36.3 / 36.3	115.0 / 117.0
	B5	36.5 / 36.9	42.7 / 42.7	8.8 / 8.8	88.2 / 88.6
	B6	18.4 / 19.4	54.9 / 55.9	4.7 / 4.7	78.0 / 80.0
	B7	132.0 / 132.2	46.2 / 46.3	26.8 / 26.8	205.0 / 205.3
Day 7	B1	14.9 / 15.9	73.2 / 73.2	11.9 / 11.9	99.0 / 100
	B2	48.8 / 48.8	88.2 / 88.2	19.2 / 19.2	116.2 / 116.2
	B3	57.4 / 57.4	71.0 / 71.0	40.0 / 40.0	128.5 / 128.5
	B4	27.0 / 29.0	75.4 / 75.4	17.0 / 170	80.0 / 81.0
	B5	49.5 / 49.5	54.1 / 54.1	10.6 / 10.6	74.3 / 74.3
	B6	24.8 / 24.8	58.1 / 58.1	19.1 / 19.1	62.1 / 62.1
	B7	24.9 / 24.9	52.6 / 52.6	30.1 / 30.1	67.7 / 67.7
Day 14	B1	31.5 / 31.5	72.1 / 72.1	21.3 / 21.3	85.0 / 85.0
	B2	50.4 / 50.4	82.3 / 82.3	17.2 / 17.3	110.0 / 110.2
	B3	6.3 / 6.3	41.7 / 41.9	40.0 / 40.0	48.2 / 48.4
	B4	13.5 / 13.5	66.8 / 66.8	29.7 / 30.7	70.3 / 71.3
	B5	7.3 / 7.3	31.9 / 31.9	40.0 / 40.0	39.3 / 39.3
	B6	13.6 / 13.6	44.5 / 44.5	26.3 / 26.3	44.5 / 44.5
	B7	14.5 / 14.5	17.4 / 17.4	21.5 / 22.5	14.0 / 15.0

Table 22: Concentration of total isoflavones (aglycones and glucosides) in soy Brie analogues fermented with mesophilic and *Penicillium candidum*. Results presented in the table is the average of total glucosides, total aglycones.

Age of cheese	**Cheese code**	**Total Aglycones (µg/g)**	**Total Glucosides (µg/g)**	**Total Isoflavones (µg/g)**
Day1	B1	78.8	132.5	211.3
	B2	121.1	196.3	317.5
	B3	104.7	163.4	268.1
	B4	81.2	116.0	197.3
	B5	50.6	88.4	139.0
	B6	53.3	79.0	132.4
	B7	56.1	205.1	261.2
Day 7	B1	115.9	99.5	215.5
	B2	138.6	116.2	254.9
	B3	144.1	128.5	272.6
	B4	130.4	80.5	210.9
	B5	144.9	74.3	219.3
	B6	118.9	62.1	181.1
	B7	97.9	67.7	165.6
Day 14	B1	193.4	85.0	278.5
	B2	266.4	110.1	376.5
	B3	217.6	48.3	265.9
	B4	215.7	70.3	286.1
	B5	203.4	39.3	242.8
	B6	217.7	44.5	262.3
	B7	163.3	14.5	177.9

Texture Analysis:

Cheese samples were prepared with formulations similar to soy Cheddar and soy Mozzarella analogues with the addition of 6, 30% oil or okara or both. Texture characteristics after 1, 7 and 14 days of ripening were evaluated and summarized in Table 23. Dairy Brie (external control) was included and evaluated in the same way as the soy Brie analogues were evaluated and results are included in Table 14 [page 64].

Table 23: Texture evaluation of soy Brie analogues. Results are performed twice, both values are presented in the table.

Age of Cheese	Cheese Code	Hardness (g)	Adhesiveness (g/mm)	Cohesiveness	Springiness (mm)	Gumminess (mm)	Chewiness (g/mm)
Day 1	B1	25.7 /25.7	0.22 / 0.24	0.20 / 0.22	2.95 / 2.95	3.23 / 3.25	9.56 / 9.58
	B2	15.52/15.5	-	-	4.68 / 4.70	-	-
	B3	15.25/15.2	0.37 / 0.39	0.85 / 0.85	1.94 / 1.96	16.77/16.79	32.73/32.7
	B4	14.70/14.7	0.34 / 0.35	0.66 / 0.66	0.84 / 0.86	11.26/11.28	9.58 / 9.60
	B5	10.42/10.4	-	-	10.5/10.54	-	-
	B6	18.07/18.0	2.62 / 2.66	0.30 / 0.32	1.89 / 1.91	5.23 / 5.23	9.93 / 9.95
	B7	23.92/23.9	2.69 / 2.69	0.31 / 0.33	1.73 / 1.73	11.26/11.30	19.49/19.5
	Dairy Brie	9.10/9.10	-	-	7.10 / 7.12	-	-
Day 7	B1	51.87/51.8	0.41 / 0.43	0.48 / 0.50	0.72 / 0.74	24.70 /26.7	17.4 /19.4
	B2	45.72/45.7	0.30 / 0.30	0.44 / 0.44	0.87 / 0.87	19.16 /21.16	16.0 /17.0
	B3	38.02/38.0	5.16 / 5.16	0.45 / 0.45	1.52 / 1.54	16.98 /16.98	23.04/23.0
	B4	37.97/37.9	3.90 / 3.90	0.35 / 0.37	1.53 / 1.55	12.45 /16.45	17.64/18.6
	B5	19.87/19.8	1.82 / 1.82	0.55 / 0.59	2.48 / 1.52	11.91 /11.91	35.18/35.1
	B6	33.22/33.2	4.07 / 4.07	0.30 / 0.30	2.03 / 2.05	9.22 /11.22	18.66/20.6
	B7	45.05/45.0	6.20 / 6.20	0.31 / 0.31	2.01 / 2.03	14.16 /14.20	24.7 /26.9
	Dairy Brie	39.10/39.1	1.90 / 1.90	0.34 / 0.34	1.61 / 1.61	11.91 /13.93	19.7 / 21.9
Day 14	B1	89.0/91.0	0.30 / 0.30	0.48 / 0.52	0.70 / 0.74	44.0 / 46.0	32.3/32.5
	B2	73.22/73.2	2.13 / 2.15	0.41 / 0.41	1.14 / 1.16	29.02 /31.0	34.4 /34.6
	B3	61.35/61.3	1.97 / 1.97	0.37 / 0.39	1.46 / 1.48	23.31/23.31	34.3 / 34.5
	B4	53.52/53.5	6.02 / 6.02	0.40 / 0.42	1.56 / 1.60	21.46 /23.48	34.4 / 36.6
	B5	36.17/36.1	3.53 / 3.53	0.38 / 0.40	1.41 / 1.45	13.10 / 15.1	20.16/20.1
	B6	83.07/83.0	6.25 / 6.25	0.30 / 0.32	1.99 / 1.99	25.75/25.75	51.24/51.2
	B7	68.57/68.5	9.32 / 9.34	0.31 / 0.33	2.00 / 2.02	21.94/21.94	43.09/45.0
	Dairy Brie	50.61/50.6	2.10 / 2.14	0.33 / 0.33	1.38 / 1.42	16.60 /16.80	22.38/24.3

- Hardness: Data indicated that B6 and B1 required almost a similar force value ± 7.00g to break through the surface. The greatest hardness value was observed in B1 (control) and B6 and their values were 90.00 and

83.07g respectively. While the lowest hardness values was in B5 and B4 and their values were 36.17 and 53.52g respectively. Dairy Brie (external control) required 85.65g to break its surface as in Table 23. B6 showed the closest hardness value to external control. Among all cheese analogues, C1, B1 and B6 showed the closest values to their dairy cheese (external control). This may suggest that the addition of different amounts and ratios of fat and/or okara to the analogue formulations did not enhance the hardness up to a value close to the dairy cheese (external control) since C1 and B1 showed the closest hardness to external standard.

- <u>Adhesiveness and Cohesiveness</u>: Results for adhesiveness of all cheese analogues showed a range from 0.30-9.33g/mm while dairy Brie had a value of 12.38g/mm adhesiveness value. The closest and greatest value observed in B7. For Cohesiveness, B6 with a value of 0.31 showed the closest value to the external control which showed 0.29 value. Among all cheese samples, C7, M7 and B7 exhibited a satisfactory difference in adhesiveness values when compared with dairy (external control). For cohesiveness, C1, M7, B6 showed the closest values to their dairy (external control).
- <u>Springiness, Gumminess and Chewiness</u>: Springiness values ranged from 0.72-2.01mm. Dairy Brie (external control) had a springiness value of 2.97mm. Among soy analogues the closest value to dairy Brie (external control) was B7 with 2.01mm springiness value. Gumminess values ranged from 16.70-45.00g/mm, the lowest value observed in dairy brie and the highest value was in B1. Gumminess of B6 is 25.75g/mm, it

showed the closest value to dairy Brie (external control). Chewiness values ranged from 20.16-51.24g/mm with the lowest value observed in B5 and the highest value observed in B6. The closest value to dairy Brie (external control) which had a value of 73.77g/mm was B6 which had a value of 51.24g/mm. Among all cheese analogues C3, M1, B7 showed the closest springiness values to their dairy (external control). While C1, M4, B6 showed the closest gumminess values to dairy cheese (external control). C4, M7, B6 showed the closest chewiness values to their dairy cheese (external control).

Sensory properties of soy cheese analogues:

Soy cheese analogues (control Cheddar C1, control Mozzarella M1 and control Brie B1) were prepared for sensory analysis and assessed for taste, texture, overall acceptance by Unicurd company staff. Commercial dairy cheese Cheddar low fat, Mozzarella and Brie were also included in the sensory analysis.

1) Soy Cheddar analogue:

Taste: The main problem with soy Cheddar was the taste, panellists commented that this product is "very sour". The chemical analysis showed that among all soy analogues, soy Cheddar had the lowest pH 5.47 and it also contains 0.10% acidity.

Texture: Soy Cheddar analogue did not resemble dairy Cheddar, it did not posses the appropriate texture, so soy Cheddar was excluded as a proper Cheddar analogue.

Overall acceptance: Soy Cheddar analogue was not accepted as a product resembling cheese mainly due to its sour taste.

2) Soy Mozzarella analogue:

Taste: Taste of soy Mozzarella analogue more preferred compared with soy Cheddar analogue, it was not as sour as soy cheddar. pH of soy Mozzarella analogue is 5.76, while the acidity content was 0.07%.

Texture: Texture of soy Mozzarella analogue did not resemble Mozzarella (external control). This is due to low fat content of soy Mozzarella and different type of protein and thus different curd formation.

Overall acceptance: Soy Mozzarella analogue was not accepted as a product as it did not closely resemble Mozzarella cheese.

3) Soy Brie acceptance:

Taste: Taste was acceptable, beany flavour was not strong compared with soy Cheddar, soy Mozzarella analogue. Soy Brie analogue had pH value of 5.77 and acidity content was 0.13%.

Texture: Soy Brie had a soft texture, but did not closely resemble dairy Brie.

Overall acceptance: Panellists suggested since the taste is acceptable but the texture does not resemble cheese, further research should be conducted regarding texture, and it can be processed into an intermediate product between cheese and tofu and this product might be called "tofu cheese spread".

III . 2) Conclusion on Part One (Soy cheese Analogues).

Among all cheese analogues prepared the following points were observed:

1) Cheese analogues supplemented with 30% soyoil as in C5, M5 and B5 showed:
    - Low moisture content.
    - Low total isoflavones.
    - Low pH values.
    - Low hardness values.
        - ✓ These results suggest that addition of oil at high concentration (30%) promoted syneresis in cheese curd. Moisture loss was directly related to total isoflavones loss.
        - ✓ Low pH values indicated more acid production, thus high oil concentration seemed to enhance microorganisms ability to ferment carbohydrates and produce more acid.
        - ✓ Hardness is the force necessary to attain a given deformation as in Table 10, or it resembled the first bite. Low values suggest that this formulation (30%) oil addition resulted in softer cheese curd compared to dairy control (external control).
2) Cheese samples supplemented with 30% okara as in C6, M6 and B6 showed:
    - High moisture content.
    - High pH values.
    - Low cohesiveness values.

- ✓ These results suggest that addition of okara at 30% promoted water retention in the cheese curd since okara contains (76 – 80%) moisture.
- ✓ High pH values indicate low acid production, thus high okara content did not seem to enhance microorganism's growth.
- ✓ Cohesiveness is the strength of the internal bonds making up the body of the product as in Table 10, so it seems that in these cheese analogues the strength of the internal bonds is weak due to the high moisture content.

3) After textural analysis, dairy cheeses (external control) were compared with soy analogues and the following points were observed:
   - Soy Cheddar analogues:
     - ✓ C1 showed the closest hardness, cohesiveness and gumminess to external control.
     - ✓ C3 showed the closest springiness.
     - ✓ C4 showed the closest chewiness, adhesiveness.
   - Soy Mozzarella analogues:
     - ✓ M1 showed the closest springiness value.
     - ✓ M4 showed closest gumminess. (Also dairy Brie control showed close gumminess value).
     - ✓ M6 showed closest hardness.
     - ✓ M7 showed closest adhesiveness, cohesiveness and chewiness.

- Soy Brie analogues:
  - ✓ B1 and B6 showed closest hardness.
  - ✓ B6 showed closest hardness, cohesiveness, gumminess and chewiness.
  - ✓ B7 showed closest springiness, adhesiveness.

Based on the previous results, addition of oil at low 6 or high 30% did not improve any of the texture parameters evaluated in this study. While addition of okara at low 6% as in C3 or C4 or high 30% as in M6, B6, M7 and B7 with or without oil addition seemed to improve one or more aspects of the texture parameters studied.

Control samples C1, M1 and B1 showed one or more close texture characteristic(s) to their external control. Thus, in order to obtain a cheese analogue resembling the traditional basic cheese from textural and compositional aspects, further modifications of curd or formulations should be investigated.

The quality of cheese is best assessed by texture and sensory analysis. The international dairy federation have suggested a quality grading system for evaluation of cheese. This system involves evaluation of "test" cheese to a reference cheese selected as a "standard" and rating the cheese in terms of a list of defects. Another way is the descriptive evaluation by a panel in which assessors taste a range of cheeses and during group discussions agree on and then define a list of descriptors that encompass the sensory attributes of the cheese or assessors can use a personal list of descriptors where they are trained to quantify the intensity of each descriptor and score their perceptions by placing marks on a live scale with defined upper and lower limits (Fox *et al* 2000).

III . 3) Soy Yoghurt:

Yoghurt samples fortified with $CaCl_2$ showed improper curdling and severe syneresis (it formed a partially gelled upper layer and "whey" as a lower layer). Therefore, these samples were excluded as an acceptable product and only four samples were further studied.

Composition of yoghurt products:

Dairy milk based yoghurt contained higher total solids 13.46% than fruit fortified soymilk based yoghurt as shown in Table 24, this is in agreement with Lee *et al* (1990). However, soymilk-based yoghurt (control) contained (14.83%) higher total solids than dairy milk-based yoghurt.

Table 24: Chemical analysis of dairy yoghurt and soymilk based yoghurt. Results are performed twice, both values are presented in the table.

Yoghurt Product	pH	Acidity %	Total Solids
Soymilk Yoghurt (control)	4.40 / 4.40	0.43 / 0.45	13.83 / 15.83
Soymilk Yoghurt (fortified with 5% papaya)	4.25 / 4.25	0.76 / 0.76	12.09 / 14.09
Soymilk Yoghurt (fortified with 10% papaya)	4.24 / 4.24	0.64 / 0.66	11.64 / 13.84
Dairy milk based yoghurt	4.09 / 4.09	1.10 / 1.10	13.46 / 13.46

As shown in Table 24, the yoghurt bacteria cultures developed a substantially higher titratable acidity (1.10% lactic acid) and slightly lower pH (4.09) in the dairy milk based yoghurt compared with soymilk based yoghurt products which showed (0.44 – 0.76%) titratable acidity and pH (4.24 – 4.40) values. The lower concentration of lactic acid in soymilk based yoghurt products is due to the fact that those products do not contain any lactose. Lee *et al* (1990) stated that it is also possible that soybean derived sugars, raffinose and stachyose, may have an inhibitory effect on the growth and acid production of yoghurt bacteria culture. The fermentation of soymilk with yoghurt bacteria culture resulted in reduced content of stachyose as presented in table 25. The content of stachyose showed the largest magnitude of reduction from 36.20 mg/100ml in soymilk to 13.50, 11.60 and 6.33 mg/100mg in soymilk yoghurt (control), yoghurt with 5% papaya, yoghurt with 10% papaya respectively. On the other hand, contents of glucose and galactose in yoghurt samples were increased compared with soymilk. Since stachyose and raffinose are responsible for flatulence caused by soymilk, therefore fermentation of soymilk with yoghurt starter culture may help to reduce this problem.

Table 25: Sugar concentration mg/100ml. Results are performed twice, both values are presented in the table. Results are expressed on wet weight basis.

Sugar	**Dairy Milk**	**Soymilk**	**Soymilk yoghurt (control)**	**Yoghurt (5% papaya)**	**Yoghurt (10% papaya)**	**Dairy milk based yoghurt**
Glucose	0.12/0.14	0.36 /0.38	1.10 / 1.30	0.48 / 0.50	0.78 / 0.80	0.24 / 0.26
Galactose	___	5.75/5.75	11.65 / 11.67	9.18 / 11.20	7.19 / 9.19	1.23 / 1.25
Stachyose	___	36.10/36.30	6.31 / 6.35	10.62/12.64	13.56/13.56	___
Raffinose	___	0.66 / 0.68	0.76 / 0.80	1.01 / 1.05	1.23 / 1.25	___
Lactose	3.2/3.4	___	___	___	___	0.24 / 0.25
Total Sugar	3.43/3.43	41.99/43.99	18.96 / 20.98	22.33/24.35	22.77/24.79	1.73 / 1.75

Isoflavones Content:

The total concentration of isoflavones aglycones found in soymilk based yoghurt (control) was 67.8 μg/g (ppm). The aglycones contributed 34% of the total isoflavones content, whilst the glucosides forms contributed the greatest concentration of isoflavones isomers in soymilk based yoghurt (66% of total). In yoghurt fortified with 5% papaya, total aglycones forms contributed 23% of total isoflavones while glucosides forms contributed 77% of total isoflavones. In yoghurt fortified with 10% papaya, total aglycones forms contributed 8.1%, while the glucosides contributed 91.8%. Tsangalis *et al* (2003) reported that isoflavones aglycones are absorbed faster and in higher amounts than the glucosides forms. Of the bioactive aglycones forms in soymilk and all soy milk yoghurt products, the concentration of daidzein was significantly higher than both genistein and glycitein as shown in Figures 35 - 38. Whereas genistin showed the greatest concentration among glucosides forms (daidzin, glycitin) in all soymilk yoghurt products and daidzin showed the lowest concentration among glucosides isomers while in soymilk, glycitin showed the highest concentration among glucosides and genistin was the lowest. The concentration of isoflavones aglycones (daidzein, genistein, glycitein), glucosides (daidzin, genistin, glycitin) in soymilk (Figures 35-A, 35-B) compared with soymilk yoghurt products showed significant reduction in glucosides and significant increase in aglycones in soymilk yoghurt products. This change in concentration may be due to possible deconjugating of the isoflavones glucoside by yoghurt bacteria culture, this is in agreement with Tsangalis *et al* (2003) who also noticed such an effect with soymilk when inoculated with *Bifidobacterium* species (*Bifidus* is one of the species in the yoghurt bacteria culture used in this study). However, as it was mentioned earlier and as shown in Figures 35 - 38, 33% of total isoflavones in soymilk based yoghurt (control) is present as aglycones, in yoghurt fortified with 5% papaya, the aglycones contributed 23%, and in yoghurt supplemented with 10% papaya, the aglycones contributed only 8%. This may indicate that in samples where fruit is added, a lower pH was obtained accompanied with a higher lactic acid content and a lower total aglycones content, this suggest that acidic conditions affects the transformation of glucosides to aglycones and it is in agreement with Shi *et al* 2002. The highest isoflavones content were observed in soymilk based yoghurt (control) followed by yoghurt (with 5% papaya) and then yoghurt (with 10% papaya). This can be linked to pH of these samples, yoghurt with the lowest pH also showed the lowest total isoflavones while soymilk yoghurt (control) showed the highest pH value and the highest total isoflavones content.

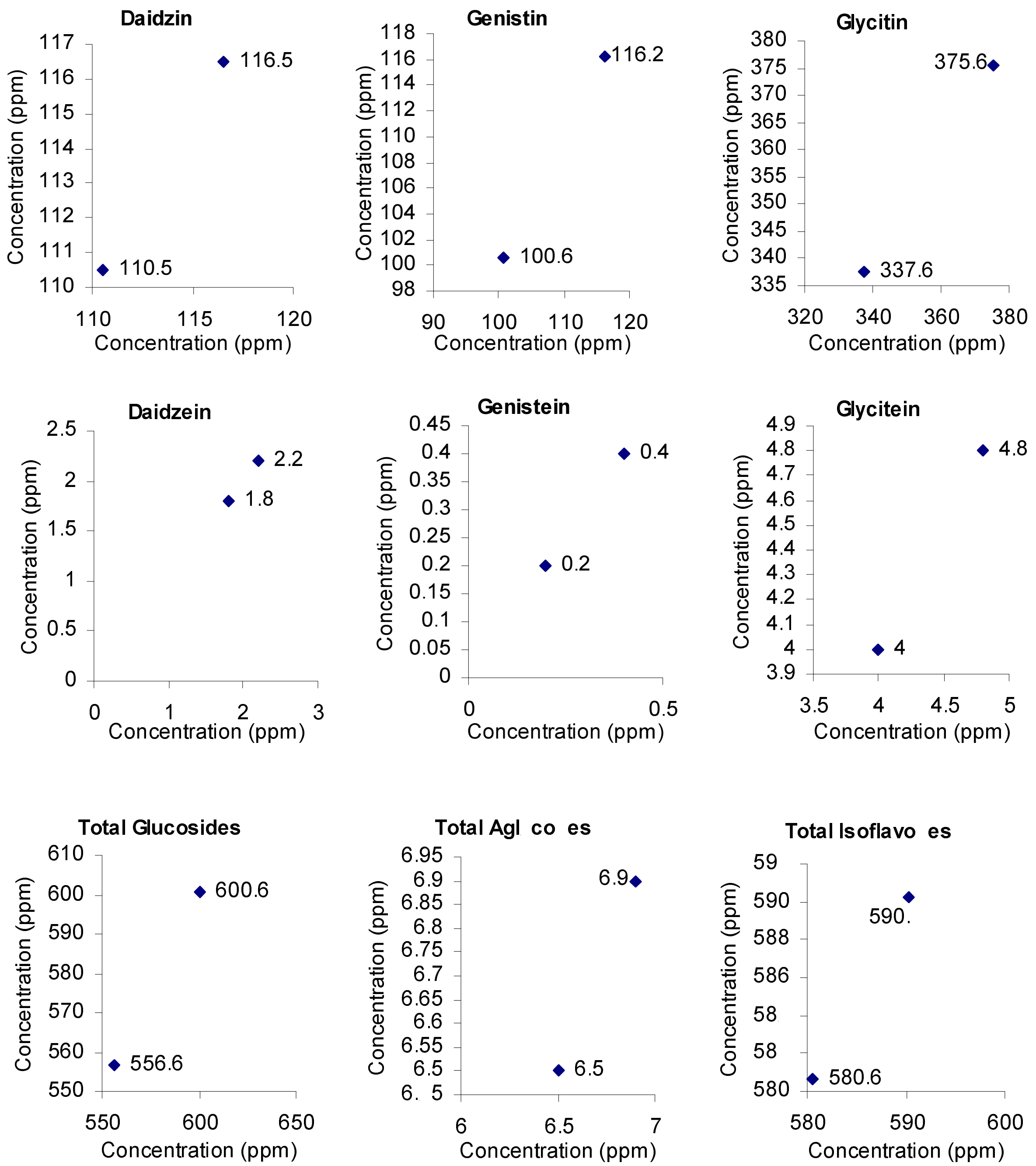


Figure 35: Isoflavones content of soymilk. Results are performed twice, each scatter plot represent both values.

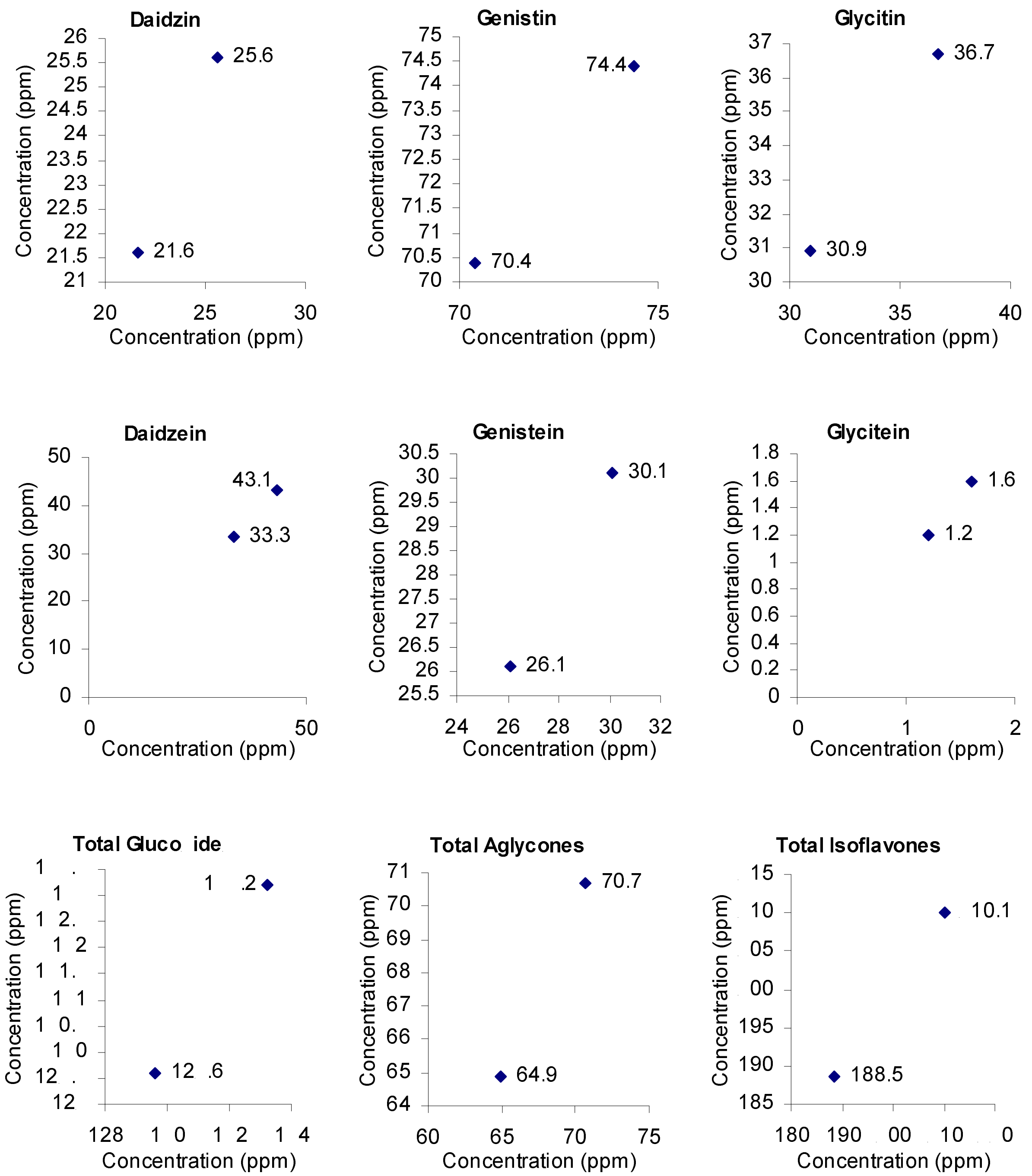


Figure 36: Isoflavones content of soymilk yoghurt (control). Results are performed twice, each scatter plot represent both values.

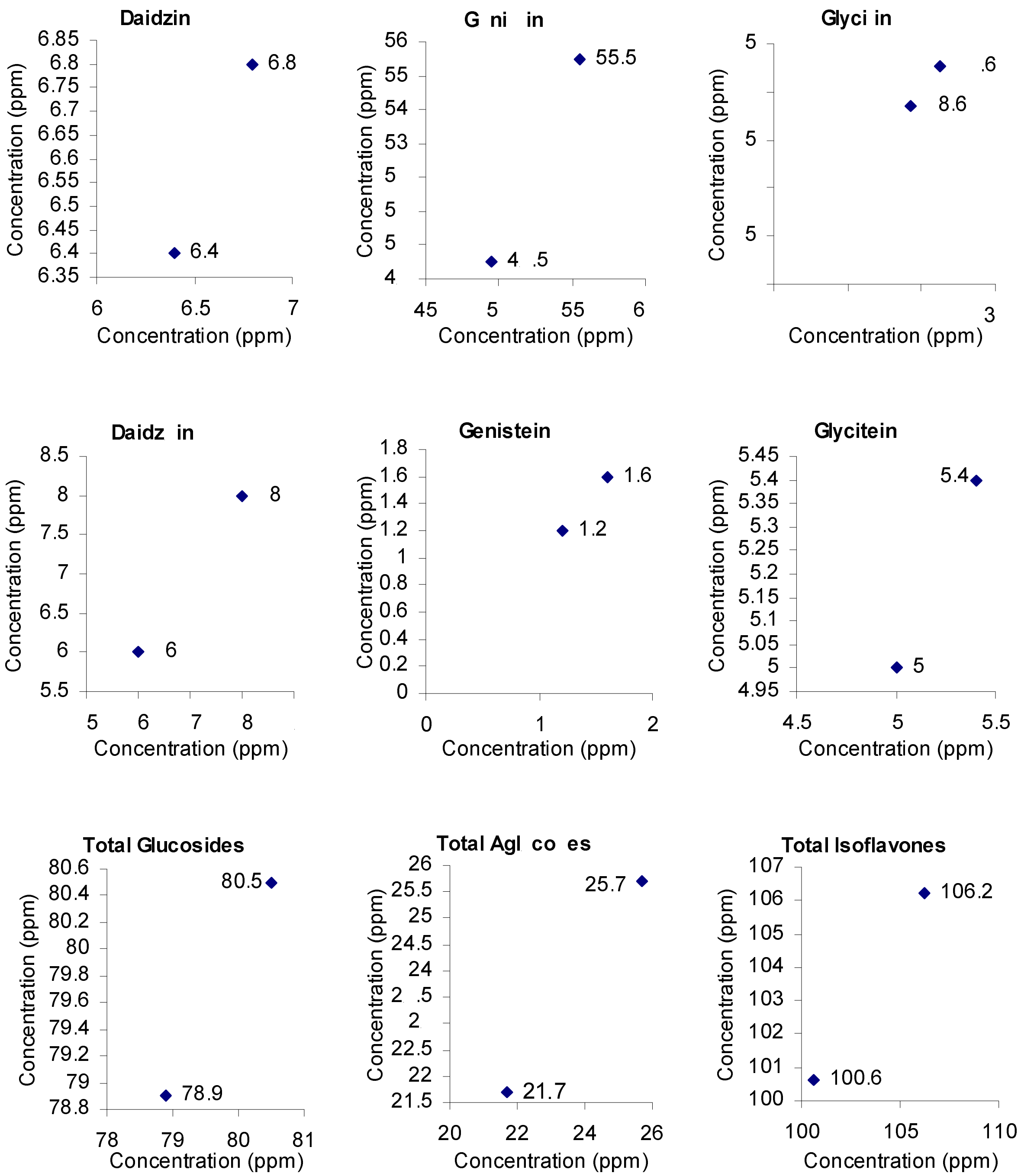


Figure 37: Isoflavones content of soymilk yoghurt (with 5% papaya). Results are performed twice, each scatter plot represent both values.

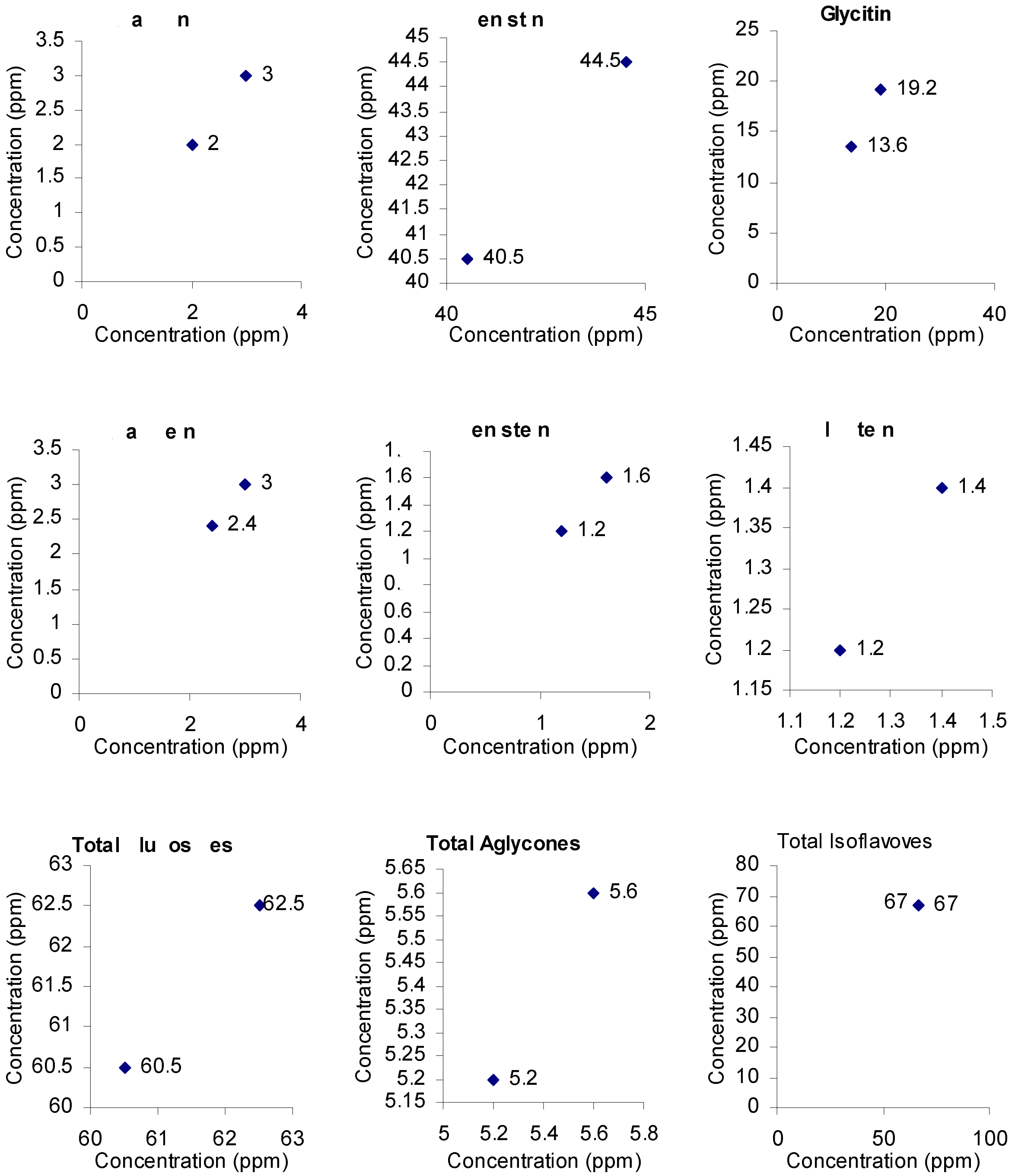


Figure 38: Isoflavones content of soymilk yoghurt (with 10 % papaya). Results are performed twice, each scatter plot represent both values.

Electrophoresis of protein constituents:

Electrophoretic patterns of total soybean protein from soymilk and soy yoghurt are shown in Figure 39. The protein bands were similar among all soy yoghurt products. The molecular weight values of ά, α and β-subunits of the 7S fraction were approximately 80, 75 and 50 kDa respectively. The subunit with the molecular weight of ~36 kDa is an acidic A3 polypeptide and the group of acidic polypeptides (A_{1a}, A_{1b}, A_2, A_4). The protein bands with MW~15 kDa are basic components of the 11S fraction. The resolution in this region of soy yoghurt products is not clear. However, it can be seen in soymilk sample although the band is not resolved properly.

Results showed that yoghurt starter culture was effective in degrading all soy proteins into small peptides and amino acids not resolved properly as in Figure 39, in particular, native β -conglycinin subunits (α, ά and β). Soy yoghurt enriched with 10% papaya profile showed more hydrolysis of 7S (α, ά and β) subunits and 11S subunits compared with soymilk based yoghurt enriched with 5% papaya and soy yoghurt (control). This may attributed to the more acidic medium of soy yoghurt enriched with papaya which enhanced the hydrolysis of protein.

Figure 40 , shows the electrophoretic patterns of protein in dairy milk and dairy milk based yoghurt. Bovine casein consists of four types of protein: αs_1, αs_2, β and κ-casein. These make up 38%, 10%, 34% and 15% respectively. However, there is no difference in the location of protein bands between soymilk and soymilk yoghurt products prepared, however, electrophoretic pattern showed faint bands of soymilk yoghurt samples compared to soymilk, which suggest more hydrolysis of protein in soymilk yoghurt samples.

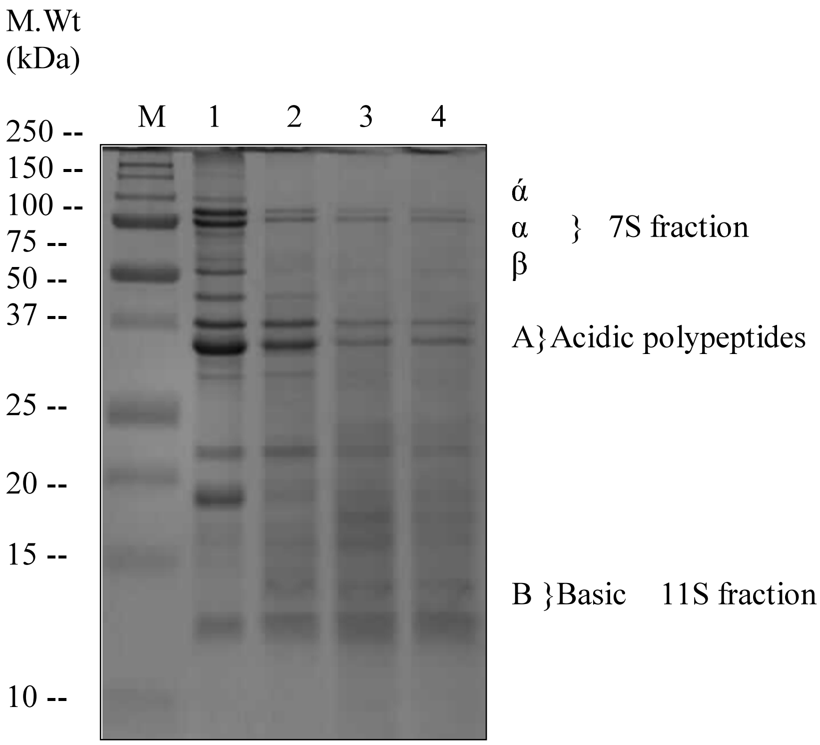


Figure 39: UREA-PAGE profile of total proteins from soymilk based yoghurt samples: Lane 1: soymilk; Lane 2: soymilk yoghurt (control); Lane 3: soy yoghurt with 5 % papaya; Lane 4: soy yoghurt with 10 % papaya. Lane marked M contains standard molecular weight marker.

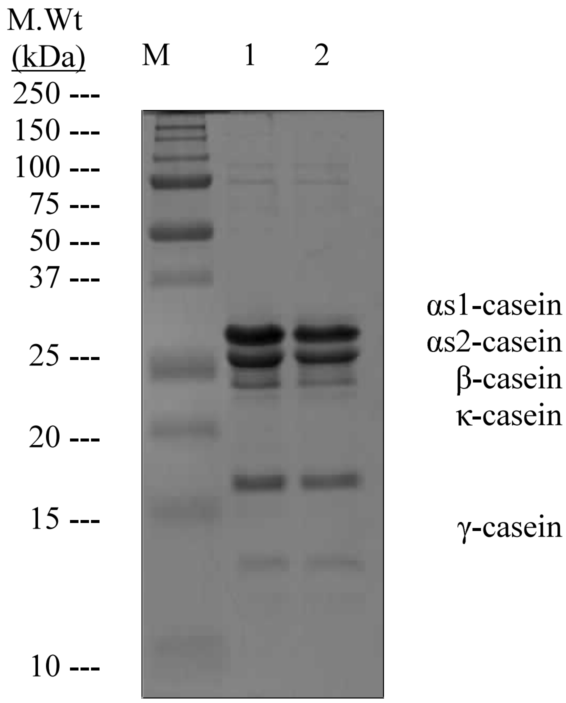


Figure 40: UREA-PAGE profile of total proteins from dairy milk based yoghurt samples: Lane 1: dairy milk; Lane 2: dairy milk yoghurt (control). Lane marked M contains standard molecular weight marker.

Texture analysis of yoghurt products:

Texture analysis as shown in Table 26 showed soy yoghurt analogues were firmer than dairy milk-based yoghurt. These results are in agreement with Cheng *et al* 1990 who prepared three yoghurt samples, one was prepared with soy milk, the second one was prepared from dairy milk and the third was prepared from soymilk but enriched with lactose. The highest penetration force through yoghurt surface was in soymilk based yoghurt, followed by soymilk enriched with lactose. The lowest penetration force was in dairy milk based yoghurt. In this study the highest penetration force was in soy yoghurt enriched with 10% papaya with 17.76g value, followed by soy yoghurt enriched with 5% papaya with a value of 17.54g. Soymilk based yoghurt (control) showed a value of 14.17g. Dairy milk showed value of 5.23g. Cheng *et al* 1990 stated that the firmer texture for soymilk based yoghurt than dairy milk yoghurt is due to the proteolytic effect of the starter culture which can break polypeptide chains and make them shorter, thus resulting in a more compact network of coagulated protein.

Table 26: Texture analysis of yoghurt products. Results are performed twice, both values are presented in the table.

Yoghurt Product	**Penetration Force (g)**
Soymilk based yoghurt (control)	11.07 / 17.27
Soymilk yoghurt (fortified with 5% papaya)	13.74 / 21.34
Soymilk yoghurt (fortified with 10% papaya)	12.26 / 23.26
Dairy milk based yoghurt	4.73 / 5.73

Sensory Properties of Yoghurt Products

Yoghurt samples were evaluated for flavour and overall acceptability by internal panellists (in the food science lab at NUS). Soymilk based yoghurt were much preferred than dairy milk yoghurt principally due to the sour taste of dairy milk based yoghurt. The beany flavour was quite obvious in soymilk based yogurt (control). However, the beany taste is less detected in papaya flavoured yoghurt. Yoghurt with 10% papaya showed the lowest beany flavour. Both yoghurt fortified with 5% and 10% papaya were equally preferred by internal panellists. Another sensory analysis was conducted by Unicurd company staff and results are as follows:

1. Soymilk yoghurt (control):

Taste: The panellists commented that this yoghurt is good but has a "sharp" or "edge" taste.

Texture: The texture is firm because it is a set yoghurt, it resembles "soy dessert".

Overall acceptance: Most acceptable among soy yoghurt samples.

2. Yoghurt enriched with 5 % papaya:

Taste: The panellists commented that this yoghurt sample is good, however it was not suitable as a product.

Texture: The texture is firm because it is a set yoghurt, stirred yoghurt can be tried.

Overall acceptance: Panellists commented that this yoghurt sample is firmer than soymilk based yoghurt (control).

3. Yoghurt enriched with 10 % papaya:

Taste: The panellists commented that this yoghurt sample is very sour.

Texture: The panellists commented that this yoghurt sample got the firmest texture among soymilk based yoghurt.

Overall acceptance: Not acceptable, very sour.

4. Dairy milk yoghurt (control):

Taste: The panellists commented that this yoghurt sample got a smooth "round" mouth feel.

Texture: The panellists commented that this yoghurt sample got a soft, creamy texture.

Overall acceptance: This product was prepared for comparison, it was good as a dairy yoghurt.

However, overall acceptance of soy yoghurt samples as a product was not high, but Unicurd staff suggested the following:

- Stirred yoghurt may be tried besides set yoghurt for comparison.
- Use of other fruits as guava (it can be used as puree), since guava has a pleasant flavour and it is acceptable to the local market.

III . 4) Conclusion on Part Two (Soy Yoghurt Analogues):

Results from experiments indicated that fortification of soymilk with fruit (papaya) stimulated yoghurt culture growth and acid production compared with soymilk yoghurt (control) which provided less stimulation of the yoghurt culture bacteria as presented by pH and titratable acidity results. Therefore, fortification of soymilk with fruit (as an extra source of sugar) improved acid production more than control soymilk. However, concentration of stachyose and raffinose which are responsible for flatulence were lower in yoghurt fortified with papaya than soymilk based yoghurt (control), it may be due to acid production which inhibited bacterial growth or dilution caused by the fruit papaya. Although the overall reduction in stacyhose in proposed soymilk yoghurt products indicated that fermentation of soymilk with yoghurt starter culture may be a practical approach to overcome the flatulence factor in soybeans.

Starter cultures are primarily responsible for the production of the flavour compounds which contribute to the aroma of yoghurt. These compounds may be divided into four main categories as follows:

- Non-volatile acids (*e.g.* Lactic acid).
- Volatile acids (*e.g.* Formic acid).
- Carbonyl compounds (*e.g.* acetaldehyde).
- Miscellaneous compounds (certain amino acids and/or constituents formed by thermal degradation of protein, fat or lactose). (Tamime and Robinson 1999).

However, these flavour compounds which are typical for dairy yoghurt are different from flavour compounds arising from soy yoghurt. In this study, flavour compounds were not evaluated chemically, it was assessed only through sensory analysis. Although studying of the flavour compounds arising from soymilk yoghurt will help to provide better understanding of the relationship between flavour compounds of soy yoghurt and sensory analysis (taste and overall acceptance).

CONCLUSION

The first aim of the study was to test the applicability of basic cheese making and yoghurt making processes to soymilk as a means of producing a cheese substitute and yoghurt substitute acceptable to traditional dairy products consumers. Pasteurized soymilk (Brix 12) was used and appeared to be a suitable medium to support spontaneous growth of different types of bacteria (mainly LAB). However, in order to prepare fermented products (cheese, yoghurt), control of the spontaneous microbial growth is vital.

In the first part of this study, soy cheese analogues were prepared. Soymilk was inoculated with mesophilic bacteria (*Lactococcus lactis subsp lactis*) to prepare Cheddar cheese analogue and thermophilic bacteria (*Streptococcus thermophilus, Lactobacillus delbrueckii subsp Lactis, Lactobacillus helveticus*) used to prepare Mozzarella analogue and two microorganisms (mesophilic bacteria and mould; *Penicilium candidum*) used to prepare Brie cheese analogue. Growth of these different microorganisms in soymilk did not show significant difference in growth patterns, in other words; it did not give rise to three different products as (Cheddar, Mozzarella and Brie cheese) although there was some differences between these three products; the sour taste “pH and lactic acid content” as recorded in Cheddar and the white surface as in soy Brie, besides soy Brie showed the lowest beany flavour. Sensory analysis of cheese products suggested that soy Cheddar analogue and soy Mozzarella analogue were not acceptable from taste and texture evaluation while Brie cheese showed better taste acceptance compared with soy Cheddar and soy Mozzarella but further improvement of the texture should be investigated.

Soy oil and okara have been used as a means to improve chemical and textural characteristics of cheese samples. Soy oil and okara were added at two different concentrations in soymilk (6 and/or 30%), although such fortification did improve one or more chemical or textural characteristics, it did not enhance the overall quality of cheese analogues compared with control cheese analogues (C1, M1, B1).

In the second part of the study, soy yoghurt was prepared with starter culture containing *Bifidus* and *Acidophilus.* Soymilk supported the growth of yoghurt starter and resulted in a yoghurt like product but it resembled "soy dessert" more than a yoghurt product, since the yoghurt prepared was a set type yoghurt not a stirred type yoghurt. However, more yoghurt samples were prepared, fortified with papaya at two concentrations (5 and 10 %) and $CaCl_2$ at 0.5%. All samples fortified with $CaCl_2$ resulted in syneresis, with two layers upper layer (gel like layer) and a lower layer (whey), so these products were excluded as suitable products. While samples enriched with papaya enhanced the growth of microorganisms more than samples without papaya as recorded by chemical analysis (pH, lactic acid content).

Generally soymilk supported the spontaneous growth of cheese and yoghurt starter culture but the cheese curd and the yoghurt curd formed did not resemble traditional cheese curd and yoghurt curd. This can be explained by the different constituents of soymilk and dairy milk which are the basics in curd formation. However, the potential of soymilk use to prepare dairy analogues is significant due to its unique health benefits and its composition with high protein content and low fat content which presents great opportunities for expanding soybean uses as food. Therefore, further research needs to be carried out.

V SUGGESTION FOR FUTURE STUDIES

The general conclusions of this study indicate that soymilk regardless of its different constituents compared with dairy milk supported spontaneous bacterial growth due to its nutritious medium and give rise to "cheese like product" and "yoghurt like product". However, these products were not acceptable due to textural and organoleptic defects. These parameters (texture and sensory analysis) are the basics of quality, it is worth to investigate ways to improve them.

Cheese and yoghurt texture is a function of its composition and physiochemical properties which includes parameters (such as level of protein, fat, degree of proteolysis, etc). Thus, it is interesting to investigate curd formation in soy dairy analogues and understand the effect of different processing conditions on the subsequent curd formed in greater depth. (Amino acid sequencing studies provide a good tool to monitor changes in protein folding during processing).

On the other hand, it is interesting to investigate flavour compounds arising from soy dairy analogues chemically and through sensory analysis conducted by trained panellists. This study suggests further investigation of soy dairy analogues needs to be carried out.

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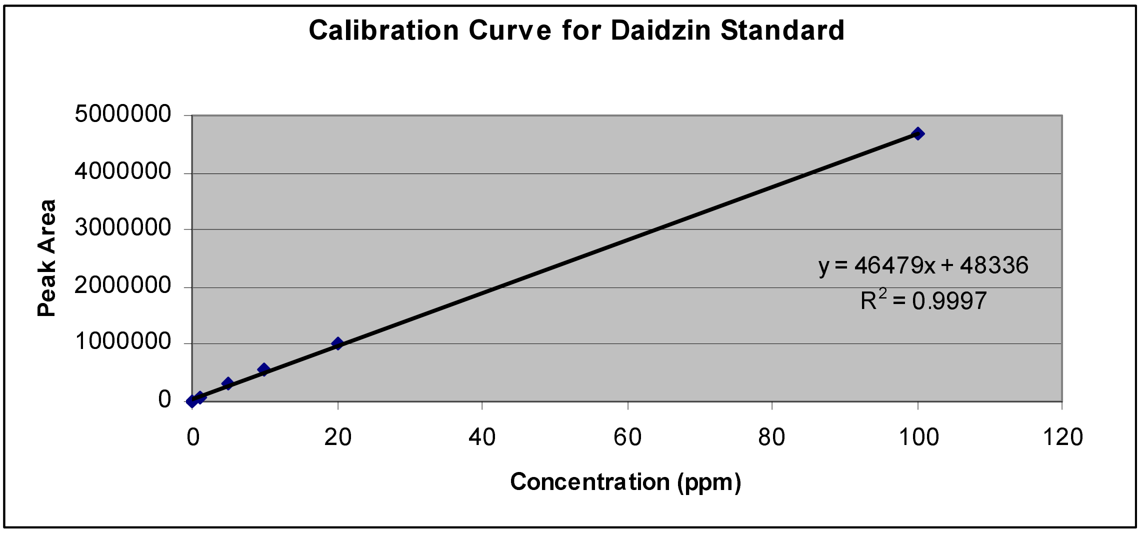
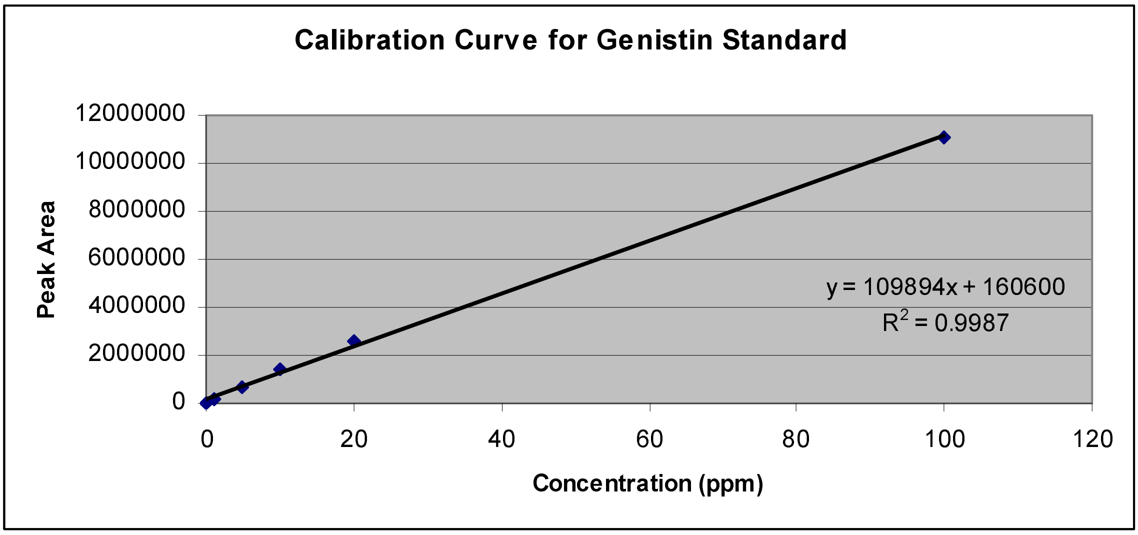
ANNEXES

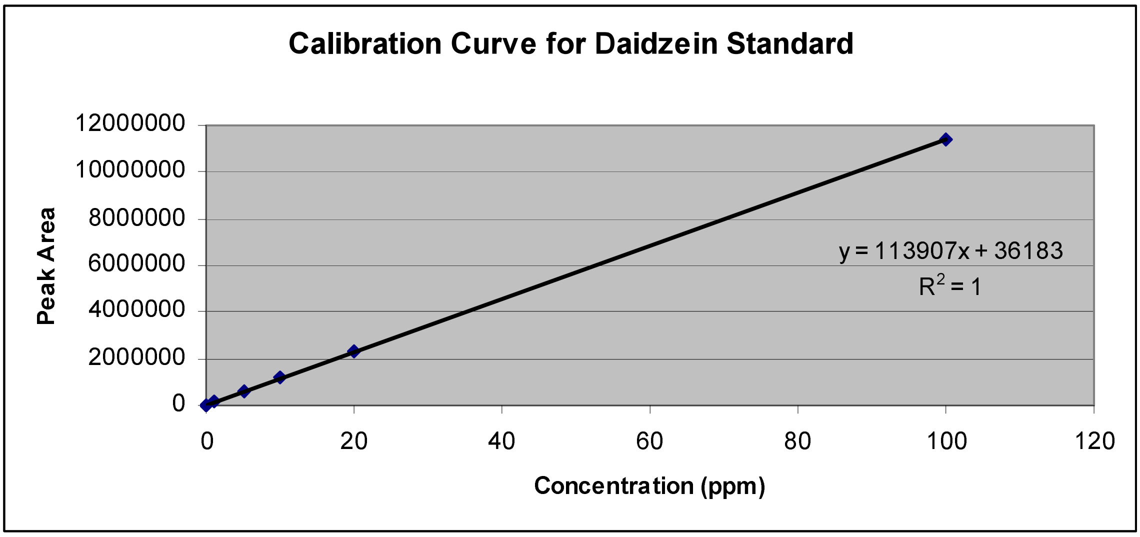
Annexe 1: Calibration Curves for Isoflavones Standards



Daidzin standard calibration curve.



Genistin standard calibration curve.



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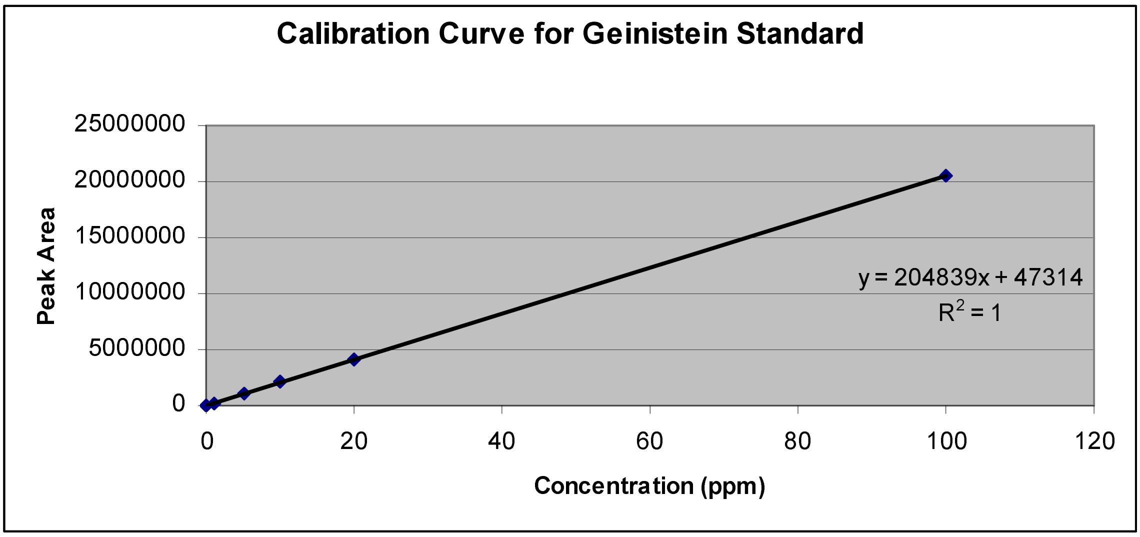
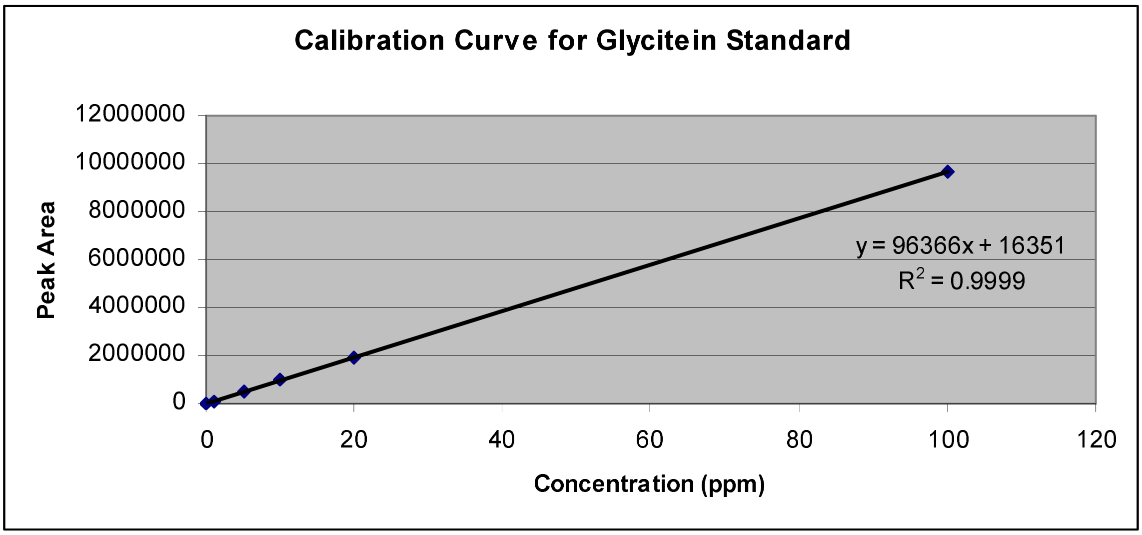
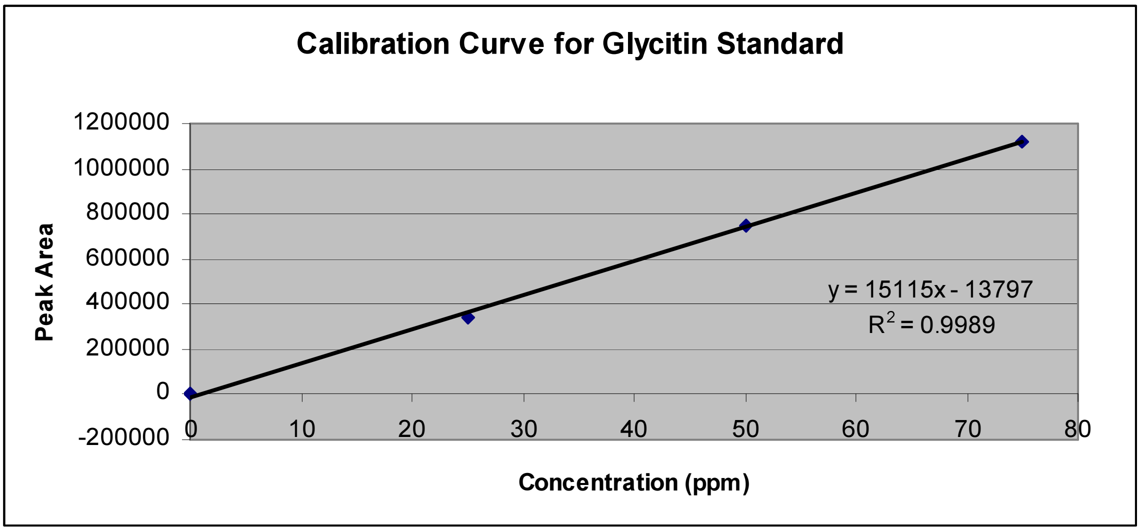


Geinstein standard calibration curve.

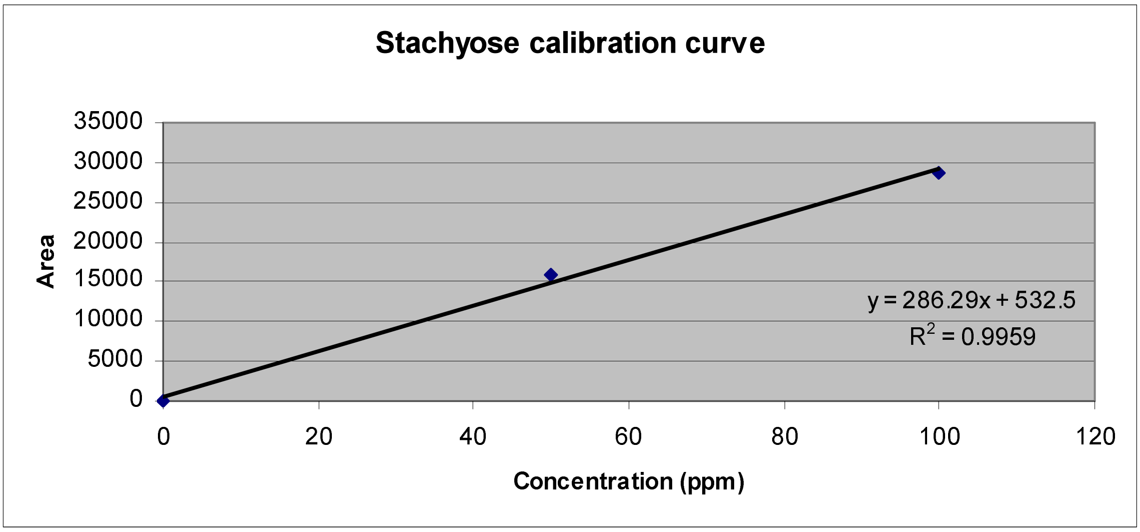


Glycitein standard calibration curve.

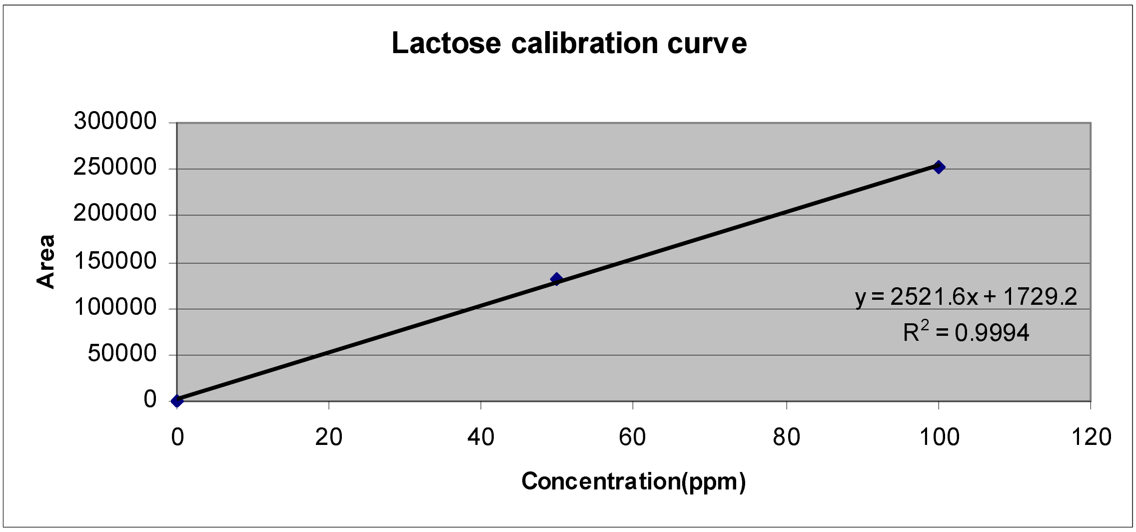


Glycitin standard calibration curve.

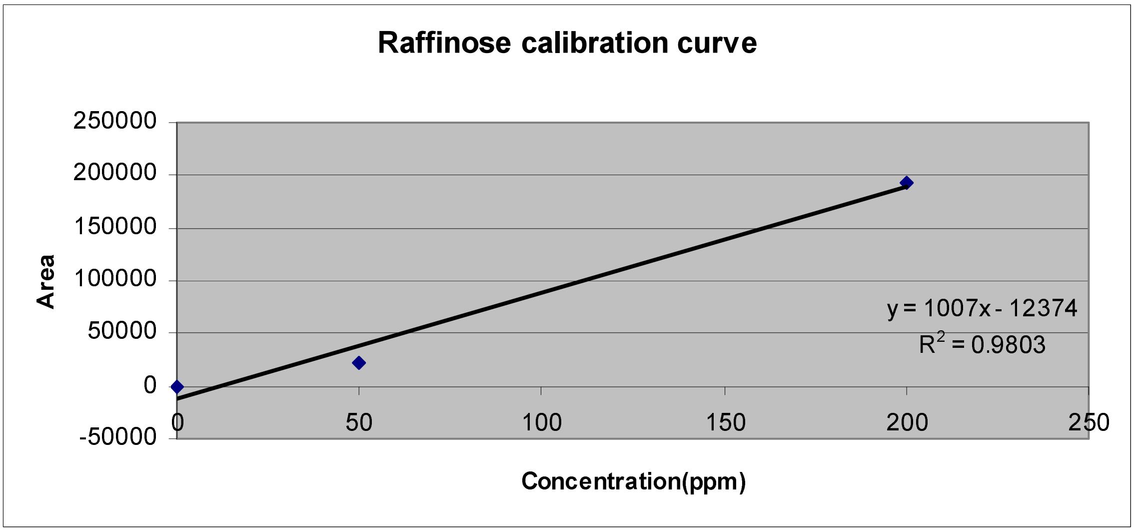
Annexe 2: Calibration Curves for Sugar Standards



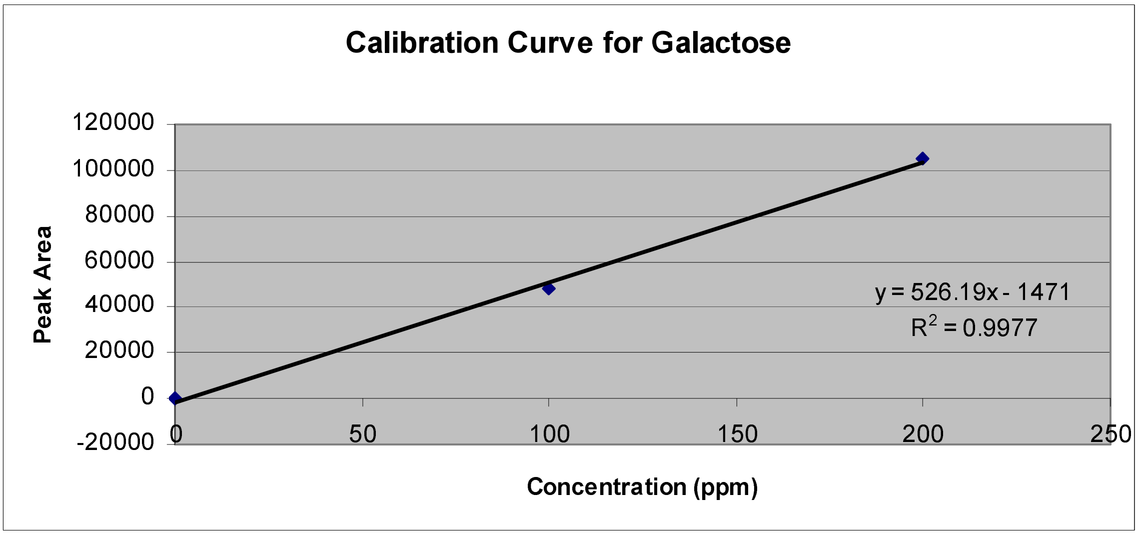
Stachyose standard calibration curve.



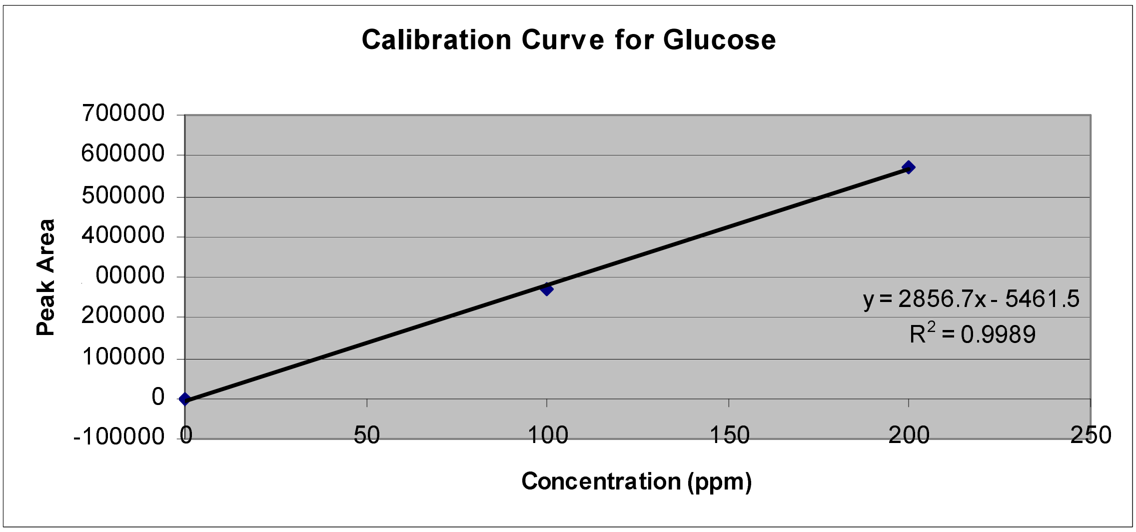
Lactose standard calibration curve.



Raffinose standard calibration curve.



Galactose standard calibration curve.



Glucose standard calibration curve.