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Production of Arabinoxylan from Sorghum By-products and Its Application in Agroindustry

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Author's Declaration

'Declaration: I confirm that this is my own work and the use of all material from other sources has been properly and fully acknowledged'.

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Abstract

Lignocellulosic residues comprise heterogeneous materials that attract much research interest as renewable resources. In Indonesia, sorghum-derived biomass is estimated at around 30-40 tons/hectare on an annual basis. There is an issue in sorghum plantation concerning the discard of the upper parts of the plant (stalks and panicles) after harvesting the grain. In large amounts, these residues can raise environmental, financial and management problems. The aim of the current study was to investigate the fractionation of sorghum processing by-products, and the utilisation of the subsequent hemicellulose, lignin and cellulose fractions in agricultural and packaging applications. Sorghum bran, stalk, and panicles were investigated as suitable starting materials, originating from an Indonesian variety of *Sorghum bicolor*, namely Kawali. A sequential alkaline extraction with varying concentrations of NaOH (0.75, 1, and 1.5 M) was applied, resulting in the generation of three fractions, those of residue (R), hemicellulose (H), and alkali lignin (L). The fractionation of the sorghum stalks with 1 M NaOH at 50^{o}C for 3 hours obtained ~64% (w/w) glucose in residue fraction. Moreover, 52 % (w/w) of xylose was predominant in the hemicellulose fraction of the stalks, which was further characterises as low branched xylan, and ~2% (w/w) of lignin was present in the liquid lignin fraction. On the other hand, in the bran, the fractionation with 0.75 M NaOH at 50^{o}C for 3 hours obtained ~40% (w/w) glucose in the residue fraction which corresponded to the cellulose. Approximately 76% of the glucose was available in hemicellulosic fraction that was further characterised as xyloglucan (XG), while nearly 2% (w/w) lignin was extracted in the lignin fraction. Thermal analysis determined by Differential Scanning Calorimetry (DSC) and X-Ray Diffraction (XRD) showed that the hemicellulose fraction exhibited lower thermal stability and higher crystallinity compared to that of cellulose. Subsequently, the sorghum-derived hemicellulosic fractions were used for the development of films and their potential use in agricultural applications as fertilising agents and food packaging materials was investigated. The addition of plasticiser (glycerol) improved the mechanical properties of bran-extracted hemicellulose films.

Moreover, the incorporation of long chain anhydride groups (2-octenyl succinic anhydride, 2-OSA) to sorghum hemicellulose extracts improved the performance (no brittleness), mechanical properties (tensile strength and total work), and barrier properties of the developed films. The films were tested as carriers of fertiliser (NPK) in soil trials and demonstrated quick release of the fertiliser and biodegradable properties. Furthermore, the application of 2-OSA-HS films as sealed-wrapping materials demonstrated adequate moisture barrier properties and reduced the weight loss of fruits after 7 days of storage in room temperature and under chilled conditions. Finally, biodegradable food trays were developed via thermopressing, using modified and unmodified corn starches with the addition of extracted fractions (cellulose, hemicellulose and lignin) from sorghum by-products (stalk and bran). The inclusion increment of cellulose fraction from 2% to 5% and hemicellulose fraction from 1% to 10% had a significant impact on the trays' colour and mechanical properties, water absorption capacity, microstructure and crystallinity. All trays exhibited no colour and structural changes during storage at 25 °C and 4 °C for 7 days. The food tray formulation that exhibited the best properties contained 2% of cellulose and 1% of hemicellulose, had a maximal resistance of 0.77 MPa and 9.46 mJ of total work, attributes which corresponded to a compact, homogenous, and dense microstructure. Overall, the extracted fractions derived from sorghum by-products have the potential to be used as starting materials for the production of a range of agricultural and packaging products. Further development of this process to improve the purity of each fractions, technology, and the properties of end products could contribute in the development of agroindustry and establish a viable route for the valorisation of sorghum-derived by-products.

Keywords: sorghum by-products, cellulose, hemicellulose, lignin, arabinoxylan, alkaline extraction, biofoam, biodegradable, film.

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Chapter 1: Introduction

Agri-food wastes and by-products are receiving significant interest due to the potential applications of lignocellulosic materials in food and non-food industries which seek to produce biochemicals, biofuels and biomaterials to replace petrochemically derived chemicals and materials with products derived from abundant biomass sources. These materials which consist of organic compounds including cellulose, hemicellulose and lignin represent an abundant, inexpensive and readily available source of renewable biomass. The valorisation of these biomaterials will stimulate the circular bioeconomy and contribute towards supporting the zero waste agenda across the food supply chain.

Cereal by-products including cereal straw and bran constitute a biomass resource for significant potential for valorisation. Among the different types of cereals sorghum is the fifth most important in the world after maize, rice, wheat, and barley, and its production has increased considerably over the years, particularly in developing countries. In Indonesia, sorghum production is considered a priority and supports the country's efforts to enhance food security and its bioenergy production. Overall, there is considerably less information and knowledge on the compositional and physicochemical characteristics of sorghum by-products, such as bran, the stalks and the panicles (particular of the latter two) compared to other cereal by-products. This information is necessary in order to be able to design effective and scalable multi-step processing schemes for the valorisation of these materials and their conversion into added value components that market potential to a variety of industrial sectors, including the food, agriculture and the packaging sectors. This information is vital which may provide its applicability as well as economic feasibility of processing in industry.

The aim of this research work was to develop an efficient process for the separation a sorghum processing by-products into various fractions containing the key macromolecules present (hemicellulose, cellulose and lignin) and investigate potential applications of these fractions as biomaterials for agricultural and food packaging applications. The objectives of this work were to:

1 Carry out in-depth compositional analysis of the different sorghum by-products (bran, stalk, panicles) including proximal and polysaccharide analysis.

2 Investigate the fractionation by alkaline extraction of the sorghum by-products into key macromolecules (hemicellulose, cellulose, and lignin) and optimise the process conditions (alkali strength, temperature, time) to maximise process yields.

3 Carry out work on the production of hemicelluloses based films, particularly investigating the type and concentration of plasticiser, on the basis of which further formulation experiments will be designed.

4 Develop sorghum derived hemicellulose films that can be potentially used for food packaging applications as well as for the controlled release of chemicals (e.g. fertilisers) into the soil and investigate their physicochemical properties.

5 Develop starch-based biodegradable foams containing sorghum derived fractions (cellulose, hemicellulose, lignin), with the aim to develop alternatives to polystyrene foams, and investigate their barrier and mechanical properties.

The thesis is structured into five chapters. Following this Introductory chapter, chapter 2 provides a literature review covering sorghum processing in Indonesia, composition of sorghum and sorghum by-products (focusing on cellulose, xylan, xyloglucan, lignin), fractionation technologies and valorisation schemes, as well as applications of polysaccharides for the production of novel films for agricultural applications and biodegradable foam materials. Chapter 3 presents the results from the compositional analysis sorghum by-products and the development of an alkaline method for its fractionation, as well as detailed compositional analysis of the fractions and their properties. Chapter 4 presents the results from the work investigating the development of films based on sorghum-derived hemicellulose fractions, analysis of their physicochemical properties and studies on their application as food packaging materials and for the release of chemical fertilisers in model systems. Chapter 5 presents the results from the work investigating the development of starch-based biodegradable foams incorporating sorghum-derived fractions (cellulose, hemicellulose, and lignin) and the study of a variety of key properties including their mechanical properties, water absorption capacity, microstructure, crystallinity and shelf life. Chapters 6 provides a general discussion on the key findings of this research within the context of potential impact and commercialisation potential, as well as recommendations for future research.

Chapter 2: Literature Review

2.1. Introduction

Sorghum (Sorghum bicolor (L.) Moench) is the fifth most important cereal in the world after maize, rice, wheat, and barley (Taylor & Duodu, 2010). The hierarchical system for the biological classification of sorghum is shown in Table 2-1. Sorghum is an established industrial crop which has a high biomass yield (Dien et al., 2009). It is drought-tolerant and resistant to water-logging and requires little input during growth (FAO and ICRISAT, 1996). Almodares, Taheri, Chung and Fathi (2008) classified sorghum into sweet, grain and forage sorghum. Sweet sorghum can be utilised for producing feed, liquid sugar or hay while non-sweet sorghum produces grain and is a staple food for several regions in Africa and Asia. For instance, chapatti and roti are common sorghum-based, non-fermented types of bread in India (Nandini & Salimath, 2001). In other parts of the world, sorghum is also used to produce various types of traditional food and beverages. Additionally, forage sorghum has attracted interest as a potential energy crop since it can grow under conditions that are unfavourable for corn production. Although sorghum is mainly utilised for food, it can also have an important role as raw material for ethanol production (Almodares & Hadi, 2009; Dien et al., 2009; Freita et al., 2014; Li et al., 2014; Suryaningsih & Irhas, 2014), or for the extraction of valuable chemical compounds such as fibre and speciality chemicals.

Lignocellulosic biomass can be used in biorefinery operations in which a variety of products can be produced through multi-step process schemes. These could include an initial step for biomass fractionation through physicochemical treatments followed by hydrolysis and fermentation to produce the desired products. Possible products include biofuels (e.g. ethanol, acetone, butanol), platform chemicals for the chemical industry (e.g. succinic acid) and speciality chemicals (oligo-saccharides, surfactants, amino acids, polyphenols, etc) that can have applications in many industries such as food, cosmetics and personal care, homecare and pharmaceutical.

Table 2-1 Hierarchical system for the biological classification of sorghum (adapted from (United Stated Department of Agriculture, 2015))

Category	Name
Kingdom	Plantae
Subkingdom	Tracheobionta
Super Division	Spermatophyta
Divisio	Magnoliophyta
Class	Liliopsida
Sub-Class	Commelinidae
Ordo	Cyperales
Family	Poaceae (Grass)
Genus	*Sorghum* Moench
Species	*Sorghum bicolor* (L.) Moench

This literature review will present and discuss the activities taking place currently in Indonesia with regards to the cultivation of sorghum and valorisation of its by-products, the compositional characteristics of sorghum and sorghum by-products, the available technologies for fractionating sorghum by-products with a focus on polysaccharides, and the potential uses of sorghum derived polysaccharides in the food and agriculture sectors.

2.1.1. Sorghum in Indonesia

The sorghum plant was introduced to Indonesia in 1925 and originates from Africa. There are several varieties of sorghum in Indonesia and most of them are cultivated in the east of Indonesia. Currently, due to several potential applications of sorghum, the government of the Republic of Indonesia intends to develop it as one of the most important crops to enhance food security and bioenergy production. However, its cultivation and development are still very limited to areas such as Java, West Nusa Tenggara, and East Nusa Tenggara (Suryaningsih & Irhas, 2014).

The production yield for sorghum in Indonesia is approximately 30-40 tons, per hectare per year (ICERI, 2018). This amount would be higher if Good Agricultural Practises (GAP) were applied. According to Rego, Nageswara Rao, Seeling, Pardhasaradhi, & Kumar Rao (2003), the most important factor that can increase sorghum production yield is balanced fertilisation. Nitrogen fertilisers promote the sucrose content, protein concentration and growth rate in sweet sorghum (Tsialtas & Maslaris, 2005, 2013). Nitrogen has a significant role in plant growth i.e., through cell division whereas potassium is required for the efficient transformation of solar energy into chemical energy. Accurate application of nitrogen and potassium fertilisers can stimulate biomass and the carbohydrate content of sweet sorghum which is considered as an essential determinant for food and industrial uses. Hence, these practices can increase crop productivity and yield.

In Indonesia, the most important cereals are rice and maize. Currently, amongst the different cereal crops, sorghum is not on the national priority list. Therefore, the production is lower than rice and maize. Moreover, the data on sorghum production after 2012 have not been officially released. Thus, in Table 2-2, some assumptions have been made based on the plantation areas for sorghum (the growth of sorghum plantation area is approximately 5000 Ha/year) and sorghum productivity (4 tonnes/year). The production of sorghum in Indonesia is still fluctuating dependent on government policy. For example, sorghum production increased significantly from 7695 tonnes to 131,530 tonnes from 2011 to 2014 (Table 2-2) due to the government's policy to plant sorghum in one of the largest national company plantations as well as in several marginal areas. Furthermore, sorghum is regarded by the government to be the crop with the biggest potential to substitute rice due to its nutrition and ease of cultivation.

The Indonesian Cereals Research Institute (ICERI) under the Ministry of Agriculture, Republic of Indonesia released a report in 2015 which included information on the varieties of sorghum cultivated in Indonesia (Table 2-3). Most of them do not originate from Indonesia, although there are some indigenous sorghum plants. Most of the varieties presented in Table 2-3 are the sweet sorghum types. Sweet sorghum (Sorghum bicolor. L. Moench) is recognised for its high sugar content in the stalk juice (Ali et al., 2008). The advantages of the sweet sorghum types are that can they adapt to a wide range of climatic and soil conditions, tolerate drought and high-temperature stress, salt tolerance, and give higher biomass yield per hectare (Almodares & Hadi, 2009; Almodares, Hadi, & Kharazian, 2009; Almodares et al., 2008). Among these sweet sorghum varieties, Kawali and Numbu are the most popular varieties in Indonesia. In fact, one national company (PTPN XII) has been instructed by the government to grow sorghum var. Kawali in their plantation area. These sweet sorghum varieties have short harvesting times of around 100-110 days, a very high carbohydrate content (>80%), and a low tannin content (0.18-0.21%) (Table 2-3). Thus, these two sorghum varieties have the potential to be cultivated at a larger scale.

Table 2-2 Production of most important cereals in Indonesia

Cereals	Year (tonnes)							
	2010	2011	2012	2013	2014	2015	2016	2017
Rice[a]	66,469,94	65,756,904	69,056,126	71,279,709	70,846,465	75,397,841	79,141,325	77.000.000
Maize[a]	17,629,748	18,327,636	17,643,250	19,387,022	18,511,853	19,833,289	23,500,000	24,500,000
Sorghum	5,723[b]	7,695[b]	92,071[c]	105,244[d]	131,530[e]	100,000[f]	120000[g]	120000[h]

[a] Central Agency of Statistics Indonesia, 2016
[b] (Directorate General of Food Crops, Ministry of Agriculture, cited in Subagio, 2013)
[c] assumption: sorghum area was 26306 Ha (H Subagio, Penelitian, & Serealia, 2013) and productivity was 3.5 tonnes/Ha
[d,e] assumption: sorghum area was 26306 Ha and productivity was 4 tonnes/Ha
[f] assumption: sorghum area was 25000 Ha and productivity was 4 tonnes/Ha
[g] assumption: sorghum area was 30000 Ha and productivity was 4 tonnes/Ha
[h] assumption: sorghum area was 35000 Ha and productivity was 4 tonnes/Ha

In sorghum plantations, one of the main cause of concern is the disposable of stalks and panicles after the grain is harvested. In large amounts, these residues cause problems such as pollution, storage, transportation, and financial issues. Agriculture and forestry activities are likely to produce pollution due to the smoke from biomass combustion. Practices such as the burning of agriculture wastes produce emissions of CO2, CH4, CO, N2O, NOX, SO2 and large amounts of particulates which cause adverse effects on human health (Gadde, Bonnet, Menke, & Garivait, 2009). The disposal or utilisation of sorghum by-products is therefore an important part of effective crop management. Storage is the main issue concerning the logistics of sorghum production and utilisation, especially as sorghum production is influenced by high seasonal availability (Rentizelas, Tolis, & Tatsiopoulos, 2009). The sorghum supply chain includes ground preparation and planting, cultivation, harvesting, handling, storage, in-field/forest transportation, road transportation and utilization of the product and by-products (residues) during the whole supply chain. It is crucial that each process is managed effectively. Due to the development of several sorghum plantations, there is an opportunity to convert sorghum biomass (residue) to value-added materials that can be used to develop the agro-industry in Indonesia. Agro-industrial development currently plays an important role in driving the Indonesian economy and is in line with the government's policy to achieve zero waste in agricultural processing thus making the utilisation of sorghum residues even more significant. The price of sorghum by-products such as bagasse (the fibrous matter that remains after sorghum stalks are crushed to extract their juice and is high in sugar) according to sorghum farmers is approximately 0.5-1 £/kg (personal communication with Indonesian Agency for Agricultural Research and Development (IAARD). However, this price depends on the market and can therefore fluctuate.

Table 2-3 Characteristics of sorghum varieties in Indonesia (Indonesian Cereals Research Institute, 2015)

Variety	Origin	Harves-ting time	Average yield	Protein content	Fat content	Carbohyd-rate content	Tannin content	Sugar content	Weight of straw-biomass	Potency of straw-biomass
		(days)	(tonnes/Ha)	(%)	(%)	(%)	(%)	(brix)	(tonnes/Ha)	(tonnes/Ha)
Super-2	ICRISAT	115-120	3.03	9.22	3.09	75.62	0.27	12.65	20.66	39.3
Super-1	East Nusa Tenggara, Indonesia	105-110	2.66	12.96	2.21	71.32	0.11	13.47	17.05	38.7
Kawali	India	100-110	2.96	8.81	1.97	87.87	0.21	na	na	na
Numbu	India	100-105	3.11	9.12	3.94	84.58	0.18	na	na	na
Sangkur	Thailand	82-96	3.6-4.0	11	3.5	61.5	na	na	na	na
Mandau	IRRI, Philippines	91	4.5-5.0	12	3	76	0.16	na	na	na
Hegari Genjah	Nebraska, The United States	81	3.0-4.0	9.44	4.9	24.3	0.13	na	na	na
KD4	North Sumatra, Indonesia	55-67	4	9.92	4.9	60.5	0.2	na	na	na
Badik	Thailand	49-55	3.0-3.5	9.25	4	26.1	0.124	na	na	na
UPCA-S1	Philippines	90-100	4	9.06	5.7	66.5	0.22	na	na	na
UPCA-S2	Philippines	105-110	4.0-4.9	9.25	3.6	64.25	0.35	na	na	na
6C	Purdue, The United States	96-106	4.6-6.0	9.7-10.4	2.67	26.4	1.19	na	na	na

*na: not available

2.1.2. Sorghum Processing

2.1.2.1. Grain and Flour Processing

The sorghum plant and the sorghum kernels are depicted in Figure 2-1. The kernels are highly exposed to the elements of nature as they are located on panicles (Figure 1a). Consequently, they are highly susceptible to pre-harvest losses from birds, insects, moulds, and unfavourable weather (Mcneill & Montross, 2002).When it is time to harvest, farmers cut the panicles from the stalks using a trimmer and then dry them to reduce the moisture content and avoid grain losses. Subsequently, threshing or shelling aims to separate the grains from the panicles and this takes place by using a thresher machine (cylinder/rotor speed and concave clearance), a separator (chaffer and sieves), and a cleaner (fan speed), similar to what they use for rice. The grains are placed in the storage room while the panicles are commonly left by the farmers in the field to dry. The required moisture content in order for the grain to have good storage stability is 13.5% or lower. The grain remains in the storage room for no more than one month (Mcneill & Montross, 2002).

(a) (b)

Figure 2-1 Sorghum grain in the panicles (a) Sorghum kernels (b)

The technology of milling sorghum grain into sorghum flour has been modified from wheat and rice milling technology although in Indonesia, sorghum milling is still carried out using traditional methods which involve manual handling and less sophisticated equipment.

However, recently industrialised milling technologies have been introduced which include decortication (or polishing) as the first step of the milling process; the aim is to remove the bran layers (pericarp and germ), in order to reduce the tannin and phytic acid contents (Delcour, Poutanen, Jiménez-Colmenero, & Delgado-Pando, 2013). Figure 2-2 depicts a sorghum polishing machine, which has a capacity of 100 kg/hour. Polished grains are normally obtained after 3-4 times of decortication.

Figure 2-2 Sorghum polishing machine at Indonesian Agency for Agricultural Research and Development (IAARD)

After decortication, sorghum grains milled and sieved, resulting in the production of flour (Aqil, 2012). In some regions in Indonesia, polished sorghum grain has become a staple food, as in the case of rice. The government of Indonesia encourages the diversification of staple food and sorghum is a good alternative (Food Security Agency Ministry of Agriculture Republic of Indonesia, 2013). However, while sorghum flour has been utilised to substitute wheat flour, not all types of sorghum are suitable for making flour. Sorghum with a low tannin content should be used to avoid the bitter taste of tannins. This is why high tannin sorghum is commonly used as animal feed.

2.1.2.2. By-products of Sorghum Processing

Images of the sorghum by-products are presented in Figure 2-3. They include stalks (Fig 2-3a), bran (Fig 2-3b), dried panicles (Fig 2-3c) and bagasse (Fig 2-3d). Bran is the main by-product of the sorghum milling operation and accounts for 10-11.5% of the grain (Ayala-Soto, Serna-Saldívar, Welti-Chanes, & Gutierrez-Uribe, 2015; Corredor, Bean, & Wang, 2007). It is derived from the decortication process which separates the grain and the aleurone to obtain high-quality food products or materials for special purposes (e.g. sorghum bran, gluten free sorghum bran, breakfast cereals). The stalks are pressed to obtain sugar juice, whereas the empty panicles are considered waste and are normally burnt. However, they can be used as natural fertilisers through land spreading. The bagasse is obtained by squeezing the stalks using a sugar cane roller mill (Figure 2-3d), and then dried in an oven (Fig. 2-3e), cut into small pieces and milled to get dried bagasse (Fig. 2-3f). Bagasse represents about 30% of the fresh stalks (Thanapimmetha, Vuttibunchon, Saisriyoot, & Srinophakun, 2011) and is an important biomass source that can be used for bioethanol production through saccharification and fermentation. After threshing, the panicles are separated from the grain, dried in the fields, and utilised as a fertiliser.

A potential valorisation scheme for sorghum is depicted in Figure 2-4. Such a scheme has been implemented in Indonesia to some extent, although at a smaller scale. The National Company uses the stalks to produce liquid and crystal sugar as well as bioethanol through small scale fermentation due to limited infrastructure, whereas the sorghum flour has long been used as a food ingredient, e.g. in cakes and cookies. In addition, bagasse is currently used as animal feed and fertiliser. Based on this scheme, it can be deduced that the valorisation of sorghum by-products, in particular, bran, panicles and bagasse could potentially lead to the production of a range of added value compounds with applications in various industrial sectors.

Figure 2-3 Sorghum by-products: fresh stalks (a); bran (b); dried panicles (c); squeezing stalk using sugar cane roller (d); wet bagasse (e); and dried bagasse (f).

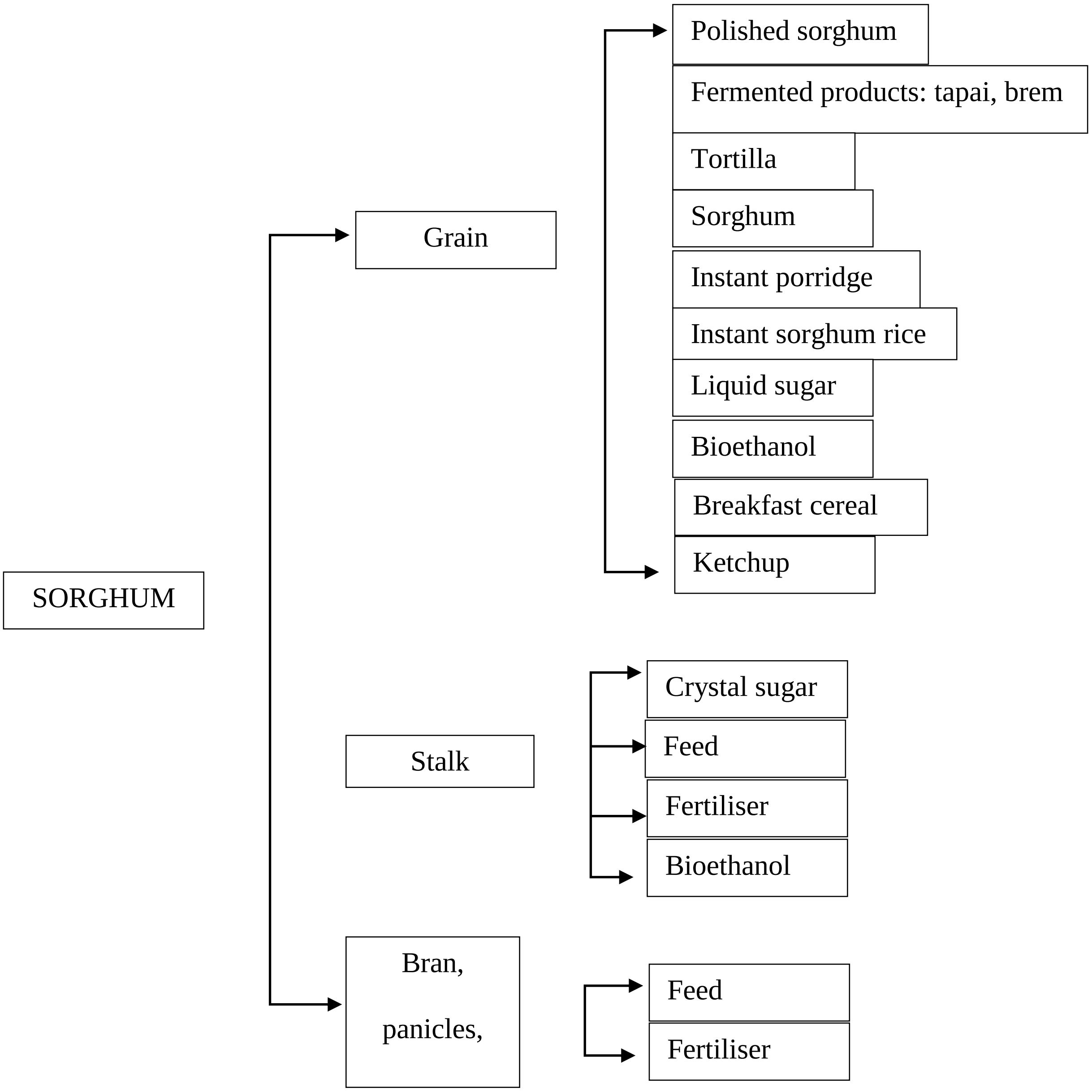

Figure 2-4 Scheme for sorghum valorisation in Indonesia

2.1.2.3. Proximate Composition of Sorghum in Relation to Other Cereals

The chemical composition of sorghum in comparison to other cereals is presented in Table 2-4. The sorghum grain contains non-structural (starch) and structural carbohydrates (cellulose, hemicelluloses). Starch is the main non-structural carbohydrate and represents the main source of energy required for germination. Around 50–75 % of total sorghum grain weight is starch, which is made up of straight-linked amylose chains (glucose unit held together by α, 1–4 glycosidic bonds) and amylopectin (glucose unit held together by α-1,4 glycosidic bonds and additional branched α-1,6 glycosidic bonds). The starch content in sorghum grain is similar to other cereals such as wheat, barley, maize, and brown rice (Table 2-4). Generally, sorghum has a lower starch digestibility than maize (Taylor and Emmambux, 2010) due to the high phytochemical content, particularly tannins which can inhibit the enzymatic hydrolysis of starch (Taylor & Anyango, 2011). On the basis of this, it has been suggested that sorghum may be a particularly suitable food for diabetic and obese people (Dicko et al., 2006). Furthermore, sorghum has higher fibre content than other cereals, except barley.

Sorghum is a gluten-free cereal (Ciacci et al., 2007; Ferreira et al., 2015) that has a lower protein content than wheat, barley, and corn but has a higher protein content than brown rice. Kafirins are the prolamins in sorghum (Taylor & Anyango, 2011). Sorghum contains various phytochemicals (phenolic compounds, tannins, plant sterols and policosanols) that are secondary plant metabolites or integral cellular components. These components are reported to positively affect human health (Ferreira et al., 2015; Taylor, Schober, & Bean, 2006). The tannin content in sorghum is the highest among other cereals; the high phenolic and tannin levels of certain sorghum genotypes are associated with enzyme inhibition in vivo and strong phenolic-starch interactions, which decrease starch digestibility, increase resistant starch and decrease the Glycemic Index (GI) of foods (Lemlioglu-Austin, Turner, McDonough, & Rooney, 2012; Mkandawire et al., 2013; Siller, 2006; Awika & Rooney, 2004).

Sorghum varieties are white, red and brown in colour. The resulting colour of sorghum products, however, cannot be predicted by the colour of the parent grain. The colour of sorghum-based products depends on the colour of the pericarp and endosperm, the presence of a pigmented or non-pigmented testa layer as well as on the production process. The pigmented testa layer of the kernel in some sorghum varieties contains condensed tannins which contribute to the dark colour (Awika & Rooney, 2004). Tannins are the most important bioactive components of sorghum since they possess high antioxidant activity (Awika, Rooney, Wu, Prior, & Cisneros-Zevallos, 2003). However, the tannin content in sorghum grain also has its drawbacks since it can bind protein and enzymes in the digestive tract, and therefore, reduce the nutritional value of sorghum grain (Awika & Rooney, 2004).

The lipid content in sorghum is higher than in wheat, barley, and brown rice but is lower than maize. The majority of lipids in sorghum are in the form of neutral triglycerides (triacylglycerols) and the predominant fatty acids are linoleic (C18:2; 38-49%, w/w) and oleic (C18:1; 31-38% w/w) (Serna-Saldivar and Rooney, 1995).

Table 2-4 Composition of sorghum and other grain cereals

Component (g/100 g edible portion)	**Sorghum**	**Wheat**	**Barley**	**Corn**	**Brown rice**
Starch	50	61.8	51.4	59.3	69.1
Protein	8.3	10.6	9.3	9.8	7.3
Lipids	3.9	1.9	2.8	4.9	2.2
Crude fibre	13.8	10.5	16.4	9.0	3.3
Phytic acid	1.0	1.0	1.1	0.9	0.9
Tannin	1.6	0.4	0.6	0.4	0.01

Source: Juliano (1999)

2.1.2.4. Composition of Sorghum by-products and Other Cereal by-products

The composition of various cereal by-products and residues is shown in Table 2-5. This information is very important in order to identify and develop suitable approaches for the full utilisation of these abundant materials and convert them into added-value products. Few studies have reported the composition of sorghum bran, stalks and panicles. It has been shown that sorghum bran contains more hemicellulose and protein and less cellulose and lignin than the stalks. These differences in composition are possibly related to the function of these macromolecules in the different parts of the sorghum plant (Ebringerová & Heinze, 2000). Compared to other cereal by-products such as the maize bran, sorghum bran contains less lignin, cellulose and hemicellulose but more starch. This is probably as a result of the differences in processing technologies used for extraction and due to the intrinsic differences in grain composition between maize and sorghum. The starch content in sorghum bran (~30%) is relatively high, most likely because of the considerable portion of the endosperm extracted during the decortication process.

The sorghum bran contains more protein than the stalk (~4.5%) (Table 2-5) and the main proteins are prolamins which are known as kafirins. Sorghum kafirins contain high amounts of glutamic acid/glutamine, leucine, alanine, proline and aspartic acid/asparagine; the content of lysine, an essential amino acid, in sorghum is relatively low (Verbruggen, 1996). Despite their low content in lysine, due to their overall high protein content, sorghum by-products are currently used as animal feed. Sorghum by-products could also be potentially used to produce biodegradable film materials (Yang, Lee, Won, & Bin, 2016). More specifically, previous works have reported that proteins extracted from by-products of wheat and maize processing can be used for the production of film packaging materials. In order to improve the physical properties of the films, plasticisers and cross-linking agents are normally added to the film casting solution (Irissin-Mangata, Bauduin, Boutevin, & Gontard, 2001; Wittaya, 2012).

In terms of cell wall polysaccharides in the sorghum plant and its by-products, they mainly consist of cellulose and hemicellulose. Cellulose is a polysaccharide in the form of a linear chain of several hundred to many thousands of β (1,4) linked D-glucose units and is an important structural component of the cell wall. Hemicelluloses are a heterogenous group of polysaccharides of different structures containing glucose, xylose, mannose, galactose, arabinose, fucose, glucuronic acid, and galacturonic acid in various amounts, depending on the natural source. Hemicelluloses have a relatively low degree of polymerization, typically between 80 and 200 (Ghoneum, Badr El-Din, Ali, & El-Dein, 2014), and are divided into four general classes of structurally different polysaccharides: xylans, mannans, glucans and xyloglucans. In sorghum by-products, the bran contains less cellulose and hemicellulose than the stalk. Likewise, maize bran and rice bran also contain less cellulose and hemicellulose than the straw (Table 2-5). However, the amount of cellulose and hemicellulose in other cereal by-products, such as barley, wheat andoat varies. The large range observed in the chemical composition of cereal by-products (Table 2-5) reflects the differences in the source of the raw material (crop variety, primary processing) as well as the extraction method used to quantify the various components.

Table 2-5 Composition of various by-products from cereal plants (in %, w/w dry weight)

Material	**Cellulose**	**Hemi-cellulose**	**β-Glucan**	**Lignin**	**Protein**	**Ash**	**Starch** (%, w/w of total carbohydrate)	**Sugar**	**Arabinoxylan** (%, w/w of total carbohydrate)	**References**
Sorghum stalk	30-32.5	27.5-30	na	7-20	4.55	0.7-7.24	na	32.81	na	(Li et al., 2014; Massoud & M. Abd El-Razek, 2011)
Sorghum bran	11	18	na	<1	10	2.7	30	60		(Corredor, Bean, & Wang, 2007b)
Wheat bran	9.0-48.1	39.8	2.2-2.6	3.0-8.6	na	5.5-6.7	4.2-16.7	41	6-22.1	(Gebruers et al., 2008; Huang et al., 2010; Zhong X. Lu, Walker, Muir, Mascara, & O'Dea, 2000; Wang, Sun, Liu, & Zhang, 2014; Y. Zhang et al., 2011; Zhou et al., 2010)
Wheat straw	30.0-60.0	20-40	na	10.0-30.0	na	5.5	na	66.2	23.11	(Xie, Liu, Ni, Zhang, & Wang, 2011); (Akpinar, Erdogan, & Bostanci, 2009); (Escarnot, Aguedo, Agneessens, Wathelet, & Paquot, 2011a)
Rye bran	60.2	12.6	2.8-3.4	4.6	10.8-18	1.4-2.8	49.6	na	24.2-50	(Delcour et al., 2013; Mansberger et al., 2014; Roubroeks & Andersson, 2000; Sárossy, Tenkanen, Pitkänen, Bjerre, & Plackett, 2013)
Rye straw	43.6	23.5	na	12.1-18.6	na	3.4	na	na	na	(Xiao, Sun, & Sun, 2001); (Franceschin et al., 2011)
Oat bran	57.0	5.6	6.6-7.5	4.5	12.0-26.0	2.0-9.0	47.0-53.0	na	3.8-13.2	(Arendt & Zannini, 2013; Mansberger et al., 2014; Sárossy et al., 2013)

Barley bran	na	na	7.7	na	na	3.7	51.1-54.4	na	14.21-21.06	(Bhatty, 1993; Gong, Jin, Wu, & Zhang, 2012)
Corn Bran	12-18	33-35	na	8-10	11.5	2.6-6	13.5-23	na	40.52	(Corredor et al., 2007b; Hromádková, Košt'álová, & Ebringerová, 2008)
Corn cob	45	39	na	17.8	na	1.2	na	82.9	33.4	(Silva, Oliveira, Neto, Pimentel, & Santos, 2015); (Nwadiogbu, Okoye, Ajiwe, & Nnaji, 2014)
Maize stem	40	23.2	na	11.7-17.3	na	na	na	na	na	(Xiao et al., 2001);(Sipponen, Laakso, & Baumberger, 2014)
Rice bran	5.9-9.0	4.6-16.9	1.8	2.8-3.9	12.32	8.73	na	na	4.54-5.11	(Xiao et al., 2001); (Gul, Yousuf, Singh, Singh, & Wani, 2015); (Kahlon, Chow, Knuckles, & Chiu, 1993); (Z. Zhang, Smith, & Li, 2014)
Rice straw	39.6	18.6	na	10.1-20.9	na	13.7	na	na	15.9	(Xiao et al., 2001); (Sharma et al., 2015)
Barley straw	36.6	21.1	na	22.4	na	4.22	na	na	na	(M. Yang et al., 2015); (Kim, Yu, Han, Choi, & Chung, 2011)
Spelt hull	30.2	35.5	na	5.7	7.0	12.6	na	42.6	20.04	(Escarnot, Aguedo, Agneessens, Wathelet, & Paquot, 2011b)

*na: not available

2.2. Treatment strategies for sorghum by-products

There are several methods that have been used to extract hemicelluloses, in particular, xylans, from cereals; these are compiled in Table 2-6. In addition to the more traditional methods, such as alkaline extraction, hot compressed air and steam explosion, ‘greener’ technologies, such as microwave and ultrasound that require less energy and utilise less amounts of harsh chemicals and thus minimising the environmental impact (Chemat, Zill-E-Huma, & Khan, 2011). The enzymatic strategies may constitute viable alternatives to such chemical methods and are more acceptable from an environmental point of view. It has been proven that the enzymatic methods using endoxylanases for the conversion of water-unextractable AX into solubilised AX were as efficient as the chemical ones (Beaugrand, Crônier, Debeire, & Chabbert, 2004). Physical treatments such as ultrasonication need to be conducted subsequently in order to get a pure and a high yield of arabinoxylan. Nevertheless, the most common method to extract xylans is alkaline treatment. Alkali can cleave most bonds in plant tissues including α-ether bonds between lignin and hemicelluloses and ester bonds between lignin and/or hemicelluloses and hydroxycinnamic acids, such as p- coumaric and ferulic acids (Xiao et al., 2001).

Table 2-6 Methods for extracting xylans from cereal by-products

Extraction method	Material	Catalyst/Conditions	Time	Temperature	Yield	References
Alkaline	Sweet sorghum stem	NaOH	2 h	90 °C	60.6 % Alkali-Soluble Hemicellulose (ASH)	Sun, Wen, Ma, Song, & Sun (2014)
	Spent husk	NaOH; $NaClO_2$–KOH	5-16 h	45°C	18.9% AX[a]	Escarnot et al. (2011a)
	Rye bran	NaOH	2 h	55 °C	62.2-68.9% AX	Mansberger et al. (2014)
	Maize stems, rice straw, rye straw	NaOH	18 h	30 °C	72.1-84.6% AX	Xiao et al. (2001)
	Distiller's grain	NaOH	3 h	50 °C	57.7% HC	Xiang, Watson, Tobimatsu, & Runge (2014)
	Rice straw	NaOH	15 min	121 °C	10-14.9% xylan	Park, Arakane, Shiroma, Ike, & Tokuyasu (2010)
Enzymatic	Wheat flour AX	α-L-arabinofuranosidase (GH51), α-L-arabinofuranosidase (GH43)	48 h	40 °C	66% WAX and 86% WAX[c]	Heikkinen et al. (2013)
	Wheat bran	endoxylanase	30 min-2 h	30 °C	35-40% AX	Maes, Vangeneugden, & Delcour (2004)
Microwave-assisted	Wheat straw	5% of NaOH, power 1000 W	10 min	140°C	73% Xylan	Janker-Obermeier, Sieber, Faulstich, & Schieder (2012)
Ultrasound-assisted	Buckwheat husks	Ratio liquid/ solid 10, power 100 W, intensity 8 Wcm^{-2}	10 min	60 °C	11.2 % HC	Hromádková & Ebringerová (2003)
	Wheat bran	50 g/l, enzyme dose, 4.5 g/l, Power 180 W, 50 °C.	70 min	50 °C	14.26 % HC	Wang, Sun, Liu, & Zhang (2014)

Steam explosion	Rice hulls, corn stalks	2% NaOH, pressure 1.5 MPa	1 min	250 ^{o}C	90% hemicelluloses	Schultz, Curry Templeton, Biermann, & McGinnis, (1984)
Hot compressed water	Corn stover	Pressure 2.1-2.4 MPa,	16 min	220 ^{o}C	90% Xylan	Liu & Wyman, 2003
	Rye straw	Pressure 50 bar	9 min	215 ^{o}C	95% Xylose	Ingram, Rogalinski, Bockemühl, Antranikian, & Brunner (2009)

[a]Arabinoxylan; [b]Hemicellulose; [c]Water extractable arabinoxylan

2.2.1. Alkaline Treatment

Alkaline extraction disrupts the hydrogen and covalent bonds and loosens up the cell wall matrix, consequently releasing various polysaccharides (Fincher & Stone, 1986). Hydroxyl ions disrupt the hydrogen bonds between cellulose and hemicellulose, break ester linkages, and hence solubilise part of the hemicellulose material (Cyran, Courtin, & Delcour, 2003). An increase in the strength of alkali results in more hemicelluloses being released. The yield of alkali-extracted xylan by cereal by-products is varied ranging between 16-31% (w/w) (Zhang, Smith, and Li, 2014).

Alkali treatment of lignocellulosic materials such as cereal straw, occurs by hydrolysing uronic and acetic esters, and by the swelling of cellulose. This decreases the crystallinity of cellulose. In addition, the treatment also cleaves the α-ether linkages between lignin and hemicelluloses and the ester bonds between lignin and/or hemicelluloses and hydroxycinnamic acids such as p-coumaric and ferulic acids (Xiao et al., 2001). By this process, straw and stems can be fractionated into alkali-soluble lignin, hemicelluloses and residues, which makes it easier to utilise them for the production of added value products.

The isolated hemicelluloses can be further purified to obtain homogeneous hemicellulosic fractions. Hemicelluloses can be recovered after neutralization and precipitation with ethanol, a process that was originally established in 1926 (Peng, Peng, Xu, & Sun, 2012). Moreover, the use of organic solvents could help in improving the separation following on from the alkaline treatment. For example, Xu et al. (2007) used 95% ethanol, which led to the separation of residual lignin from hemicellulose following alkali treated with ryegrass. The results showed that over 90% of residual lignin was dissolved in ethanol and was separated from hemicellulose.

The addition of three volumes of ethanol can precipitate more than 70% of xylan from basic hydrolysates as reported by Deutschmann and Dekker (2012). Dervilly, Saulnier, Roger and Thibault (2000) reported that the Ara/Xyl ratio and the amount of xylose residues that were di-substituted increased with ethanol concentration. Peng et al. (2012) also reported the Ara/Xyl ratio increased with increasing ethanol concentration. On the other hand, low ethanol concentration precipitates xylans of longer chain lengths and more branched polymers. Furthermore, by increasing the ethanol concentration, shorter xylan chain lengths and less substituted Xylo-oligosaccharides (XOS) were obtained (Dervilly et al., 2000). The differences observed between the various research studies are probably due to the different hydrolysis methods and conditions applied as well as to differences in the raw materials.

2.2.2. Physical treatments

As shown in Table 2-6, several types of physical treatments have been applied to extract arabinoxylans from cereal by-products, including ultrasound treatment, hot-compressed water, extrusion, steam explosion and microwave treatment. According to Chemat et al. (2011), ultrasound-assisted extraction (UAE) is more advantageous than other physical methods, such as microwave, since it may be simpler and faster than the latter due to increased mass transfer. Moreover, the types of solvent used, the raw materials, and the moisture content do not limit the use of UAE. In recent years, ultrasound has been the subject of research and development in the food industry due to its low environmental impact, high efficiency and low processing time. Additionally, practical and reliable ultrasound equipment is now available for industrial use. Ultrasound is a technology that uses sound waves of frequency > 20 kHz and generates cavitation effects as a result of the hydrodynamic shear forces in the aqueous phase. The main goal of such treatment is to rupture the cell wall and to facilitate the release of intracellular matter in the aqueous phase for subsequent depolymerisation (Khanal, Grewell, Sung, & van Leeuwen, 2007). Ultrasound has been applied in food processing, preservation and extraction (Chemat, Huma & Khan, 2011).

The application of ultrasound for the extraction of natural products has received much attention due to the advantages mentioned above. More specifically, ultrasound extraction can potentially be completed in minutes with high reproducibility, reducing the consumption of solvent, simplifying manipulation and work-up, giving higher purity of the final product, eliminating post-treatment of waste water and consuming only a fraction of the fossil energy normally needed for a conventional extraction method such as a Soxhlet extraction, maceration or steam distillation (Chemat et al., 2011). Several classes of food components such as aromas, pigments, antioxidants and other organic and mineral compounds have been extracted from a variety of matrices, such as animal tissues, food and plant materials. More specifically, ultrasound has been applied for the extraction of hemicelluloses from various plant cell walls, such as maize bran (Ebringerová & Hromádková, 2002), buckwheat (Hromádková & Ebringerová, 2003) and wheat bran (Hromádková et al., 2008).

Ultrasound can be combined with other treatments, such as the presence of alkali [NaOH, Ca(OH)2]. For example, ultrasound pre-treatment in combination with NaOH (5%) improved the yield of xylan (28%–36%) obtained from corn cobs (Ebringerová et al., 1998). In addition, Hromádková & Ebringerová (2003) reported that ultrasound used in combination with NaOH (1% and 5%) can increase the extraction of hemicellulose from buckwheat hulls by 10%–40%, whereas the whole extraction time can be reduced from 60 minutes to 40 minutes. The above indicates that there is significant potential for developing an efficient and environmentally friendly process based on alkaline extraction and potentially ultrasonication for the extraction of xylans from sorghum by-products targeting high extraction yields (> 60%).

2.3. **Value-added products from sorghum by-products**

A detailed schematic process for the potential production of various added-value products from sorghum by-products is given in Figure 2-5. The end-products include bioethanol, oil, biopolymers, food thickeners, activated carbon, phenol, binders, prebiotic oligosaccharides as well as packaging materials. These products can be derived from the chemical and enzymatic fractionation of sorghum stalk bran and panicles to obtain key macromolecules, including xylan, xyloglucan, mixed-linkage β-glucan and lignin. Xylan can be produced from sorghum stalk whereas xyloglucan and mixed-linkage β-glucan can be produced from sorghum bran

(Verbuggen, Beldman, & Voragen, 1995). Lignin and oligosaccharides with potential prebiotic activity can be potentially obtained both from sorghum stalk and bran as well as panicles. In addition, protein and oil can be produced from sorghum bran.

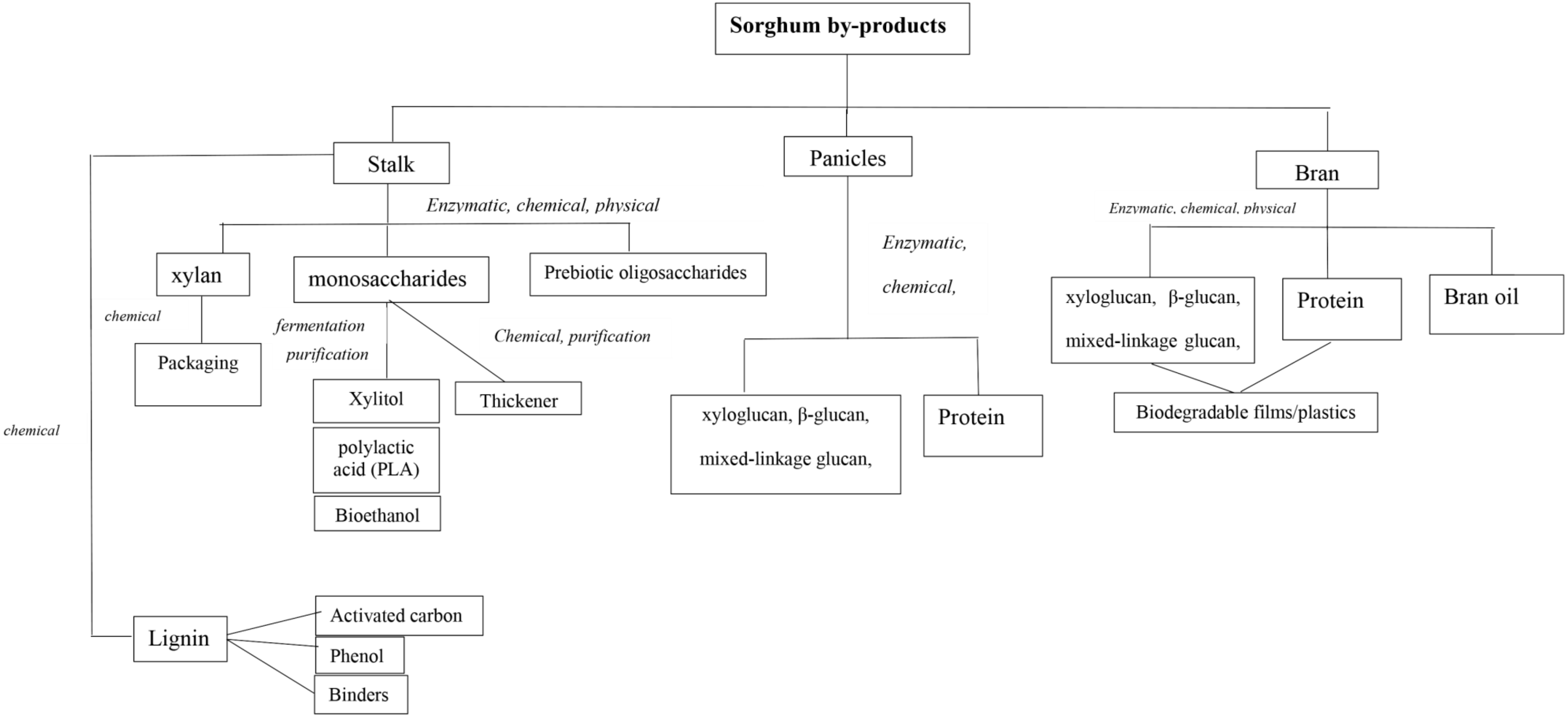

Figure 2-5 Valorisation of sorghum by-products based on a biorefinery strategy

2.3.1. Xylan

Xylan is the main hemicellulose in cereal plants, consisting of a linear backbone of (1,4)-linked β-D-xylopyranose backbone with varying degrees and types of substitution. Xylans form hydrogen bonds with cellulose, covalent bonds (mainly a-benzyl ether linkages) with lignin and ester linkages with acetyl units and hydroxyl cinnamic acids. A common xylan structure in cereal plants is presented in Figure 2-6. The main chain can be substituted in positions 2 or 3 or both with R-L-arabinofuranosyl, D-glupyranosyluronic acid, or D-xylopyranosyl residues. The xylopyranosyl units can be mono- or di-substituted and the arabinofuranosyl units can be further substituted with other sugars. Some naturally occurring xylans, particularly in wheat, also carry O-acetyl and feruloyl substituents. The degree of substitution varies significantly between xylans from different species, but also between different parts of the plant and between plant materials grown in different seasons. For example, the xylans in sorghum, finger millet, rice and maize have more complex side chains than in wheat, oat and barley, which include xylopyranose, galactopyranose, and α-D-glucuronic acid or 4-O-methyl-α-D-glucuronic residues (Zhang, Smith, & Li, 2014).

The degree and type of substitution have a great impact on the physicochemical properties of the xylan polymer (e.g. solubility, viscosity, water binding capacity) and consequently its applications. Currently, arabinosylated xylans (AXs), primarily from wheat and maize, are used as thickening agents and as improvers of dough properties in bread processing. AXs are added since they have water-binding property (Mansberger et al., 2014). Moreover, AXs have also been suggested to exert potential biological activities, in particular being effective hypocholesterolemic agents, and effective agents to suppress blood pressure, bind hydrophobic mutagens in diet, and potentially inhibit the growth rate of tumours (Ebringerová & Heinze, 2000). The functional and biological characteristics of AXs are a result of their molecular weight and ferulic acid content (Zhang et al., 2014). For instance, modified wheat bran AXs with low molecular weight (MW) (6.6 × 104 Da) was shown to exert potential prebiotic properties in vitro (Hughes, Shewry, Li, Gibson, Sanz & Rastall, 2007), whereas enzymatic modification rice bran hemicellulose (MW 30–50 kDa) using carbohydrate hydrolysing enzymes from shiitake mushrooms, exerted immune-modulating activities in in vitro and in vivo studies (Ghoneum, 1998; Ghoneum & Brown, 1999; Ghoneum & Matsuura, 2004). On the other hand, high molecular weight AXs (220-300 kDa) have demonstrated the ability to lower the post-prandial glycaemic response in vivo (Lu, Walker, Muir, Mascara et al., 2000; Lu, Walker, Muir and O'Dea, 2004). The above studies demonstrate that the macromolecular characteristics such as the variation in the degree of branching, molecular weight and spatial arrangement of AXs recovered by different methods can have a significant effect on their biological activity (Saulnier, Sado, Branlard, Charmet, & Guillon, 2007).

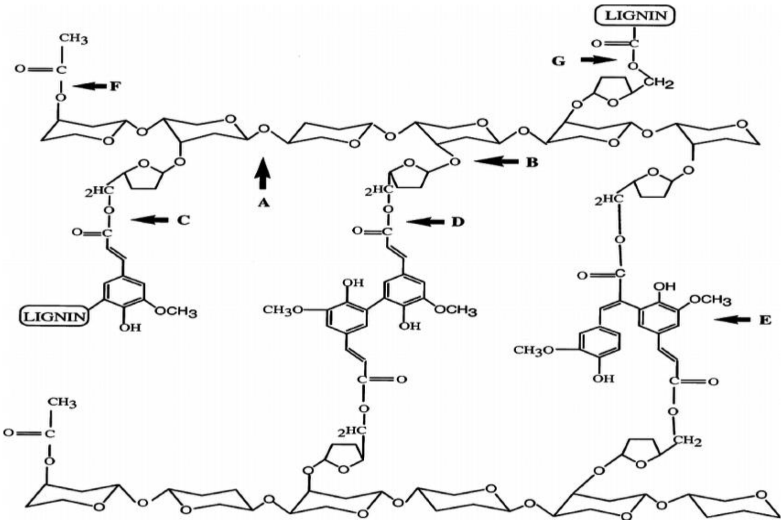

Figure 2-6 Generalised structure of arabinoxylan.

A: β -(1-4) linked xylan backbone, B: Xylose-arabinose linkage, C: 5-O-feruloyl lignin, D: 5-O-diferuloyl group (5-5 linked dimer), E: 5-O-diferuloyl group(8-5 dimer), F: 3-O-acetyl group, G: arabinose-lignin.

2.3.2. Xyloglucan and mixed-linkage β-glucan

Xyloglucan (XG) is typically hemicellulose in dicots plant. However, previous studies have found that monocotyledon plants, i.e, cereals, also contain xyloglucan and mixed-glucan linkages; for example these can be found in rice bran (Shibuya & Iwasaki, 1985) and rye bran

(Sárossy, Tenkanen, Pitkänen, Bjerre, & Plackett, 2013a)a well as in rice seeds (Kato, Ito, Iki & Matsuda, 1982). In the plant structure, xyloglucan (XG) is synthesised in the Golgi and exported to the apoplasm in soluble form. During growth, the content of XG decreases and has a function in cell enlargement which in turn becomes a key component in primary walls. XG binds to the surface of cellulose microfibrils and is thereby incorporated into the xyloglucan/cellulose network, which then forms a major load-bearing structure in the primary cell walls. There has been limited information on the availability of XG in sorghum bran. A previous study reported by Woolard, Rathbone, & Novellie (1977) revealed that β-glucan and glucoarabinoxylan were isolated from the sorghum bran while Verbruggen et al. (1993) reported that mixed-linkage glucan and xyloglucan were found in the sorghum endosperm.

The sorghum flour, bran, stalk and panicles may consist of different types of hemicelluloses. The content and structure of hemicelluloses in various plant species differ depending on the type of plant tissue and organ (Li et al., 2015). A previous study reported that the water-extractable material of sorghum flour consists mainly in the form of (1,3), (1,4)- β-D-glucans and a small amount of xyloglucan (Verbruggen et al., 1998). However, in the water-unextractable cell wall material, it was reported that glucose was co-extracted and linked to arabinoxylan. As a result of the high glucose concentration, the hemicellulose of sorghum flour consists primarily of glucoarabinoxylan (GAX) (Ayala-Soto et al., 2015) while sorghum stalks contain L-arabino-(4-O-methyl-D-glucurono)-D-xylans (Li et al., 2015). A backbone of (1-4)-β-linked D-xylopyranose residues with single arabinofuranose units are attached at O-3 or at both O-2 and O-3 of certain xylose units. Other structures such as glucuronic acid and 4-O-methylgucuronic acid are attached at O-2 of xyloses and arabinose residues, single or as chains at O-3.

The structure of XG has glucose as a backbone which is 75% substituted with (1,6)-linked xylopyranose branches that in turn may be further derivatised by β-(1,2)-linked galactopyranosyl residues such as in tamarind seeds (Mishra & Malhotra, 2009). α-Xylose residues are linked to the O-6 of the β-glucose residues and the terminal galactose or arabinose is linked to the O-2 site of xylose residues with β-bonds. However, the XG in monocotyledons including in sorghum plants may contain less xylose, and generally, terminal galactose does not exist while a small amount of arabinose is available which is lower than those from dicotyledons (Hayashi & Kaida, 2011).

Molecular interactions between hemicelluloses and β-glucans in the cell wall matrix are essential from a technological perspective. There is evidence of spontaneous and strong intermolecular association between unsubstituted regions of the hemicellulose chains and cellulose-like β-(1,4)-linked fragments from the β-glucan chains (Najmudin et al., 2006). Such non-covalent associations, as well as the extent of cross-interactions between β- glucans and hemicelluloses in the plant cell walls, might contribute to the water-unextractability cell wall materials (WUS), and the solubility and enzymatic indigestibility of polysaccharides. In addition, xyloglucans are interconnected with hydroxyproline-rich protein by covalent bonds, whereas hydrogen bonds are interconnected by cellulose and xyloglucan.

The extraction of hemicelluloses from plant cell walls is commonly conducted using alkaline conditions. The xyloglucans are mostly extracted with strong alkali, as shown for pea and Arabidopsis (Hayashi & Kaida, 2011). However, there is no appropriate method to extract with a 100% yield hemicelluloses without contamination from other components of the cell wall. Moreover, hemicelluloses tend to degrade during the extraction/isolation process depending on the alkali type used and the processing conditions (Sun & Ren, 2010). Further research in developing process methodologies that lead to higher yields and purity of the hemicellulose fractions, such as xyloglucan, in cereal by-products is important and should be explored. Besides, β-glucans are extractable with aqueous solvents or alkali solutions after the removal of protein and starch using hydrolytic enzymes and/or selective adsorption and precipitation of β-glucans from purified solutions with alcohol and freeze drying or alternatively drum or spray drying of the extracts (Izydorczyk & Dexter, 2008).

Xyloglucans have a wide range of utilisations either in food or non-food applications, as they can be used as thickening, gelling and stabilising agents in food products, and as adhesive and binding agents for the textile sector (Mishra & Malhotra, 2009). In terms of food applications, previous research works have investigated the physicochemical characteristics of xyloglucans, including their flow behaviour, water-holding ability, as well as resistance against heat, salt, and various pH (Mishra & Malhotra, 2009). On the other hand, β-glucans are already well-known for their health benefits in terms of reducing serum cholesterol levels and attenuating postprandial blood glucose and insulin responses (Du, Zhu, & Xu, 2014). Moreover, the physical properties of β-glucan, such as water solubility, viscosity, and gelation have been used in food and beverage products throughout the world (Izydorczyk & Dexter, 2008). Apart from the application of β-glucan in the food and pharmaceutical industry, β-glucan also has been applied in cosmetics for its soothing, moisturising and anti-irritant properties as well as other cosmetic and personal care products which benefit from its inclusion.

2.3.3. Cellulose

Cellulose is a linear homopolymer composed of D-glucopyranose units linked by β-1,4-glycosidic bonds. Cellulose exists as the main component of the cell walls in lignocellulosic plants and ranges from 23-53% on a dry-weight basis depending on the plant species, their maturity and the environmental conditions (Wahyudiono, Sasaki, & Goto, 2008). In straw plants, including sorghum plants, approximately 35-45% of the total mass is cellulose (Sun & Ren, 2010). The cellulose exists as microfibrils and is linked to hemicellulose, lignin and other polymers in a gel matrix. The microfibrils have an indefinite length and various degrees of crystallinity which contribute to the mechanical strength of the cell wall. Furthermore, it is insoluble and resistant to alkaline conditions.

The fractionation of highly pure cellulose has been the subject of extensive studies for many years due to the complexity of its cell wall structure. The combination of chemical and mechanical treatments are necessary for the dissolution of lignin, hemicellulose and other noncellulosic substances. Usually for materials rich in lignin the initial step involves treating the material with sodium chlorite to remove the lignin and subsequently treating the material with alkali agents. However, in the case of straws with a low lignin content, the delignification treatment is not usually required. Alkali extraction is the most common method to dissolve the non-cellulosic materials (e.g. hemicellulose and lignin) in cereal straws and obtain the cellulose fraction as a residue. For instance, the cellulose from dewaxed maize stem, rye straw and rice straw were isolated by treatment with 1 M NaOH at 30 oC for 18 h, which resulted in the recovery of 49%, 54%, 32% of cellulose, respectively (Xiao et al., 2001). The structure and properties of the cellulose fibers are influenced by the levels of lignin present, as these give a coarse, stiff and brownish colour to the cellulosic fibres. Hence, it is very challenging to obtain lignin-free cellulose.

Additionally, research has shown that the recovery of the cellulose fraction of sorghum bran and bagasse which was extracted using hot water (85 ^{o}C) for 1 hour and extracted by 50% (w/v) NaOH at pH 11.5 were 11.68% and 46.73%, respectively (Qiu, Yadav, & Yin, 2017). This cellulose fraction from the sorghum bran was very rich in insoluble dietary fiber (IDF), approximately 84% (w/w). Moreover, it had water holding capacity (WHC) which was 23 to 35 times higher than that of the original material before extraction, and was also significantly higher than the cellulose extracted from other cereals such as wheat bran (Qiu et al., 2017). This high WHC value could be attributed to the high porosity of the sorghum derived cellulose, the amorphous structure of cellulose, and the spray-drying method which was used.

Both cellulose and hemicellulose can be used as feedstocks for the production of ethanol. However, the main challenge of using agricultural and forestry lignocellulosic residues as fermentation feedstocks, is the efficient saccharification of the different types of polysaccharides present such as cellulose, xylans, starch, glucans, arabinogalactans and glucomannans and their bioconversion through microbial fermentation (Deutschmann &

Dekker, 2012). Apart from fermentation feedstocks, cellulose fibre could also be used as a reinforcement agent in packaging applications (Bergeret & Benezet, 2011).

2.3.4. Lignin

Lignin is a non-polysaccharide cell wall component that is often bound to cellulosic fibre. Cereal bran generally contains very low amounts of lignin whereas the stalk contains higher amounts of lignin, i.e 7-20% (Table 2-5). There are three monomers in lignin, methoxylated to varying degrees: p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol (Adapa, Karunakaran, Tabil, & Schoenau, 2009). These are incorporated into lignin in the form of phenylpropanoids p-hydroxyphenyl (H), guaiacyl (G) and syringyl (S), respectively (Adapa et al., 2009). The lignin of most grasses consists mostly of guaiacyl while some plants have syringl, such as in the case of sorghum (She et al., 2010). Lignin has a number of applications including for the production of fuels, as a source for the production of aromatic compounds that are used in chemical synthesis by the chemical industry and as an intrinsic resin to produce binder-less boards (Li, Zhao, Wang, Huber, & Zhang, 2015).

2.4. Potential uses of polysaccharides from sorghum residues in agricultural industry

This part of the literature review will focus on two potential applications of the polysaccharids (hemicellulose, cellulose) fractions extracted from sorghum processing by-products, namely for the development of films for agricultural applications and for the production of biodegradable foam packaging materials.

2.4.1. Film Applications of Sorghum Derived Hemicellulose

The potential exploitation of hemicellulosic materials extracted from natural resources is attracting significant interest nowadays due to their funtional properties. Another added advantage is that they can be found in abundance in nature and thus their exploitation opens up a variety of potential applications in a number of fields (e.g. food, agriculture, pharmaceuticals, cosmetics). The properties of hemicellulose such as its solubility, thermal behaviour, rheological behaviour, surface tension, molecular weight and biological activity depends to a large extent on its structure (Sun & Ren, 2010). The latter is influenced by a number of factors including the plant species (e.g. woody or non-wood plants, monocots or dicots plants), the plant tissues, and the methodologies used for its extraction and purification (Verbruggen, Beldman, & Voragen, 1995; Xiao, Sun, & Sun, 2001).

A possible application, is towards the development of polymeric films and coating, which has become a focus area in polymer-based research. A film is a stand-alone thin layer of materials composed of a polymer matrix providing structural integrity. The formulation of films requires one polymer component being capable of forming a structural matrix with adequate cohesiveness. High molecular weight polymers are normally needed in order to develop cohesive strength and coalescence and produce a strong film structure (Rhim & Ng, 2007). The film properties, such as density and compactness, porosity and permeability, flexibility and brittleness are influenced by the degree of cohesion. The mechanism for producing polysaccharide films is based on cleaving the polymer segments by using alkali and reforming the polymer chain into a film matrix or gel by evaporating the solvent, thus creating hydrophilic and hydrogen bonding (Bourtoom, 2008; Rhim & Ng, 2007). Xylan-based films have been explored targeting food packaging applications, although these have been limited due to the high water permeability of these films. This is an advantage though for agricultural applications if their disintegration can be controlled. In this case, a disadvantage of polysaccharide-based films is their low elasticity (Bourtoom, 2008; Gordobil, Egüés, Urruzola, & Labidi, 2014; Rhim & Ng, 2007), whichcauses brittle films. To overcome the low elasticity of polysaccharide films, a plasticiser can be used to modify its physical properties. Plasticisers are low molecular weight agents such as glycerol and sorbitol, which are used to increase the flexibility and processibility of the films (Vieira, da Silva, dos Santos, & Beppu, 2011). The addition of plasticisers decreases the crystalline regions as a result of an increase in the free volume of the polymer structure as well as decreases the glass transition temperature.

Potential applications of polysaccharides in the agriculture and horticulture sectors include the development of polymeric films which can be used for the controlled release of fertilisers into the soil. The advantage of a polymer-based fertilisers is that the active ingredients (fertiliser) are released at a much longer period of time, as compared to simple, non-conditioned fertilisers, which are released very quickly into the soil. Slower release is advantageous for the growth of plants. Fertiliser coating, also called fertiliser packing, is a universal controlled-release method. The fertiliser is encapsulated by a coating material and dissolved slowly into the soil. Slow-release fertilisers are classified into four types: (1) low-soluble inorganic materials, such as metal ammonium phosphates; (2) low-soluble and chemically or biologically degradable materials, such as urea–formaldehyde; (3) relatively soluble materials that decompose gradually in soil and (4) water-soluble fertilisers controlled by physical barriers (e.g. coating and matrix formation) (Jin et al., 2013).

Fertilisers can be coated with various materials. The coating materials should be cheap and show significant coating properties (Han, Chen, & Hu, 2009). Furthermore, they should be tested for degradation in the soil since the type of coating would have an effect on the encapsulated elements. The fertiliser diffuses into the soil through the pores or is released as a result of the coating's erosion and degradation. Recently, super-absorbent polymers (SAP) have been developed such as hydrophilic polymers with a crosslinked three-dimensional network structure; examples include the use of hydrogels in diapers and incontinence garments. The degree of crosslinking is defined as the number density of junctions joining the chains into a permanent structure (Mark, 2007). Such materials have also been utilised to improve the soil's physical properties in agriculture including its water-holding capacity, the nutrient retention of sandy soils, the water permeability and the structure of soil (Jin et al., 2013). Early super-absorbents were made from chemically modified starch and cellulose and other polymers such as polyvinyl alcohol (PVA) and polyethylene oxide (PEO), all of which are hydrophilic and have a high affinity for water. When lightly cross-linked, chemically or physically, these polymers become water swellable but not water-soluble. However, a drawback of using superabsorbents is the fact that they are not easily degradable in the soil. Therefore, extracted hemicellulose (e.g. xylan) from agri-food residues (e.g. sorghum by-products) can potentially substitute the usage of super-absorbents by itself or by modification.

Taking into account the current literature, this research area has not been fully explored although some preliminary works have been reported (Alekhina, Mikkonen, Alén, Tenkanen, & Sixta, 2014; Gordobil, Egues, Urruzola, & Labidi, 2014; Heikkinen et al., 2013; Mikkonen et al., 2012; Simkovic, Gedeon, Uhliariková, Mendichi, & Kirschnerová, 2011). The latest published articles regarding hemicellulose based films from various agricultural sources, including their preparation and the applied conditions are summarised in Table 2-7. In order to evaluate the potential application of hemicellulose-based films, key aspects that need to be investigated include the films' thickness, water vapour permeability, solubility, mechanical properties, thermal behaviour, and crystallinity. Moreover, soil studies should also be conducted to demonstrate the applicability of this type formulations for the release of fertilisers.

Table 2-7 Research works on the development of hemicellulose based films using hemicellulose derived from agricultural residues

Material	**Film preparation process**	**Process conditions**		**Drying method**	**References**
		Temperature (°C)	**Time**		
Xylan from beech holocellulose	Dissolved 0.1-2 g samples in 5-30 ml dH_2O.	60	6 h	Room temperature	(Šimkovic, Gedeon, Uhliariková, Mendichi, & Kirschnerová, 2011b)
Arabinoxylan from rye endosperm	Heated and homogenized using an Ultra-Turrax at 9,500 rpm	60	10 min	Dried at 23 °C and 50% relative humidity (RH).	(Mikkonen et al., 2012)

Sulfation of xylan from beech sawdust holocellulose	Samples (0.1–2 g) mixed with 5–30 ml of deionized water	100	Heated up to boiling point.	Room temperature	(Šimkovic et al., 2011b)
Wheat Arabinoxylan (WAX)	WAX dissolved in deionized water (20 g/l)	90–95	15 min	Dried at 23^{o}C and 50% RH.	(Heikkinen et al., 2013)
Arabinoxylan extracted from maize bran	Dissolved 6 g of AX in 100 ml osmosed water	75	40 min	Dried at a ventilated chamber for ~4 h at 40% relative humidity and 30 °C.	(Péroval, Debeaufort, Despré, & Voilley, 2002)
Arabinoxylan from wheat flour	Dissolved 3g of AX in 100 ml deionized water	95	30 min	Dried for 5 h in a ventilated cupboard at 40 ^{o}C and 30% RH.	(The, Debeaufort, Voilley, & Luu, 2009)
Distiller grain-hemicelluloses (DG-HC)	DG-HC dissolved in water (10%)	90	30 min	Room temperature	(Xiang et al., 2014)
Xyloglucan from tamarind seeds	XG mixed dissolved in deionised water	60	125 min	Dried at 35^{o}C 50%RH	(Bergstr̈m, Salmén, Kochumalayil, & Berglund, 2012)
Xylan from birchwood (X) and wheat gluten (G)	10% (w/w) of composite mixture dispersed in water containing 2% (w/w) glycerol.	70	30 min	80 ^{o}C, RH 35% and 20 ^{o}C, RH 35%	(Kayserilioğlu, Bakir, Yilmaz, & Akkaş, 2003)
Water-extractable hemicellulose (WEH) fractions from rye bran	Freeze-dried WEH fractions were mixed into water at a concentration of 10 g/l.	40	24 hour	23 ^{o}C and RH 50%	(Sárossy et al., 2013)

2.4.2. Bio-degradable foams for packaging applications (composite foams)

Globally, in the last few years, more environmentally friendly packaging has emerged in the packaging market in an effort to reduce the negative environmental impact of synthetic polymers, such as polyethylene (PE), polypropylene (PP), polystyrene (PS), poly vinyl chloride (PVC) and polyethylene terephthalate (PET). The material with the highest potential to replace synthetic polymers is starch. As such, several studies have been conducted to develop starch-based biodegradable foams. Biodegradable foam (or biofoam) is an alternative for extruded polystyrene foam; a commercial example for the latter includes Styrofoam which is produced from Dow Chemicals. Biodegradable foams can be used for packaging applications (e.g. food trays) but also in combination with biodegradable fillers, such as lignin, for the production of biocomposites; the latter has applications in a variety of industries, including food, agricultural, automotive and chemical industries.

Usually a number of additional components are added in the formulations including fibre and plasticisers, as starch itself is brittle and water sensitive and thus needs to be modified or blended with other components (Soykeabkaew, Thanomsilp, & Suwantong, 2015). In order to enhance its water resistance, starch can be modified using chemical components such as polylactic acid (PLA), long chain succinic anhydrides, latex and other hydrophobic polymers. Previous studies have also used natural ligno-cellulosic fibers, aspen, soft wood, wheat bran, and Kraft cellulose to reinforce starch foams (Table 2-8 presents some of these studies). To this end, sorghum by-products are potentially suitable materials for the development of starch-based biofoams as they have a high content in cellulose which is needed to exert strong mechanical properties and water resistance to biofoams, and also contain hemicellulose and lignin. Key factors in developing biodegradable foams include the selection of input ingredients (starch, blend, additives/fillers, etc.) and the appropriate production method (Soykeabkaew et al., 2015) which can be modified in order to design foams for particular applications. More specifically, key aspects that need to be investigated during the product and process development include the influence of the fibre type and the production method on the three-dimensional structure of the biofoams, their properties (crystallinity, density, hardness, morphology structure, water absorption, and mechanical properties) as well as their shelf life. A filler such as lignin which has antioxidant, antimicrobial properties could also potentially be added into the formulation to increase the shelf life of the biofoam.

Table 2-8 Research works on the development of biodegradable foams for packaging applications (food trays)

Foam formulation	Processing	Outcomes	References
Cassava starch, cellulose fibre, sunflower protein, protein isolate,	thermopressing	The addition of fibre improved mechanical properties and reduced the post-pressing moisture content, but increased the water absorption capacity of the material.	(Salgado, Schmidt, Molina Ortiz, Mauri, & Laurindo, 2008)
Corn starch, aspen fibre, guar gum, magnesium stearate, water.	thermopressing	The increased addition of fibre into the batter resulted in longer baking times, improved the tensile strength of the trays.	(Lawton, Shogren, & Tiefenbacher, 2004)
Corn starch, Aspen fibre, guar gum, magnesium stearate, water.	thermopressing	The addition of kraft, zein, and gluten increased the compressive strength and water absorption of trays.	(Kaisangsri, Kerdchoechuen, & Laohakunjit, 2014)
cassava starch, zein, protein gluten, soy protein, kraft fibre by weight of starch, palm oil was mixed with Tween® 20 (w/w of palm oil content).	thermopressing	The addition of fibre increased the tensile strength whereas the addition of monostearyl citrate improved the water resistance.	(Shogren, Lawton, & Tiefenbacher, 2002)
Acetylated high amylose corn starch, Monostearyl citrate, wood fibre, poly vinyl alcohol.	thermopressing	The increase of cellulose decreased the density and the tensile strength.	(Schmidt & Laurindo, 2010)

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Chapter 3: Fractionation of Sorghum (*Sorghum bicolor* (L.) Moench) By-products and Characterisation of Isolated Fractions

Abstract

Sorghum by-products like bran, stalks, and panicles are currently being discarded and this often has a significant impact on the environment. However, there are some potential valorisation strategies that could be developed in agriculture and food packaging sectors to minimise the impact. The aims of this study were to develop a multi-step process based on an initial alkali extraction for the fractionation of sorghum by-products and to characterise them with regards to their physicochemical properties. A sequential alkaline extraction with varying concentrations of NaOH (0.75, 1, and 1.5 M) was applied, resulting in the generation of three fractions, those of residue (R), hemicellulose (H), and alkali lignin (L). The fractionation of the sorghum stalks with 1 M NaOH at 50oC for 3 hours obtained ~64% (w/w) glucose in residue fraction. This corresponded to the cellulose and 52 % (w/w) of xylose which was predominant in the hemicellulose fraction of the stalks and ~2% (w/w) lignin. This treatment recovered 100% of xylose from the sorghum stalks. On the other hand, in the bran, the fractionation with 0.75 M NaOH at 50oC for 3 hours obtained ~40% (w/w) glucose in R fraction which corresponded to the cellulose. Approximately 76% of the glucose was available in hemicellulosic fraction reflecting the presence of mixed-linkage glucan, while nearly 2% (w/w) lignin was extracted. This treatment recovered 100% of the glucose from the sorghum bran. Fourier Transform Infrared (FTIR) and Nuclear Magnetic Resonance (NMR) spectroscopy analysis of the sorghum bran revealed that the predominant hemicellulosic compound was xyloglucan (XG) while in the stalk low branching xylan was predominant. Thermal analysis determined by Differential Scanning Calorimetry (DSC) and X-Ray Diffraction (XRD) showed that the hemicellulose fraction exhibited a lower thermal stability compared to that of cellulose. This is probably due to the lack of crystallinity of the former. Stalk-derived lignin fraction contained more phenolic compounds, (mainly p-coumaric and ferulic acid) and had a higher lignin content and antioxidant capacity compared to bran-derived lignin fraction. A mass balance analysis determined that the extraction process was effective since more than 80% material from the initial sorghum by-products could be recovered. Overall, this study provides a comprehensive understanding of the chemistry of sorghum by-products, the added value after fractionation, their properties, and the potential utilisation that could play an important role in agro-industry.

Keywords: Sorghum stalk, sorghum bran, cellulose, hemicellulose, lignin, alkaline extraction

3.1. **Introduction**

The depletion of fossil fuels, global environmental issues and the increasing interest for utilising renewable resources as feedstock are the main drivers for the valorisation of agricultural food waste and by-products. Cereal grain by-products such as straw account for approximately 3000 million tons per year worldwide (Sun & Ren, 2010), with a significant proportion being produced in developing countries, such as India and Indonesia. In Indonesia, agricultural waste and by-products are estimated to be approximately 147 million tons of biomass per year, of which the major cereal plantations are rice, maize and sugar cane (Tajalli, 2015). Sorghum (Sorghum bicolor (L.) Moench) represents the fifth most important cereal in the world after maize, rice, wheat and barley (Taylor & Duodu, 2010). As reported by the Indonesian Agency for Agricultural Research and Development (IAARD), Ministry of Agriculture, Republic of Indonesia, sorghum has become an important crop in recent years and its plantation area has continued to increase on an annual basis (Sumarno, Damardjati, Syam, & Hermanto, 2013).

In Indonesia, the main types of sorghum that are planted are the sweet sorghum varieties which are mainly cultivated in Java, West Nusa Tenggara, and East Nusa Tenggara (Suryaningsih & Irhas, 2014). The superiority of sweet sorghum over other types of sorghum and cereal crops such as grass sorghums and grain is that its stalk contains higher amounts of sugar and thus it can be used for the simultaneous production of food and energy products. Furthermore, sweet sorghum is adaptable to a wide range of climatic and soil conditions such as drought, high-temperature, stress, and salt as well as providing a higher yield of biomass per hectare (Almodares & Hadi, 2009; Almodares, Hadi, & Kharazian, 2009; Almodares et al., 2008). Among the sweet sorghum varieties, Kawali and Numbu are the most popular ones cultivated in Indonesia. According to the Indonesian Cereals Research Institute (ICERI), Indonesian Ministry of Agriculture, these sweet sorghum varieties have a short harvesting time of around 100-110 days, a very high carbohydrate content (>80%), and a low tannin content (0.18-0.21%) (H Subagio & Aqil, 2013). Moreover, their production generates approximately 4 to 10 million tons of biomass (including bran, stalk and panicles) per year. In Indonesia, sorghum biomass is usually used for feed and for land spreading but not so much for electricity production, and in some cases they are either discarded or burnt (Sumarno et al., 2013). However, particulate combustion from burning biomass is associated with health and environmental issues (Gadde, Bonnet, Menke, & Garivait, 2009; Sun & Ren, 2010). Therefore, developing strategies for the valorisation of sorghum by-products, such as bran, stalks and panicles is a priority in order to minimise the environmental impact of the current disposal methods as well as to generate additional economic value. Sorghum bran, stalks and panicles are lignocellulosic materials that contain high amounts of cellulose, hemicellulose, lignin, and polyphenols. Its application could potentially be found in various sectors including the food, agricultural and packaging.

Bran is the main by-product of the sorghum milling operation, accounting for 10-11.5% of the whole grain (Ayala-Soto et al., 2015; Corredor et al., 2007). It is derived from the decortication process which separates the kernel of sorghum and the aleurone layer to obtain high-quality food products such as flour, kafirin or products for other purposes, such as livestock feed (L. S. Da Silva & Taylor, 2004; Hoseney Carl, 1994). The stalk, the above-ground part of the sorghum plant that remain after the leaves and the panicles are removed and pressed to obtain sugar juice. On the other hand, the empty panicles are considered a waste and are normally burnt although they can be used as natural fertilisers through land spreading. The dried stalks are obtained by squeezing the juice out using a sugar cane roller mill, drying them in an oven and then milling them down into dried stalks. The dried stalks represent around 30% of the fresh stalks (Thanapimmetha et al., 2011) and are an important biomass source that can be used for bioethanol production (Yu, Zhang, Zhong, Zhang, & Tan, 2012).

Hemicelluloses are heterogenous polysaccharides present in the cell wall of the plant which are linked with cellulose through hydrogen bonds. Hemicelluloses are connected with lignin by a covalent bonding of α-benzyl ether linkages, and also with hydroxycinnamic acids and acetyl units through ester linkages, in Gramineae plant cell walls (Sun & Ren, 2010). Hemicelluloses have a relatively low degree of polymerization, typically between 80 and 200 units (Ghoneum, Badr El-Din, Ali, & El-Dein, 2014). The hemicellulosic fractions in cereals may consist of arabinoxylans, as in the case of barley husks (Krawczyk, Persson, Andersson, & Jo, 2008), sweet sorghum stem (Li et al., 2015; Sun, Wen, Ma, & Sun, 2013), sorghum flour (Nandini & Salimath, 2001),wheat spent grains and wheat bran (Mandalari et al., 2005). They may also consist of galactoarabinoxylans and arabinogalactans as in the case of sorghum flour (Nandini & Salimath, 2001); glucuronoarabinoxylans as in the case of sorghum grain (Verbruggen, Beldman, Voragen, & Hollemans, 1993), xyloglucan as in the case of rice bran (Shibuya & Iwasaki, 1985) and β-glucans as in the case of oat and barley grains (Hoseney Carl, 1994). In comparison to other cereals, however, there is relatively little information on the hemicellulose content of sorghum by-products, such as bran, stalks and panicles. Hemicelluloses are the second most abundant lignocellulosic material in plantsafter celluloses. The structure of hemicelluloses is unique in terms of the degree of polymerisation, crystallinity, and complexity components in the cell wall (Sun & Ren, 2010), which brings a great impact on the physicochemical properties of the polymer and consequently its applications. In addition, the isolation of this material is challenging due to the strong linkage to other polysaccharides in the cell wall. Hemicelluloses form hydrogen bonds with cellulose, covalent bonds (mainly a-benzyl ether linkages) with lignin and ester linkages with acetyl units and hydroxyl cinnamic acids. In recent years, the applications of this material include as a prebiotic, a thickening agent, an improver of dough properties in bread processing and in medicine as a hypocholesterolemic agent, suppressing blood pressure, binding hydrophobic mutagens in diet, and inhibiting the growth rate of tumours (Ebringerová & Heinze, 2000). Therefore, given their potential and the numerous characteristics and applications of hemicelluloses in food and non-food industries, many scientists and industries have been attracted to study them further.

The variations observed in the chemical composition of cereal by-products reflect the differences in the source of the raw material (crop variety, primary processing) and the extraction methods used to separate the various components. This study focuses on the development of an efficient process for the separation of sorghum processing by-products, namely bran, stalks and panicles, into various fractions. It also emphasizes on the characterisation of their physicochemical properties, with the view to identify potentially added-value applications in the food and agricultural sectors.

3.2. Materials and Methods

3.2.1. Materials

The sorghum varieties used in this study were Sorghum bicolor var. Kawali and var. Numbu. The sorghum bran, which constitutes a milling by-product, was obtained from the Indonesian Cereals Research Institute, whereas the sorghum stalks and panicles were obtained from the farm of a national Indonesian company, [PTPN XII (PERSERO)]. The bran was screened through a 60 mesh size sieve. The leaves and the panicles were then removed from the stalks. After removal of the leaves, heads and the squeezing of the juice using a roller mill, the panicles and stalks were dried under sunlight and then cut into small pieces. They were then grounded and screened through a 60 mesh size sieve. All chemicals used were of analytical grade including ethanol (95%), hydrochloric acid (HCl) (37%), H_2SO_4 (72%, v/v), CaCO3 powder (98%, ≤50 μm particle size) and were purchased from Sigma Aldrich (UK). Sodium hydroxide (≥97%, pellets) was purchased from Fischer Scientific (UK).

3.2.2. Methods

3.2.2.1. Proximate Analysis

Protein and lipid analyses were performed using standard AOAC methods (Nielsen, 2010). More specifically, the protein content was measured using the Kjeldahl method and by multiplying the nitrogen (N) content by 5.7 (Mosse, 1990). The lipid content was determined gravimetrically on the Soxhlet method (Horwitz, Willam and latimer, 2005). Briefly, 1 g of sample was placed into pre-weighted, pre-dried extraction thimbles which were closed using a fat-free piece of cotton. The thimbles were then placed into the Soxhlet extractor apparatus and the lipids were extracted with petroleum ether for 4 hours. Samples were dried overnight at 100 ^oC, cooled in a desiccator and then weighed.

The ash content was measured by weighing 5 g of sample into a pre-weighted crucible and placing it in a furnace at 600 ^oC for 4 hours. Samples were cooled down in a desiccator, and the weight of the samples was measured to calculate the ash content (Escarnot, Aguedo, Agneessens, Wathelet, & Paquot, 2011).

3.2.2.2. Carbohydrate Analysis

A carbohydrate analysis was performed according to the Laboratory Analytical Procedure (LAP) protocol by the National Renewable Energy Laboratory (NREL) (Sluiter et al., 2004). Approximately 300 mg of dried samples were mixed with 3 mL (72%, v/v) of H_2SO_4 and incubated at 30 ^oC for 1 hour. The mixture was then diluted to 4% (v/v) H_2SO_4 with deionised water and autoclaved at 121 ^oC for 30 minutes. Samples were left to cool down and filtered. The pH of the supernatants were adjusted to pH 5.0 using anhydrous solid CaCO3 (1-2 g) and the supernatants were filtered through 0.2 μm filters into 2 mL vials. Monosaccharide analysis was carried out by High Pressure Liquid Chromatography (HPLC) using an Agilent Infinity HPLC system (Agilent 1200 series), equipped with an Aminex HPX-87H analytical column at 65°C and a refractive index (RI) detector. The mobile phase used was 0.005 M sulphuric acid and the flow rate was set at 0.6 mL/min. For sugar quantification, known concentrations of sugars and sugar acids (glucose, xylose, arabinose, uronic acid) were used to construct calibration curves.

3.2.2.3. Lignin Analysis

The acid soluble lignin content of the samples was determined according to a published protocol (Sluiter et al., 2004). Approximately 50 mL of the acid hydrolysed solution produced from the analysis of monosaccharides were vacuum filtered and the absorbance of the aliquot was measured at 320 nm using a spectrophotometer. The acid soluble lignin (ASL) was then calculated and determined by Sluiter et al.(2004) as:

ASL (%)= [UVabs x Volume filtrate x Dilution / ε x ODWsample × Pathlength] X 100,

where:

UVabs = average UV-V is absorbance for the sample at an appropriate wavelength

Volume hydrolysis liquor = volume of filtrate, 86.73 mL
Dilution = (Volume sample + Volume diluting solvent)/ Volume sample
ε = Absorptivity of biomass at specific wavelength (see table below)
ODWsample= weight of sample in milligrams
Pathlength=pathlength of UV-V is cell in cm

3.2.2.4. Starch and Mixed β-glucan Analysis

The starch content was measured enzymatically using the total starch test kit from Megazyme, which is based on the use of thermostable α-amylase and amyloglucosidase. This method originally came from the AOAC (Official Method 996.11) and AACC (Method 76.13.01) methods (Horwitz, Willam and latimer, 2005). Mixed-glucan analysis was measured enzymatically using the β-glucan test kit from Megazyme which is determined by the use of lichenase and β-glucosidase. This approach is based on the methods of AOAC (Method 995.16), AACC (Method 32-23.01), EBC (Methods 3.10.1, 4.16.1 and 8.13.1), ICC (Standard No. 166), RACI (Standard Method) and CODEX (Type II Method).

3.2.2.5. Extraction of Hemicellulose

The extraction of hemicelluloses from dried sorghum stalks and bran was performed under alkaline conditions according to previous protocols with some slight modifications (Xiang et al., 2014; Xiao et al., 2001). Briefly, 5 g of dry-milled sample were mixed with 100 mL NaOH solution (0.75, 1.0 and 1.5 M) at 50 ^{o}C, in a 1:20 solid to liquid ratio. The mixture was maintained at 50 ^{o}C and stirred at 200 rpm for 3 hours, followed by centrifugation at 17.105 x g for 20 minutes. The collected residue was washed with deionised water and freeze-dried prior to further characterisation. The alkali-soluble supernatant was adjusted to pH 5.5 using 6 M HCl and concentrated to about one-third of its original volume in a rotary evaporator. Then, three volumes of ethanol (95%, v/v) were slowly poured into the solution under constant stirring. The precipitated solid was separated and washed with 95% ethanol using a filter paper and was designated as alkali-soluble hemicellulose. The ethanol solution was designated as an alkali-lignin fraction and was collected as a solid after evaporating the ethanol. The fractionation process is depicted in Figure 3-1.

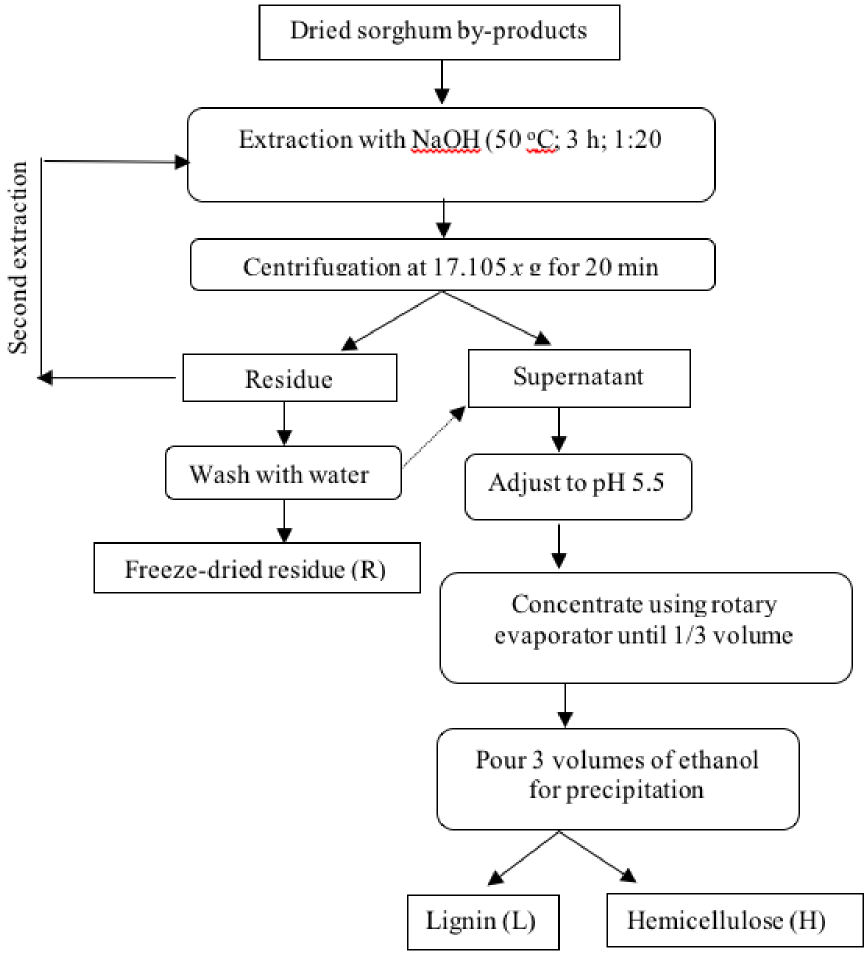

Figure 3-1 Process scheme for the fractionation of sorghum by-products.

3.2.2.6. FT-IR Spectroscopy

The functional groups of the different fractions after extraction were analysed using a Fourier Transform Infrared (FTIR) equipped with a universal Attenuated Total Reflectance (ATR) scanning accessory to remove the need for transmission cells and KBr pellets when performing measurements on liquid, semi-solid and solid materials. The Atmospheric Vapor Compensation (AVC) software was used to remove spectral interferences caused by water and carbon dioxide. The spectra were obtained with a FT-IR spectrophotometer (Perkin Elmer Spectrum 100), and were recorded in the range of 4000-600 cm^{-1} at 4 cm^{-1} resolution, with 16 scans per sample.

3.2.2.7. NMR Spectroscopy

10 mg of stalk-derived hemicelluloses was dissolved in 0.75 ml DMSO-D_6, and then transferred into 5 mm NMR tubes. The 1D NMR-1H spectra was recorded on a Bruker DPX 400 MHz NMR spectrometer (Bruker, Germany) operating at 400.07 MHz for proton observation at 70 ^{o}C. Meanwhile, solid state ^{13}C (high-power decoupled) NMR spectra of bran-derived hemicellulose were obtained on a Bruker Avance III 500 MHz spectrometer (Bruker, Karlsruhe, Germany) using the cross polarized magic angle spinning (CP-MAS) technique which contained a 90-100 ramp contact pulse of 400 µsec in order to reduce the measuring time due to long relaxation times as well as for signal/noise enhancement. Experiments were carried out with a standard bore Bruker MAS probe. All the MAS spectra were obtained at an ambient temperature with a spinning speed of 10 kHz to eliminate the broadening from dipole–dipole coupling and chemical shift anisotropy.

3.2.2.8. X-ray Diffraction

A powdered X-ray diffractometer (Bruker D8 Advance, Germany) was used to record the residue and hemicellulosic fractions from the sorghum by-products diffractogram at 25 °C. The X-ray source was Cu Kα radiation at 40 kV and 35 mA ($\lambda = 1.54$ Å). The samples were mounted on a sample holder and the pattern was recorded in reflection mode at an angle of 2θ over a range of 5.000° to 80.030° at a speed of 10°/minutes. The crystallinity index (Cr.I) was calculated based on Ciolacu, Ciolacu and Popa (2011) as follows:

$$\text{Cr.I. (\%)} = (Sc / St) \times 100,$$

where: Sc – area of the crystalline domain, St – area of the total domain

3.2.2.9. Thermal Analysis

Structural and thermal characterisation of residue and hemicellulose fractions were carried out by differential scanning calorimetry (DSC) (Perkin Elmer DSC 8000, USA) with the following parameters. Approximately 5-10 mg sample was measured for each pan. An empty pan was used as a reference. Both the samples and reference were heated at a heating rate of 10oC/min from -50 to 250°C using nitrogen flushing.

3.2.2.10. Phenolic Acid Analysis

Phenolics were analysed according to a protocol optimised for bioactive components in small grain cereals (Li, Harflett, Beale, Ward, & Shewry, 2009). Approximately 0.025 g samples were extracted using 80% methanol for 10 minutes in a sonic bath at room temperature to obtain free phenolic compounds. Following this sonication, the samples were centrifuged for 10 minutes at 17,105 x g (this step was repeated 3 times). The supernatant was evaporated to dryness (a yellow residue was obtained) using speedvac (Eppendorf, Germany). 100 µl of 2% (v/v) aqueous acetic acid was added to the dry samples containing free phenolic acids, and centrifuged for 10 minutes at 17,105 x g. Conjugated phenolic acids analysis was conducted by combining the dry samples and an added 800 µl of 2 M NaOH solution which was mixed for 30 seconds, allowing the hydrolysis to take place for 4 hours. Later, each sample was acidified by thoroughly mixing it with 160 µl of 6 M HCl to pH 2.

The esterified phenolic acids were derivatised by mixing the extract with 500 µl ethyl acetate, and then the samples were centrifuged for 10 minutes at 17105 x g (this step was repeated 3 times). Subsequently, the upper layer was removed to a clean Eppendorf tube and evaporated to obtain dry samples. 100 µl of 2% (v/v) aqueous acetic acid was then added to the dry samples, and centrifuged at 17,105 x g for 10 minutes and the supernatants were collected into HPLC vials. Meanwhile, 1600 µl of 2 M NaOH was added to the residue that was left after removing the free and conjugated phenolic acids in order to obtain bound phenolics, and then the samples were mixed for 30 seconds and incubated at room temperature for 4 hours for the hydrolysis to take place. Subsequently, the sample was centrifuged for 10 minutes at 17,105 x g and the collected supernatant was acidified by thoroughly mixing it with 160 µl of 6 M HCl to pH 2. Derivatisation of the phenolics was conducted by mixing the samples with 500 µl ethyl acetate, and then centrifuged for 10 minutes at 17,105 x g (this step was repeated 3 times). Then, the upper layer was removed into a new Eppendorf tube and evaporated using speedvac (Eppendorf, Germany). 100 µl of 2% (v/v) aqueous acetic acid was then added to the dry samples, which were centrifuged at 17,105 x g for 10 minutes, and the supernatant collected into HPLC vials.

HPLC analysis was performed with a 1260 Infinity HPLC system (Agilent Technologies, Waldbronn, Germany) equipped with a diode-array detector (DAD) using a Zorbax C18 (250 x 4.6 mm i.d., particle size 5 µm, Agilent) column at 30 oC. The mobile phase consisted of acetonitrile (solvent A) and 1% (v/v) acetic acid in water (solvent B), and the linear gradient at a flow rate of 1 mL/min was programmed as follows: (0-29 min: A 0%, B 100%); (30-49 min: A 15%, B 85%); (50-54 min: A 50%, B 50%); (55 min: A 70%, B 30%). The injection volume was 20 µL; the duration of the analysis was 55 minutes. The absorbance was measured at 320 nm. ρ-coumaric acid and trans ferulic acid were used as standards.

3.2.2.11. Antioxidant Capacity

The scavenging activity of phenolic compounds against DPPH radicals was assessed according to the method used by Blois (1958) and Khalaf, Shakya, Al-Othman, El-Agbar, and Farah (2008) but with some modifications. First, 0.2 mL of the lignin fraction (L) was mixed with 2 mL of 2 mM DPPH-methanol solution. Then the solution was incubated for 30 minutes at 25◦C in the dark and the decrease in absorbance at 517 nm was measured. Finally, 0.2 mL water mixed with 2 mL of 2 mM DPPH-methanol solution was used as the blank.

The percentage of inhibition was calculated using the following equation:

$$\text{Inhibition (\%)} = (A_o - A1)/A_o \times 100,$$

where Ao = absorbance of the blank and A1 = absorbance of the sample

3.2.2.12. Statistical Analysis

A one-way analysis of variance (ANOVA) was used to compare the mean differences of the components of various sorghum by-products. Duncan's Multiple Range Test was applied whenever a difference was detected among the factors ($p < 0.05$). The SAS 9.1.3 software was used for the statistical analysis. Results are presented as mean ± standard deviation.

3.3. Results and Discussion

3.3.1. Chemical Composition of Sorghum by-products

Compositional analysis was carried out to characterise the sorghum by-products that were used in the study (Table 1). Both varieties of the sorghum bran contained considerable amounts of starch (25-34%, w/w) and protein (12-13%, w/w), which was comparable to previous studies reporting the existence of 33% w/w starch (Qiu, Yadav, & Yin, 2017) and 9.95% w/w of protein (Ayala-soto, Serna-saldívar, Welti-chanes, & Gutierrez-uribe, 2015). The amount of starch and protein may vary depending on the species, or the genetic and environmental variations within the same species (Serna-Saldivar, 2010) as well as the de-branning process used. The total carbohydrate content of the two sorghum bran varieties was 61-68%. The Kawali bran had a higher xylose and arabinose content (15.4% and 10.4% respectively) compared to the Numbu bran (4.5% and 3.5% respectively), indicating a higher amount of arabinoxylans in the former. On the other hand, the Kawali bran had a lower glucose content (14.94%) compared to the Numbu Bran (23.6%) indicating a lower amount of cellulose and mixed β-glucan. The lignin content in both varieties was very low (< 1.5 w/w) compared to a previous report of 4% (Ayala-soto, Serna-saldívar, Welti-chanes, & Gutierrez-uribe, 2015). The lipid content in the bran was approximately 10% for both varieties. This result is higher than that reported by Qiu, Yadav, & Yin (2017) which was 5% w/w. Lipids are minor components of the sorghum bran and most of them are deposited in the germ although they do have an important role in the stability, organoleptic properties, shelf life and processing of the bran (Serna-Saldivar, 2010). The ash content was approximately 4% w/w for both bran varieties.

Ash consists mainly of minerals such as iron, and zinc, magnesium, manganese, cobalt and copper, as reported by Serna-Saldivar (2010).

The stalks of both the sorghum varieties contained small amounts of starch (2.39 to 3.85%) and lignin (2% w/w), whereas the glucose content was 28.83 to 29.43%, and the xylose and the arabinose were 20.88 to 21.54% and 2.19 to 3.48%, respectively, indicating the presence of cellulose and mixed linkage-glucan (~30% w/w) and hemicellulose polymers such as arabinoxylan and xyloglucan (~21-22%w/w) (Table 3-1). The cellulose and hemicellulose contents of the stalks were comparable to the data reported by Matsakas, Rova, & Christakopoulos (2014), i.e. 20% of cellulose, and Sun, Wen, Ma, & Sun (2013), i.e. 21.8% of hemicellulose (w/w). However, She et al., (2010) reported a higher hemicellulose content 31.4%, which was most likley due to de-waxing and de-lignification treatment prior to extraction hemicelluloses process, which removed liposoluble compounds cell wall (waxes, pigments, and chlorophyll) (Marina, Andrea, & López-galán, 2018). Therefore, the fractionation lignin and hemicelluloses will obtain a higher yield of each compound. De-waxed raw material was de-lignified in order to ensure the removal of most of the lignin. In this study, dewaxing and delignification treatments were not conducted due to the low amount of lignin in the tested cultivars. It can be deduced from the data that the hemicellulose contained less amount of arabinoxylan compared to the bran. The mixed-linkage glucan including xyloglucan as water-unextractable hemicellulosic fraction content is likely to be higher than reported by Verbruggen, Beldman, Voragen, & Hollemans (1993) who stated that mixed linkage glucans were β-(1→3)- and β (1→4)-linked- D-glucose-units as well as (1→4), α-(1→6)-D-glucans which were isolated from sorghum husk. The amounts of these major non-starch polysaccharides (NSP) in cereal grains would vary depending on the cereal species, the genotype of the individual cereal species, environmental conditions during grain development, maturation, and distribution in cereal kernels (Collins et al., 2010).

Table 3-1 Compositional analysis of sorghum by-products (%, g/100 g dry weight)

Components	Bran		Stalk		Panicles	
	var. Kawali	var. Numbu	var. Kawali	var. Numbu	var. Kawali	var. Numbu
Moisture	5.18±0.04^{b}	6.65±0.14^{a}	4.13±0.02^{a}	4.71±0.06^{a}	2.44±0.04^{a}	2.13±0.06^{a}
Ash	3.77±0.05^{b}	4.34±0.03^{a}	4.41±0.07^{a}	5.44±0.09^{b}	3.81±0.17^{a}	3.84±0.07^{a}
Protein	12.05±0.10^{a}	12.99±0.08^{a}	1.88±0.14^{a}	1.28±0.14^{b}	12.90±0.11^{a}	14.12±0.14^{a}
Fat	10.43±0.07^{a}	9.81±0.08^{a}	4.78±0.19^{a}	5.12±0.29^{a}	9.77±0.20^{a}	8.53±0.16^{b}
Total Carbohydrate	77.49±0.13^{a}	78.47±2.65^{a}	56.67±1.08^{a}	55.85±0.54^{a}	58.36±0.10^{a}	64.66±0.47^{a}
Starch	25.14±1.32^{a}	34.20±0.23^{a}	3.85±0.09^{a}	2.39±0.36^{a}	18.36±0.12^{a}	27.37±1.03^{b}
Arabinose	10.36±0.17^{a}	3.48±1.20^{a}	2.19±0.05^{a}	2.03±0.04^{a}	7.33±0.06^{a}	5.91±0.04^{b}
Xylose	15.36±0.31^{a}	4.49±0.02^{b}	20.88±0.45^{a}	21.54±0.11^{a}	14.04±0.04^{a}	11.86±0.06^{b}
Glucose*	14.94±1.7^{b}	23.60±1.69^{a}	29.43±0.68^{a}	28.83±0.04^{a}	12.67±0.04^{a}	8.51±0.67^{b}
Uronic acid	11.69±1.75^{a}	12.70±0.52^{a}	0.32±0.25^{a}	1.06±0.12^{a}	5.96±0.29^{a}	11.01±0.32^{a}
Lignin	0.92±0.14^{a}	1.44±0.01^{a}	1.89±0.14^{a}	1.67±0.01^{a}	1.58±0.03^{a}	1.77±0.19^{a}
Total	109.84±0.15^{a}	113.7±2.54^{a}	73.76±1.23^{a}	74.07±0.81^{a}	88.86±0.20^{a}	95.05±0.32^{a}

Mean ± standard deviation; Values with the same letters in the same rows did not differ significantly, based on the Duncan's new multiple range test at a 95 % significance level ($p = 0.05$); *The glucose could be derived from cellulose, mixed linkage β-glucans and xyloclucan

The panicle is the part which encloses the grains in the sorghum plant. Panicles have considerable amounts of starch (18-27%), hemicellulose (16-21%), cellulose and mixed linkage-glucan (8.5-12%), protein (12-14%), and lignin (1.58-1.77%) (Table 3-1). Overall, the composition of panicles is similar to that of bran, and as hemicellulose contains significant amounts of arabinose, it indicates the significant presence of arabinoxylan. No information could be found in the literature on the composition of panicles. The main possible reason for this is that higher value applications of panicles has not really been considered thus and the main disposal route of this by-product is incineration in the field. The compositional analysis demonstrated that there was significant potential for the valorisation of panicles leading to the production of added value products. However, the rest of this study focused on the sorghum bran and stalks, as currently there is significant activity and interest in Indonesia in these by-products. Based on the results from the two varieties, the Kawali bran was selected for further research in this study as the bran contains higher amounts of arabinoxylan and is also a more popular cultivar in Indonesia than the Numbu variety.

3.3.2. Fractionation of Sorghum Stalks and Bran by Alkaline Extraction

A multi-step process based on an initial alkaline treatment was used to fractionate the sorghum stalks and bran. Various NaOH concentrations (0.75, 1.0 and 1.5 M) were tested and following extraction and secondary processing of three fractions were generated, namely the residue (R), hemicellulose (H), and alkali lignin (L), as demonstrated in Figure 3-1. The results of the compositional analysis of these fractions are presented in Table 3-2.

R fractions of stalk and bran mainly consist of cellulose, a fraction of the hemicellulose which is non alkaline soluble such as β-glucan as well as a small amount of lignin and protein. The isolation of highly pure cellulose is a challenge due to the complexity of cell wall structure and a tight set composed of hemicelluloses, lignins, and other carbohydrate polymers in a gel matrix (Sun & Ren, 2010). It is likely that the recovered cellulosic material would have an amorphous structure. Therefore, to fractionate the cellulose is to remove the lignin and hemicelluloses using the alkaline method which is regarded as a common method. Alkaline treatment will saponify acetyl groups that are attached to the xylan backbone, and other ester-linkages will cause cellulose to swell and disrupt the hydrogen bonds between hemicelluloses and cellulose. This will result in the solubilisation of hemicelluloses and a water and alkali-unextractable cellulose-rich residue will remain (Verbruggen, Beldman, & Voragen, 1995).

The glucose content in the R fraction of the sorghum stalk increased from ~55% to 62% as the concentration of NaOH increased from 0.75 M to 1.5 M, while the content of xylose and arabinose decreased from 13% to 7% and from 1.4% to 0.9%, respectively. This result showed that alkali treatment could extract the majority of the hemicellulose and lignin present in the soghum stalks which is in line with a previous study which revealed that the residue fraction obtained from alkali extraction 0.5% and 1% NaOH at 30 °C for 3 hours were 66.9% and 57.8% (Li et al., 2015). However, the glucose content in the R fraction of the sorghum bran was relatively stable (~35 - 40 %) regardless of the employed NaOH concentration. This result is higher than that of a previous study in which cellulose-rich fractions of sorghum bran and bagasse (dried stalk) yielded 11.68% and 46.73%, respectively (Qiu et al., 2017).

The residue fraction is rich in cellulose and has the potential to be a natural fiber for textile, composite, and other fibrous applications. A recent study concerning the functional properties of cellulose fraction from the sorghum bran showed that it has a high water holding capacity (22 to 35-fold its dry weight) at room temperature due to its very porous nature and branched structure (Qiu, Yadav, & Yin, 2017). Thus, cellulose fraction has the potential to be applied in industrial products including in the agricultural sector such as regenerated cellulose, natural textile fibers, or in the form of paper and board. It is also a versatile starting material for subsequent chemical modification (ethers and esters) to be used in the form of fibers, films, and a series of cellulose derivatives food casings, membranes, sponges, natural organic sorbents (Sun and Ren, 2010) as well as biofuels such as bioethanol.

The H fraction of sorghum stalk after the extraction with 0.75 M, 1 M, and 1.5 M of NaOH at 50 °C for 3 h, consisted approximately of 49.31%, 52.09%, and 31.9% w/w arabinoxylan (AX) respectively (calculated as the sum of the arabinose and xylose content), with the ratio of A/X ranging between ~0.13-0.15. Xylose was the predominant monosaccharide in the H fractions of sorghum stalk (31.99-52.09%, w/w), followed by glucose (4.93-11.61%, w/w), arabinose (7.11-8.02%, w/w), glucuronic acid (0.02-1.27%, w/w) and galacturonic acid (0.15-0.99%, w/w). Alkali extraction demonstrated the presence of significant amounts of hemicellulose, which are likely to be alkali soluble arabinoxylans, as reported by Sun, Wen, Ma, & Sun (2013). The higher the strength of the alkali solution the more effective was the extraction of hemicellulose. This is most likely due to the fact that at higher concentrations, the alkali can effectively reduce the molecular size of the hemicellulosic polymers and cleave the hydrogen bonds, covalent bonds and ester linkages in the cell wall matrix between carbohydrates and lignin. More specifically, hydroxyl ions from alkali disrupt the hydrogen bonds between cellulose and hemicellulose, and also break the ester linkages between lignin and hemicellulose, and hence solubilise part of the hemicellulosic material (Cyran, Courtin, & Delcour, 2003). However, as the NaOH concentration was further increased to 1.5 M, the xylose content was decreased 31.99%, w/w. This indicates that higher NaOH concentrations released primarily linear hemicellulose, a fact which was supported by the low A/X ratio (0.16-0.22) of the H fraction under these extraction conditions. This result is in agreement with previous studies on sweet sorghum stalk's extraction which concluded that a higher concentration of NaOH resulted in the solubilisation of the linear hemicellulose (Ebringerová & Heinze, 2000; Hromádková & Ebringerová, 1995; Li et al., 2015). In addition, a higher concentration of NaOH resulted in more amorphous cellulose in the sorghum stalk as reported by Mittal, Katahira, Himmel, & Johnson (2011), which indicates that the aqueous NaOH treatments caused a substantial reduction in both cellulose crystallinity and crystallite size. Furthermore, high concentrations of NaOH might have led to the degradation of hemicellulosic polymers into oligosaccharides and monosaccharides, which were not recovered by ethanol precipitation (Peng et al., 2012). Alkali treatment also dissolved lignin by the cleavage of bonds such as α- aryl ether and arylglycerol-β-aryl ether, and a large proportion of hemicelluloses were exposed at the surface of residue, which resulted in an increase in the release of the hemicellulosic polymers at higher NaOH concentration. This caused cellulose to swell, and cleaved the hydrogen bonds between hemicelluloses and cellulose from the cell wall, resulting in a substantial dissolution of hemicellulosic polysaccharides.

Regarding the sorghum bran, as the concentration NaOH increased from 0.75 M, 1, and 1.5 M the glucose content of the hemicelulosic fraction (H) decreased from approximately

75.9%, to 59.1% and 40.8% w/w, with the ratio X/G equal to 0.08, 0.10, and 0.09, respectively. This ratio implied that the degree of branching was low. Sugar analysis of the H fraction of the bran revealed low amounts of arabinose and xylose and a significant amount of glucose at 0.75 M NaOH (76%, w/w). It can be inferred that the type of hemicellulose is mixed-linkage glucans since it mostly comprised of glucose, small amounts of β-glucan and is associated with arabinoxylan (AX) in the pericarp tissues as reported by Maes & Delcour (2002a). This also indicates that alkaline process most likely extracted xyloglucan from sorghum bran. The availability of mixed-linkage glucan and XG in H fraction of bran was an interesting finding since they are rarely found in graminae plants. Low levels of xyloglucan have been detected by microscopic analysis of cell wall polysaccharides in the aleurone and testa, but not the endosperm of sorghum grain. XG constitutes a hemicellulose that occurs in the primary cell wall plant, such as eudicots and non-graminaceous monocotyledonous plants (Hotchkiss et al., 2015). In the plant, the role of XG is to support the cell wall structure and function and it is linked to the cellulose microfibril. Arabinoxylans, glucuronoarabinoxylan and mixed linkage glucans (β-glucan and xyloglucan) were obtained after sequential alkali extraction of sorghum endosperm, as reported by Verbruggen, Beldman, & Voragen (1995). This result confirms the findings of the current study which suggested that arabinoxylans and mixed linkage glucans were present in the hemicellulosic fraction of sorghum bran. In addition, glucoarabinoxylans have also been reported to be present in sorghum bran (Ayala-Soto, Serna-Saldívar, Welti-Chanes, & Gutierrez-Uribe, 2015). The main hemicelluloses in the bran of other cereals, such as wheat, oat, rice and rye include arabinoxylans, β-glucan, and small levels of xyloglucan (Maes & Delcour, 2002b; Sárossy, Tenkanen, Pitkänen, Bjerre, & Plackett, 2013; Shibuya & Iwasaki, 1985). However, in this study the main hemicellulose in sorghum bran based on the compositional analysis was most likely xyloglucan, which includes a backbone of β-1,4-glucan decorated primarily with xylose residues; this was further supported by FT-IR and NMR analysis, as discussed later on.

The alkali lignin (L) fractions contained only small amount of sugars (Table 3-2). Glucose (1.9-4.62%, w/w), small amount of xylose (0.4-1.1%, w/w) and arabinose (0.82%, w/w) were detected in the lignin stalk extract. On the other hand, the lignin bran contained xylose (0.3-1%, w/w), glucose (~1.6%, w/w), and small amount of uronic acid (~0.3%, w/w). A previous study showed that as the concentration of NaOH increased from 0.5 M to 1.5 M, the arabinose content decreased from 0.1% to 0.04% (w/w), the glucose decreased from 0.18% to 0.01% (w/w), and the xylose decreased from 0.9% to 0.03% (w/w) in the isolated lignin fraction from sorghum stalk at 30 ^{o}C for 3 h (She et al., 2010). These sugars were mostly released during the alkaline treatment, which causes a breakdown of α-ether linkages between lignin and hemicelluloses and of ester bonds between lignin and hemicelluloses and hydroxycinnamic acids, such as p-coumaric and ferulic acids (Xiao et al., 2001).

Table 3-2 Content of sugars (% dry matter) of the fractions extracted from sorghum Kawali’s stalk and bran

Sugars in Fractions of Stalk (%)	**$R_{0.75}$[a]**	**R_1**	**$R_{1.5}$**	**$H_{0.75}$[b]**	**H_1**	**$H_{1.5}$**	**$L_{0.75}$[c]**	**L_1**	**$L_{1.5}$**
Xylose	12.87 ± 0.31	10.60±10.76	7.10±1.07	49.31±0.76	52.09±0.48	31.99±1.58	1.14±0.08	0.39±0.02	0.36±0.21
Arabinose	1.43±0.29	1.21±0.21	0.88±0.14	8.02±0.01	7.62±0.33	7.11±0.49	0.82±0.03	0	0
Glucose	55.4±0.13	63.78±2.26	62.46±6.87	11.40±1.28	11.61±1.19	4.93±0.03	4.62±0.08	1.99±0.13	1.93±0.23
Glucuronic acid	nd[d]	0.15±0.02	0.15±0.05	0.02±0.00	1.27±0.29	0.75±0.09	nd	nd	nd
Galacturonic acid	0.17±0.02	nd	nd	0.99±0.22	0.76±0.86	0.15±0.08	nd	nd	nd
Total sugars	69.87	75.74	70.59	69.74	73.35	44.93	6.58	2.38	2.29
A/X[e]	nc			0.16	0.15	0.22	nc		
X/G	nc	nc	nc	nc	nc	nc	nc	nc	nc
Sugars in Fractions of Bran (%)	**$R_{0.75}$**	**R_1**	**$R_{1.5}$**	**$H_{0.75}$**	**H_1**	**$H_{1.5}$**	**$L_{0.75}$**	**L_1**	**$L_{1.5}$**
Xylose	8.69±0.62	5.56±0.84	5.11±0.62	5.95±0.48	6.03±0.99	3.90±0.28	0.32±0.17	nd	0.95±0.01
Arabinose	6.94±0.60	4.15±0.62	4.13±0.40	5.54±0.57	5.97±0.11	3.52±0.24	nd	nd	nd
Glucose	39.86±0.62	35.39±5.55	37.31±2.02	75.85±0.92	59.12±0.73	40.79±3.05	nd	1.55±0.47	nd
Glucuronic acid	0.45±0	0.27±0.06	0.41±0.09	0.37±0.01	0.19±0.3	0.41±0.09			
Galacturonic acid	nd	0.57±0.07	nd	0.33±0.06	0.15±0.21	0.28±0.02	nd	0.34±0.01	nd
A/X	nc			0.93	0.99	0.90	nc		
X/G[f]				0.08	0.10	0.09			
Total	55.94	45.94	46.96	88.04	71.46	48.9	0.32	1.89	0.95

Mean ± standard deviation; [a] $R_{0,75}$, R_1, $R_{1.5}$ represent residue fractions generated by alkaline extraction with 0.75, 1, and 1.5 M NaOH; [b]$H_{0.75}$, H_1, $H_{1.5}$ represent hemicellulose-rich fractions generated by alkaline extraction with 0.75, 1, and 1.5 M NaOH, respectively; [c] $L_{0.75}$, L_1, $L_{1.5}$ represent alkali lignin fractions generated by alkaline extraction with 0.75, 1, and 1.5 M NaOH, respectively; [d] nc, not calculated; [e]Represents arabinose to xylose ratio; [f]Represents xylose to glucose ratio; [g] [e]nd, not detected

Table 3-3 lists the recovery of sugars from the sorghum stalks and bran in the extracted fractions carried out at temperatures of 50°C for 3 hours using 0.75 M, 1 M, and 1.5 M NaOH. Overall, the recovery for arabinose ranged from 33% to 49% in bran and 95% to 100% in stalk while the recovery for xylose ranged from 37% to 63% in bran and 64% to 99% in stalk. In addition, the recovery for glucose ranged from 57% to 100% in bran and 95% to 100% in stalk. Overall, the extraction process in the stalk is considered to be more effective than in the bran since almost all sugars in the former component were recovered. The stalk had a sugar higher recovery due to a simpler structure of arabinoxylan compared to that of the bran. It can be inferred from the ratio A/X that the structure of hemicelluloses was low branching which allowed NaOH to work more effectively to dissolve hemicelluloses and break the bonds between polysaccharides in the gel matrix of the cell wall. The highest recovery of arabinoxylan in the stalks was at 1 M NaOH (100% of xylose, 81% of arabinose and 96% of glucose); whereas in the bran, the highest recovery was at 0.75 M which recovered 100% of glucose, 63% of xylose, and 49% of arabinose.

Table 3-3 Recovery of neutral sugars compared to raw material - % dry weight in the residue (R), hemicellulose (H), and Lignin (L) fractions. Compared to the sugar in contents of sorghum stalk and bran.

NaOH	**Sugar**	**Recovery in Stalk (%)**			**Total recovery (%)**	**Recovery in Bran (%)**			**Total recovery (%)**
		R[a]	**H[b]**	**L[c]**		**R[a]**	**H[b]**	**L[c]**	
0.75 M	Xylose	29.52	31.07	na	63.69	19.25	43.78	0.32	63.35
	Arabinose	31.12	57.29	na	100.0	15.90	32.6	na	48.5
	Glucose	79.72	6.43	4.62	98.6	14.54	92.31	na	100.00
1 M	Xylose	26.18	76.85	na	100.00	8.22	53.06	na	61.28
	Arabinose	31.57	49.68	na	81.25	10.63	60.80	na	71.43
	Glucose	87.81	5.79	1.99	95.59	11.20	57.8	1.55	70.55
1.5 M	Xylose	15.27	57.09	na	74.49	8.13	27.54	0.95	36.62
	Arabinose	18.13	77.19	na	95.32	6.87	25.72	na	32.59
	Glucose	82.16	8.03	1.93	100.00	9.95	47.65	na	57.6

[a]R: Residue fraction; [b]H: hemicellulose fraction; [c]L=lignin fraction; na= not available.
*Sequential alkali treatment reaction was conducted at 50 °C for 3 hours.

3.3.3. FTIR Analysis of Fractions Extracted from Sorghum Stalk and Bran

It has been well established that the molecular vibrations in the IR spectra depend on interatomic distances, bond angles and bond strengths, rather than on the bulk properties of the compounds. These vibrational frequencies provide a molecular fingerprint which enables the identification of a compound when it is either in pure states or in mixture states (Adapa, Karunakaran, Tabil, & Schoenau, 2009). In this study, the vibrational spectra of the obtained fractions were recorded in the mid-IR region (4000-500 cm^{-1}). Table 3-4 shows the FT-IR spectra of the R, H and L fractions of the sorghum stalks and bran.

The spectra of the residue fractions (R) of both sorghum stalks and bran had prominent signals in the region 3600-2915 cm^{-1} which can be assigned to stretching vibrations of CH and OH (She et al., 2010). Additionally, the region 850-1100 cm^{-1} can be assigned to be anomeric and is typical of β anomers, indicating the typical structure of cellulose present in the residues (Oh, Yoo, Shin, & Seo, 2005; Xiao et al., 2001). The difference among them was that there was no peak between 1400-1350 cm^{-1} at residue fraction from the bran which was assigned as C-H deformation (methyl and methylene) in cellulose.

In the lignin fractions (L) of both sorghum stalk and bran, the region 2900-3400 cm^{-1} can be assigned to the hydroxyl groups of phenolics and the stretching of the aromatic methoxyl groups as reported by She et al. (2010) in which the major peaks at broad bands at 3426 cm$^{−1}$ were attributed to hydroxyl groups in phenolic and aliphatic structures, and the bands centred between 2925 and 2858 cm$^{−1}$ predominantly arose from C–H stretching in aliphatic C–H and aromatic methoxyl groups. The prominent peaks at 1583 cm^{-1} and 1553 cm^{-1} can be assigned to very strong aromatic ring stretches and C-O stretching, whereas the peaks at 1411 cm^{-1} and 1413 cm^{-1} also indicate aromatic C-O stretching, as reported by She et al. (2010). The lignin stalk profile had more prominent peaks at 3000-2000 cm^{-1} compared to lignin bran, which denoted C-H stretching in aliphatic C-H and aromatic methoxyl groups. These data support the chemical composition analysis which showed that stalk contained more lignin than the bran.

The FTIR spectra of the hemicellulosic fractions (H) of both sorghum stalk and bran are depicted in Figure 3-2. It can be observed that the spectral profiles and intensities were different. The absorption bands at 3307 cm^{-1} in the H fraction of sorghum stalk and at 3310 cm^{-1} in the H fraction of sorghum bran can be assigned to the stretching vibration of –OH groups. The absorption band at 2917 cm^{-1} in the H fraction of sorghum stalk was assigned as C-H stretching of aliphatic groups, while in the H fraction of sorghum bran the band at 2921 cm^{-1} was assigned to xyloglucan (XG) as reported by Singh, Malviya, & Sharma (2011). The absorption at 1589 cm^{-1} in the H fraction of sorghum stalk is associated with absorbed water, since hemicellulose are potentially easy to be hydrated (Li et al., 2015; Sun et al., 2013); however, this peak was not seen in the H fraction of sorghum bran. The peak at 1632 cm^{-1} in the H fraction of sorghum bran is assigned to the bending vibrations of the –OH groups. In addition, the peak at 1357 cm^{-1} can be assigned as CH$_2$-bend which is a prominent peak of XG and is in agreement with a previous study reported by Mukerabigwi et al. (2015).

Table 3-4 Prominent peaks from FTIR analysis of bran and stalk sorghum fractions

Wavenumbers (cm^{-1})	**Residue (R)**		**Hemicelluloses (H)**		**Alkali lignin (L)**		**Assignment**
	Stalk	**Bran**	**Stalk**	**Bran**	**Stalk**	**Bran**	
4000-3000	3429,3408	3307	3307	3310	3415	3507	Hydroxyl group in phenolic and aliphatic groups
3000-2000	2916.37	2917	2917.8	2920.7	2983,2933	2920	C-H stretching in aliphatic C-H and aromatic methoxyl groups
1650-1600	1639	1587.9		1632	-		Bending vibrations of –OH groups
1600-1550			1589		1583	1553	Absorbed water of Hemicellulose; Very strong aromatic ring stretch, aromatic C-O stretch in Lignin
1500-1420	1456	1425.7	1423				O-C-H in plane bending vibration
1420-1400	-	-			1413	1411	Very strong aromatic ring stretch, aromatic C-O stretch,
1400-1350	1363.67	-		1357	-	-	C-H deformation (methyl and methylene) in

							cellulose, CH_2-bend in xyloglucan
1300-1100	-	-	1101.3	1108, 1148.5	-	-	Acetylated hemicellulose
1100-900	-	-	1020	1022, 1061			Glycosidic C-O, C-C stretching or C-OH bending
850-1100	993	880.14	880.14	847.7			β-1,4 linkage

Additionally, a sharp peak at 1423 cm^{-1} was only available in the H fraction of the sorghum stalks and could be assigned as O-C-H-plane bending vibration of xylan. The prominent peak at 1020 cm^{-1} of the H fraction of the stalk can be assigned to glycosidic C-O, C-C stretching or C-OH bending, and is an indication of the presence of xylan (Sun et al., 2013; Xiao et al., 2001). In the H fraction of the sorghum bran, this peak type appeared at 1022, 1077 cm^{-1}. The sharp band at 880 cm^{-1} in the H fraction of the stalks indicates that most linkages between the sugar units were β-1,4 glycosidic bonds, while in the H fraction of the sorghum bran this peak came out at 847 cm^{-1} with the weak intensity. Thus, it is suggested that the H fraction of the sorghum stalk contained arabinoxylan whereas the H fraction of sorghum bran contained xyloglucan, which is in line with the compositional data presented in Table 3-4.

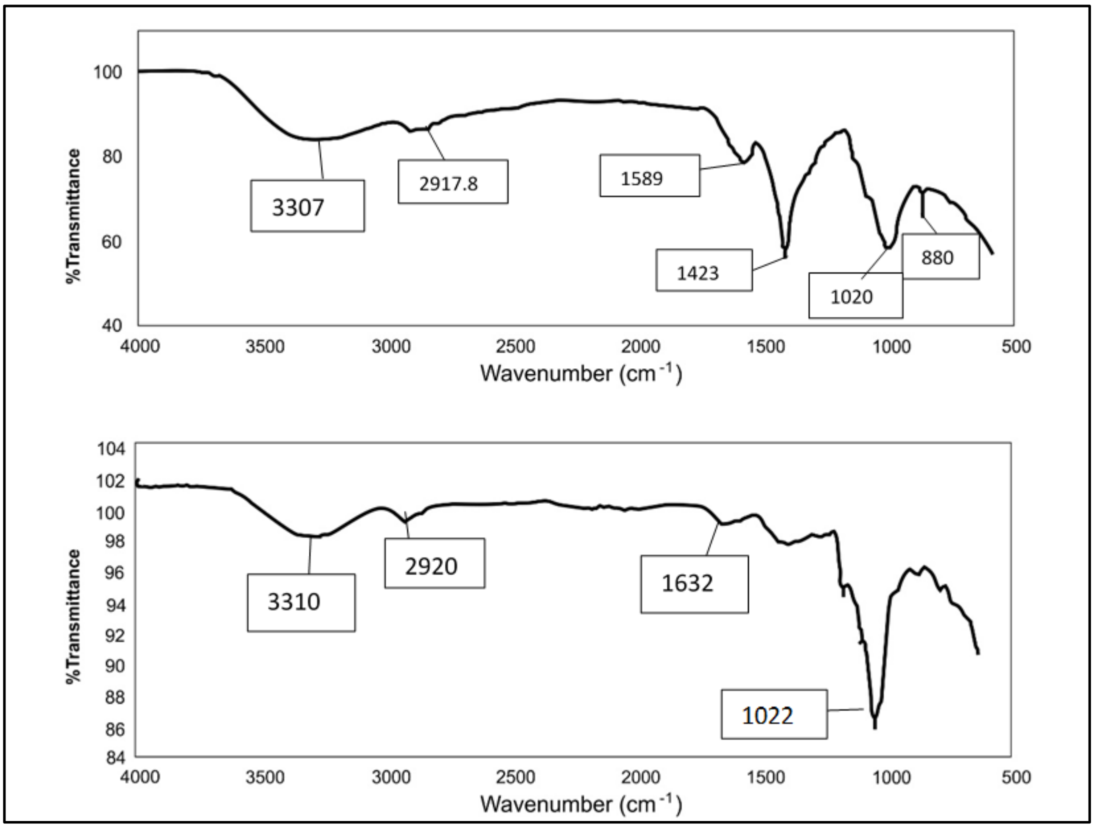

Figure 3-2 FT-IR spectra of hemicellulose fractions (H) obtained from sorghum stalk extraction with 1 M NaOH at 50 ^{o}C for 3 h (A), and sorghum bran extraction with 0.75 M NaOH at 50 ^{o}C for 3 (B).

3.3.4. NMR Spectra of Hemicellulose Fractions

^{1}H-NMR analysis was carried out in order to investigate the structure of the hemicellulose fraction (H) that was extracted from sorghum stalk (Figure 3-3 A), while the H fraction of sorghum bran was conducted by solid state NMR (Figure 3-3 B). This technique was conducted differently since the hemicellulosic fraction from sorghum bran cannot be detected by liquid NMR spectroscopy. Figure 3-3A the chemical shifts (δ) 3.05-5.35 ppm characterise the protons of arabinose and xylose residues, as reported by Li et al. (2015). Small peaks were observed at 1.74 and 2.02 ppm, which could be assigned to the methyl protons of the acetyl group and 4-O-methyl-D-glucuronic acid a, respectively (Sun & Ren, 2010). According to Sun et al. (2013), the signals at δ 3.05-4.28 ppm are assigned as the equatorial protons of the anhydroxylose units and 4-*O*-methyl-α-D- glucuronic acid units of the hemicellulose polymer. Strong signals of (1-4)-linked β-D- xylanpyranosyl (Xylp) were detected at δ: 3.05 ppm (H-1), 3.34 ppm (H-3), 3.17 ppm (H-2), 3.52 ppm (H-4) and 3.98 ppm (H-5 eq).

The region δ 4.1-4.68 ppm corresponds to the β-configuration of Xylp in the H fraction which is in line with a previous study by Li et al. (2015). Meanwhile, the signals δ at 5.01 and 5.13 ppm are assigned as α-xylp$_1$ units. Furthermore, signals δ at 5.30-5.35 ppm are assigned as α-arabinofuranosyl units. Based on the above, the extracted H fraction is likely to consist of L-arabino-(4-O-methyl-α-D-glucurono)-D-xylans backbone with branches of α-arabinofuranosyl and α-xylanpyranosyl units. This is in line with the compositional data in Table 1.

^{13}C-NMR was used to identify the structure of the hemicellulosic fraction (H) from the sorghum bran by a solid state NMR. The spectrum of this fraction is depicted in Figure 3-3 B. The chemical shift at 103 ppm indicated an amorphous material. The signals (δ) at 103, 102.3, 81.92, 72.18, and 60.94 ppm are assigned as C-1 glucose, C-1 xylose, C1-arabinose, C-4 glucose, C-4 xylose, and C-6 glucose, respectively. This result is in agreement with a previous study which explored the structure of xyloglucan in mung bean and flaxseed kernels (Bootten, Harris, Melton, & Newman, 2004; Ding et al., 2016). Based on the above, the hemicellulosic fraction of the sorghum bran can be characterized as xylogucan with α-xylose residues are linked to the O-6 of the β-glucose residues and arabinose is linked to the O-2 site of xylose residues with β-bonds. This is in line with the compositional data in Table 3-1, and indicates the different structure of the hemicellulose extracted from the sorghum bran and stalk.

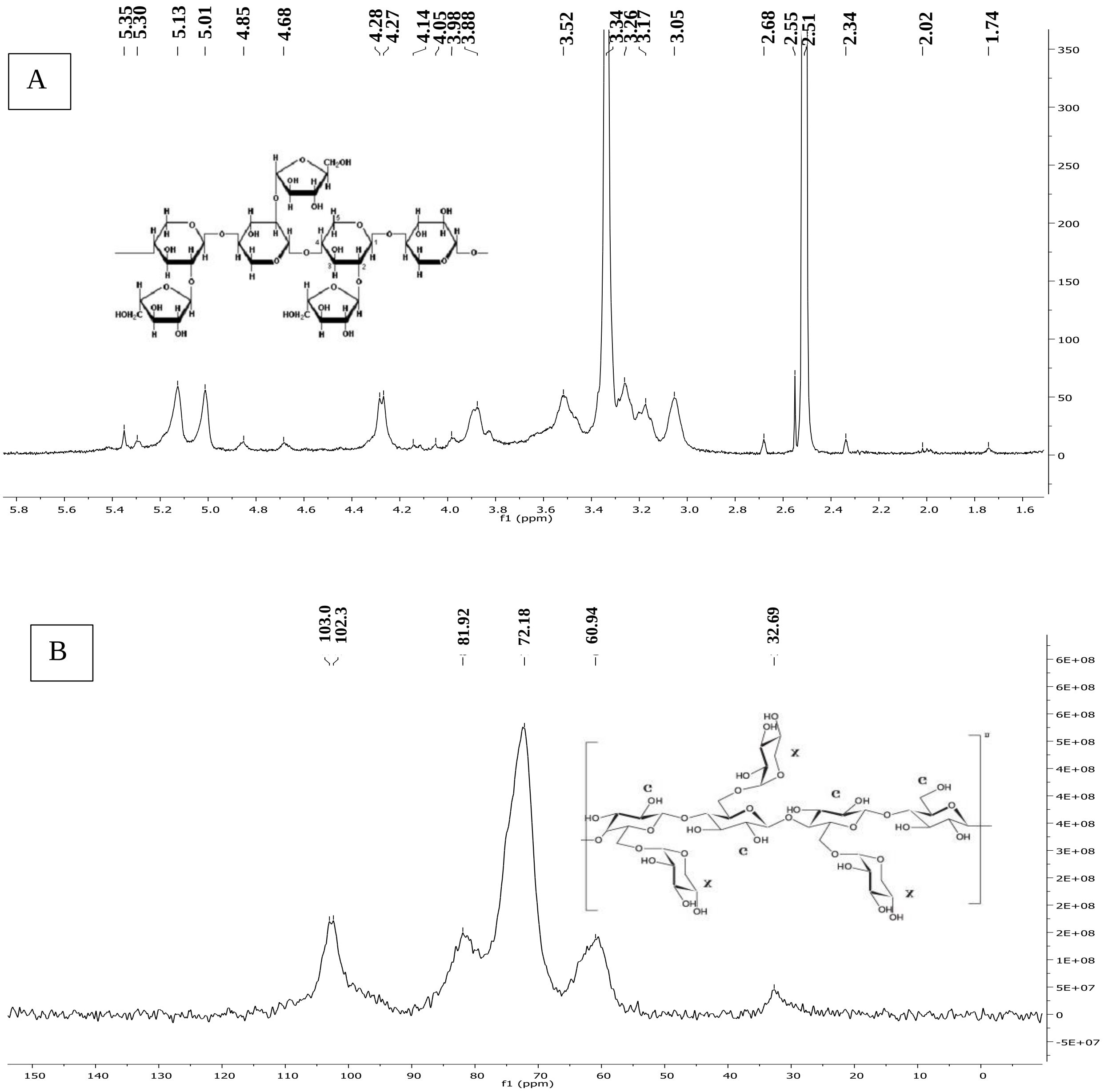

Figure 3-3 ^{1}H-NMR spectrum of hemicellulosic fraction (H) obtained from sorghum stalk after 1M NaOH extraction at 50 ^{o}C for 3 h (A), and ^{13}C-NMR spectrum of hemicellulosic fraction (H) obtained from sorghum bran after 0.75 M NaOH extraction at 50 ^{o}C for 3 h (B). Possible structures were L-arabino-(4-O-methyl-α-D-glucurono)-D-xylans (A); Xyloglucan (B).

3.3.5. Thermal Analysis of Cellulose (R) and Hemicellulose (H) Fractions from Sorghum Stalk and Bran

Further characterisation of the extracted fractions was carried out using differential scanning calorimetry (DSC). The glass transition temperature (Tg) and the melting point (Tm) of the extracted cellulosic fractions (R) were important parameters to determine their suitability for further processing and their potential end-applications. The Tg of the R stalk fraction was 52.3 ^{o}C (Table 3-5), and was very similar to Tg of R bran fraction (51.8 ^{o}C) despite the fact that there was a high difference in moisture content. The moisture content of the R fraction of bran was much higher than that of the stalk R fraction, which is most likely the reason for the lower transition of the glass temperature (Tg).

The Tg values of the H fraction of stalk and bran were 90.8 ^{o}C and 80.7 ^{o}C, respectively. The Tg of commercial arabinoxylan from the wheat bran and commercial xylan from the birchwood were also measured and were found to be 76.7 ^{o}C and 95.6 ^{o}C, respectively. The Tg value for the birchwood xylan was very similar to the Tg values of the sorghum stalk H fraction. This demonstrated that the molecular mass and structural characteristics of these samples were similar. However, the lower Tg value of fraction of the H bran fraction showed that it has a more amorphous structure than the H stalk fraction. This is most likely due to an increase degree of substitution of side chains (α-L-arabinofuranosyl) of this polymer which reduced the mobility of hydrogen bonding in polymer-polymer interactions as well as an increment in free volume (Buchanan et al., 2003). Moreover, the side chains may act as a plasticiser and therefore it has lower Tg (Hoseney Carl, 1994). It was noted that the Tg of these arabinoxylan esters decreased as the length of the side chain and the total DS increased. This occurrence is likely the result of a decrease in polymer–polymer interactions arising from hydrogen bonding.

Table 3-5 Thermal analysis properties of alkaline-derived fractions of sorghum stem and bran, as well as commercial xylan from birchwood and arabinoxylan from wheat bran.

Samples	**Moisture Content (%)**	**T_g (°C)**	**T_m (°C)**
Residue fraction (R) of stalk	5.8±0.8	52.3	101.2
Residue fraction (R) of bran	22.7±0.6	51.8	102.8
Hemicellulosic fraction (H) of stalk	2.9±0.1	90.8	100.0
Hemicellulosic fraction (H) of bran	4.5±0.1	80.7	97.8
Commercial xylan from birch wood	1.1±0.3	95.6	106.1
Commercial arabinoxylan from wheat bran	7.6±0.5	76.7	101.8

The melting point (Tm) provides insights on the characteristics of the polymer and reflects its structure. In this study, the R fraction of the sorghum stalk and bran degraded at 101.21 and 102.78oC, respectively and the Tm of H fraction of the stalks and bran were 100oC and 97.83oC, respectively. Based on the results, it is suggested that the residue and hemicellulosic fraction degraded almost at the same time although Tm values were slightly lower than the Tm of commercial xylan and arabinoxylan. These differences are probably due to difference in structure, molecular weight, degree of branching, and the more crystalline nature of the commercial polymers compared to the polymers extracted from this study.

3.3.6. X-Ray Diffraction of R and H Fractions from Sorghum Stalk and Bran

The X-ray diffraction profiles of the fractions extracted from sorghum by-products are depicted in Figure 3-4 which shows the x-ray diffractograms of the cellulose fraction (R) of the sorghum stalks and bran. The crystallinity values for cellulose fraction of the stalk and the bran were 34.6% and 28.1%, respectively. Therefore, the XRD results confirm the Tg results obtained by the DSC. In addition, the major peaks of the XRD patterns for both cellulose fractions of the stalk and the bran showed a broad diffraction peak at the same 2θ=~20°C, which confirmed that cellulose fractions in both materials were very similar, and mainly consist of glucose. The X-ray diffractograms also illustrate that these samples are amorphous. The amorphous regions (broad peaks) of cellulose fractions of the stalk and bran increased significantly from the initial stalk and bran after extraction using NaOH, by approximately 35 and 39%, respectively. This fact could explain how alkaline extraction cleaved the hydrogen bonds in the intra- and intermolecular of the cellulose and increased the porosity or surface area; hence making the cellulose structure more amorphous (Ciolacu et al., 2011; Sun & Ren, 2010). In another study, the Crystallinity Index (CI) of cellulose varied from 39% to 67% and was analysed using four different techniques including XRD (Thygesen, Oddershede, Lilholt, Thomsen, & Ståhl, 2005). Their CI result is similar to the findings of this study. Cellulose crystallinity is an important property in enzymatic hydrolysis. It has an amorphous region that is easy for enzymes to digest and a crystalline region that is difficult to digest. Information on material structure is also important for some applications for example an amorphous cellulose can be used as an absorbent; while the crystalline cellulose can be used to reinforce fillers to increase mechanical properties of products. DSC results can be used to confirm the structure of polymers by evaluating their thermal profile. Semi-crystalline materials will show peaks in XRD and can be confirmed with glass transition and melting point temperatures in DSC.

The crystallinity of the hemicellulosic fractions is depicted in Figure 4B. It can be seen that the crystallinity of commercial xylan (XC), commercial arabinoxylan (AXC), hemicellulosic fraction of bran (XB), and hemicellulosic fraction of the stalk (XS) were 43.6%; 35%; 41.8%, and 46.5%, respectively. Additionally, with regards to the hemicellulosic samples, the 2θ position of XB, XS, XC and AXC was similar at 20^{o}C. This mainly denoted an amorphous state of the material that contained small crystalline peaks in the region. The substitution pattern of arabinoxylans which is reflected by the ratio of arabinose/xylose affected the amorphous or crystalline structure of arabinoxylans, as reported by Hoije et al. (Höije, Gröndahl, Tømmeraas, & Gatenholm, 2005). For instance, when arabinoxylans is de-branched, its crystallinity increased. The use of chemical reagents and processing conditions may affect the crystal habit of an original material. Hence, can affect the physicochemical properties such as melting point, solubility, true density, dissolution profile, flowability, compressibility, structure, and thermal stability (Keraliya, Soni, Thakkar, & Gandhi, 2010). The hemicellulosic fraction of the bran had the lowest intensity among those of the tested samples. The diffractogram showed that a semi-crystalline hemicellulose peak was present, an observation similar to Höije et al., (2005). This indicated that some of the xylan was present in its crystalline form (narrow and sharp peaks) and that unsubstituted regions crystallise while substituted regions are amorphous as depicted in Figure 3-4.

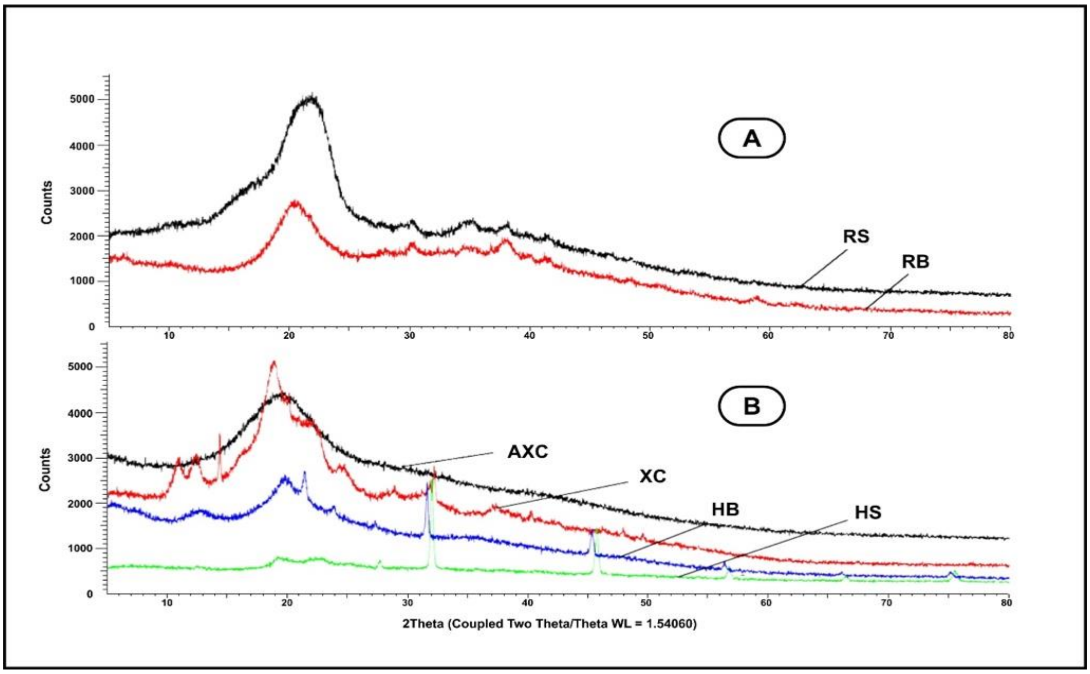

Figure 3-4 X-ray diffraction pattern of cellulose R fraction (A) and Hemicellulose H fraction (B) extracted from sorghum stalk and bran. Extraction of stalk was conducted with 1 M NaOH at 50 °C for 3 h whereas extraction of bran with 0.75 M NaOH at 50 °C for 3 h

3.3.7. Phenolic acids composition of sorghum by-products and extracted lignin fractions

In this study, the lignin fraction (L) was isolated from the soluble hemicellulose fraction (H) by ethanol precipitation. Lignin is a complex phenolic polymer produced by the oxidative combinatorial coupling of mainly three p-hydroxycinnamoyl alcohol monomers also known as monolignols (Campbell & Sederoff, 1996; Cesarino, Araújo, Pereira, Júnior, & Mazzafera, 2012). These alcohols: coniferyl, sinapyl, and 4-hydroxycinnamyl, give rise to p-hydroxyphenyl, guaiacyl, and syringyl lignins, respectively.

The concentration of phenolic acids in the initial sorghum by-products is presented in Figure 3-5. Sorghum bran Kawali contained 1.13 mg/g of free phenolics, 0.26 mg/g bound phenolics and 0.91 mg/g of phenolics were in conjugated form. The total phenolics content was 2.3 mg/g. Total phenolics in the bran (2.3 mg/g) and stalk (2.4 mg/g) were comparable. The panicles contained less amount of phenolics (1.33 mg/g) compared to the bran and stalk, and most were in free form. These results indicated that these by-products, particularly the stalks and bran, can be used as potential sources of phenolic compounds. Previous studies reported that the sorghum phenolic acids are mostly concentrated in the bran, and are mainly composed of ferulic acid (1400-2700 µg/g), sinapic acid (100-630 µg/g), and p-coumaric acid (0-970 µg/g) (Awika & Rooney, 2004; Hahn, Faubion, & Rooney, 1983). Phenolic compositions among sorghum varieties differ according to grain characteristics including plant color (white, black, red, tannin sorghum), pericarp (i.e ferulic acid ~1400–2170 µg/g), genotype and testa (pigmented testa contained high tannin) (Awika & Rooney, 2004).

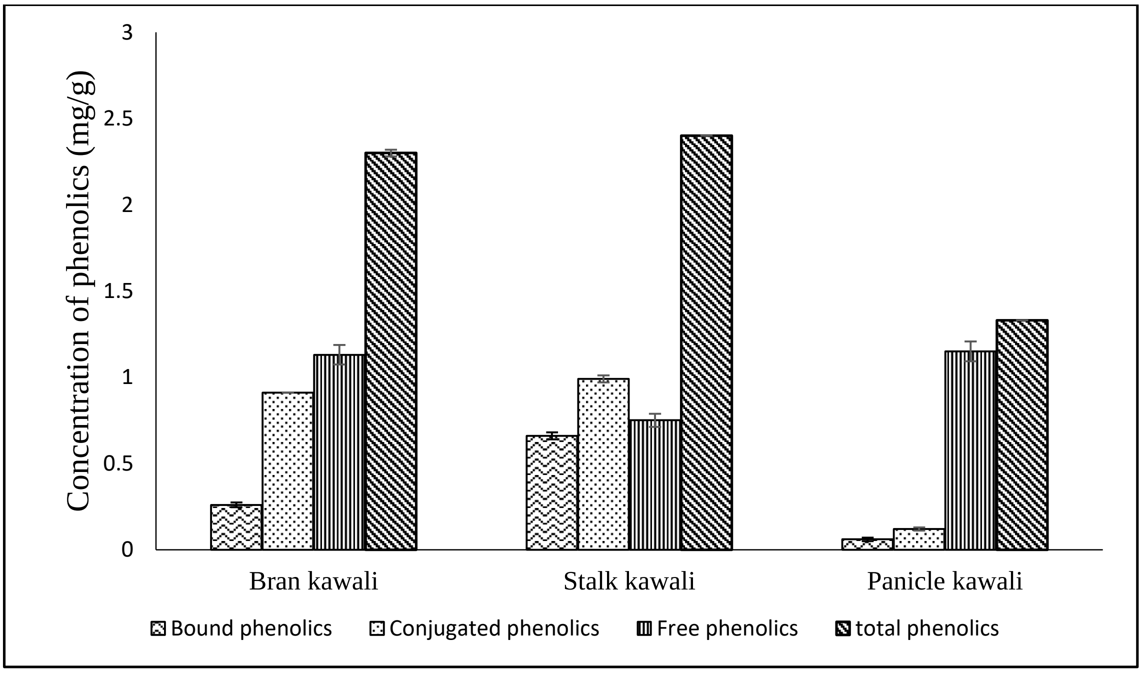

Figure 3-5 Phenolic acid content of sorghum (variety Kawali) by-products

Table 3-6 presents the results of phenolics content, protein, total phenolics content, total lignin, and antioxidant capacity of lignin bran and stalk. The lignin fraction (L) were found to contain varying amounts of phenolic compounds. The L fraction of stalk had a higher phenolic content compared to the bran (Table 3-6). In the L fraction of the stalks, p-coumaric acid was found in greater amounts than ferulic acid (Table 3-6), contrary to that of the L fraction of bran. This finding is similar to the fact that p-coumaric acid and ferulic acid are significant in plants belonging to the Poaceae family including sorghum (Cesarino, Araújo, Domingues Júnior, & Mazzafera, 2012). Ferulic acid is the major hydroxycinnamic derivative in young grass cell walls, while p-coumaric acid is an indicator of cell wall maturity, since it is mainly esterified to side chains of Syringyl units and its incorporation follows the same deposition pattern of syringyl units (Cesarino, Araújo, Domingues Júnior, et al., 2012). Moreover, the L fractions contained varying amounts of protein, which together with phenolic compounds contributed towards the antioxidant activity that these fractions exhibited (Awika & Rooney, 2004). Protein and specifically, the L fraction of the stalks had significantly higher antioxidant capacity compared to the lignin bran which was caused by a higher phenolic acid content in the stalk. A high correlation between total phenolic and antioxidant capacity has been reported by Ayala-Soto, Serna-Saldívar, Welti-Chanes and Gutierrez-Uribe (2015).

Table 3-6 Phenolics content, protein, total lignin, and antioxidant capacity of lignin fractions

Sample	Lignin Bran	Lignin Stalk
ρ-Coumaric acid (mg/g)	0.39±1.94	7.67±3.20
Ferulic acid (mg/g)	0.75±0.33	1.33±1.66
Total Lignin (mg/g)	0.05±0.19	7.78±0.35
Phenolic content (mg/g)	1.57±1.45	9.35±1.02
Protein (%)	3.09±0.16	4.13±3.09
Antioxidant capacity (%)	23.67±0.56	54.57±0.48

3.3.8. Mass balance

An effective extraction process can be defined by the overall mass balance. Figure 3-6 and Figure 3-7 show the overall process design based on a sequential alkaline pre-treatment process of sorghum stalks and bran with 1 M NaOH (sorghum stalk) and 0.75 M NaOH (sorghum bran) at 50^{o}C for 3 hours (the best treatment condition for obtaining maximum hemicellulosic fraction). The residue fraction of the stalks and the bran obtained after sequential alkaline treatment revealed that almost 90% of bran material were alkali solubilised. The overall mass balance at the end of fractionation step showed mass losses of 18% in the stalk and 12% in the bran. Part of the H fraction (42%) and most of the L fraction (85%) of the stalks were alkali solubilised; however, cellulose (almost 100%) and lignin (~16%) were recovered in the residue fraction.

In the stalks, the residue fraction mainly comprised of glucose, i.e. 63% (w/w). However, the residue still contained a noticeable amount of xylose (11% w/w), arabinose (1.4% w/w), uronic acid (~0.2% w/w), and klason lignin (~0.62% w/w). Furthermore, the R fraction also contained 1.2% w/w protein, 20% w/w ash and 2% lipid. Ash content showed that some minerals remained in the residue fraction. This observation also suggests that the alkali treatment of the stalk under the given condition resulted in a partial cleavage of these esterified or etherified linkages.

In the case of bran, cellulose (mainly glucose) left in the residue fraction was 35%. Most of the glucose remained in the supernatant, which indicated that mixed-linkage glucans were solubilised. The hemicellulosic fraction of the bran contained glucose which was three times higher than in the initial one. This has shown that alkaline treatment worked efficiently. Approximately 2.63% of β-glucan, 1.71% of protein, 18% of ash, and 1.7% of lignin were detected in the hemicellulosic fraction.

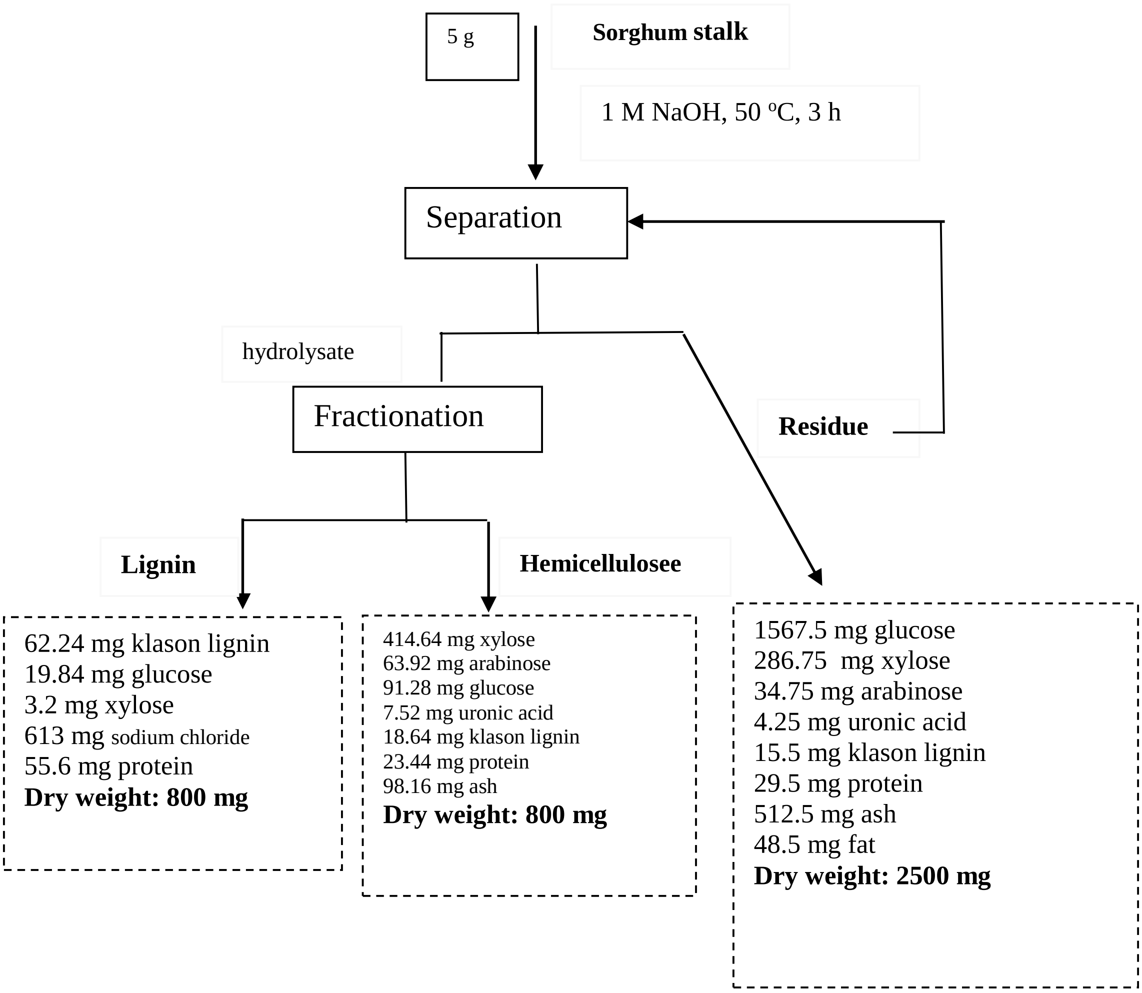

Figure 3-6 Process scheme and mass balances for the valorisation of sorghum stalk

In the plant cell wall, lignin deposited in thickened secondary cell wall with other biopolymers. The amount of lignin was an indicator of the plant’s maturity. The alkaline treatment under given conditions revealed that most of the lignin fraction (85%) was in the stalk and only 12% of the bran could be isolated. A number of sugars in lignin fraction showed the strong link to lignin. Overall, from this process 79.4% of the initial mass stalk and 89.6% of initial mass bran were recovered and distributed among the obtained three fractions. Overall, the proposed process scheme is effective to recover the main key sorghum stalks and bran components (cellulose, hemicellulose, and lignin) with relatively low losses whilst providing a viable approach for the valorisation of sorghum by-products.

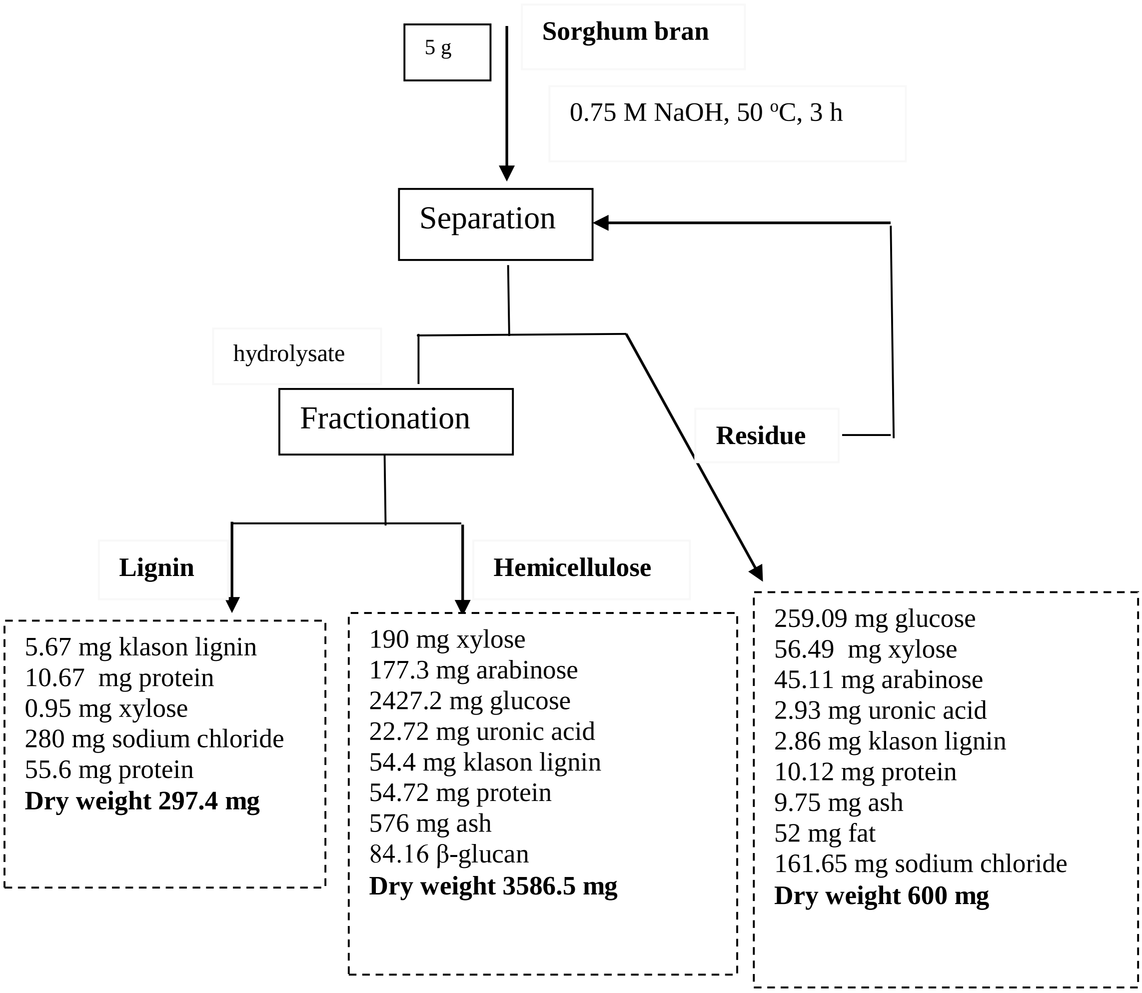

Figure 3-7 Process scheme and mass balances for the valorisation of sorghum bran

3.4. Conclusions

Stalk, bran, and panicles are underutilised lignocellulosic by-products of the sorghum plant. This study demonstrated an effective approach through sequential extractions with NaOH concentrations for the fractionation and the recovery of key-components in sorghum stalk and bran. Overall, 79.4% of the initial mass of the stalk and 89.6% of the initial mass of the bran were recovered and distributed among the obtained three fractions. The hemicellulosic fraction of sorghum bran was characterised as arabinoxylogucan, whereas that of stalk mainly consisted of arabinoxylans. Extraction residues were mainly composed of glucose (cellulose), whereas the lignin fraction of the stalk was particularly rich in lignin and phenolics. Based on the detailed characterisation of the obtained fractions, a range of potential applications can be addressed in the food and agricultural sectors.

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Chapter 4: Hemicellulosic Fractions from Sorghum By-products as Raw Materials for the Development of Biodegradable Films

Abstract

Hemicellulose-based films have limited applications in food packaging due to their susceptibility to moisture and brittleness. In this study, hemicellulosic-films were developed from sorghum by-products and their potential use in agricultural applications as fertilising agents and food packaging materials was investigated. To this end, the addition of plasticiser (glycerol) and the incorporation of long chain anhydride groups (2-octenyl succinic anhydride, 2-OSA) to sorghum hemicellulose extracts was carried out and the mechanical, physical, thermal properties of the films were determined. The plasticisation with glycerol improved the mechanical properties of bran-extracted hemicellulose films. With regards to films made with 2-OSA-stalk-derived hemicellulose, these demonstrated improved performance (no brittleness), mechanical properties (tensile strength and total work), and barrier properties compared to those without modification. The succinylation of stalk-derived hemicellulose was verified by FTIR analysis of modified films. Developed films were tested carriers of fertiliser (NPK) in soil trials and demonstrated quick release of the fertiliser and biodegradable properties. Furthermore, the application of 2-OSA-HS films as sealed-wrapping materials demonstrated adequate moisture barrier properties and reduced the weight loss of fruits after 7 days of storage in room temperature and under chilled conditions.

Keywords: hemicelluloses, sorghum by-products, films, properties

4.1. Introduction

The need to substitute non-biodegradable, petrochemical-based packaging materials with more sustainable materials has emerged in recent years and this has led to the evaluation of various renewable materials as biodegradable polymers. In addition, consumers' demand for environmental friendly packaging materials that are able to maintain high-quality products has raised concerns in developing biodegradable packaging materials as alternatives to plastics, such as polysaccharide- and protein- based polymers. Polysaccharides, such as starch and their derivatives, cellulose and its derivatives, seaweed extracts, gums, pectins and chitosan, have been used for films and coatings (Talens, Fabra, & Chiralt, 2010). Additionally, hemicellulose holds potential as a polymer suitable for film packaging (Ebringerová & Heinze (2000)) However, hemicellulose and its derivatives may vary in structure and properties, and as such its commercial applications are still minor compared to starch and cellulose (Mikkonen & Tenkanen, 2012).

Sorghum by-products such as bran and stalk represent abundant, inexpensive, and readily available sources of renewable, lignocellulosic biomass. In Indonesia, these by-products are primarily used as animal feed, landfilling or they are burnt, and therefore have a low market value and, in the case of burning, a negative environmental impact (Gadde, Bonnet, Menke, & Garivait, 2009). These raw materials contain large amounts of polysaccharides, in particular hemicellulose, cellulose, and lignin which if efficiently extracted, can be utilised for the development of a range of added-value products.

Dried sorghum stalk is an agricultural by-product which derives from squeezed sorghum stalks. On the other hand, sorghum bran is the main by-product of the sorghum milling operation and accounts for 10-11.5% (w/w) of the parent grain (Ayala-Soto, Serna-Saldívar, Welti-Chanes, & Gutierrez-Uribe, 2015). It derives from the decortication process which separates the grain and the aleurone to obtain high-quality food products or other special purposes (gluten free sorghum bran and breakfast cereals). These two by-products represent promising biomass sources for sustainable applications in the food and agricultural sector.

Hemicellulosic materials from cereal by-products, namely wheat bran, rye, corn hulls and bran, have been previously investigated in film applications (Alekhina, Mikkonen, Alén, Tenkanen, & Sixta, 2014; Ebringerová & Heinze, 2000). Specifically, hemicellulose-based films from cereal by-products have been studied in food packaging applications, where their high water permeability and low elasticity has been reported as a challenging property (Ebringerová & Heinze (2000) and Sárossy, Tenkanen, Pitkänen, Bjerre, & Plackett (2013b). Low elasticity leads to brittle hemicellulose-based films, particularly in the case of xylans that exhibit low arabinosylation in their structure. To overcome this challenge, a plasticiser can be used to modify the physical properties of hemicellulosic films. Plasticiser, usually a hydrophilic compound, plays a role in increasing the mobility and reducing the intermolecular forces of the polymer chains or acts as water binding agent during film development (Pommet, Redl, Guilbert, & Morel, 2005) . On the other hand, water permeability affects the degree of barrier properties of film against water vapour as well as its solubility.

Internal plasticisation has also been studied to develop high quality of hemicellulose films using hydrophobic carbon chains (2-octenylsuccinic anhydride half-ester groups) (Zhong, Peng, Yang, Cao, & Sun, 2013). This compound has a function to substitute the hydroxyl groups of hemicellulose as an attempt to improve its hydrophobicity. This study focused on the utilisation of sorghum-derived hemicellulosic fractions for the development of films containing soil fertilisers. Additionally, their potential in food packaging applications was also investigated though the evaluation of their key physical and mechanical properties.

4.2. Materials and Methods

4.2.1. Materials

Sorghum bran was obtained from the Indonesian Cereals Research Institute, as a milling by-product, and sorghum stalk was obtained from the farm of a national company in Indonesia, [PTPN XII (PERSERO)]. The bran was screened through 60 mesh size sieve. The leaves and the panicles were stripped out from the stalks. The stalks, after squeezing their juice using a roller mill, were dried in sunlight and then cut into small pieces, ground down and screened through 60 mesh size sieve. Commercial arabinoxylan (wheat bran AX) was obtained from Megazyme (Ireland) and commercial xylan from birchwood was obtained from Universal Biological (UK). All chemicals were of analytical or reagent grade including ethanol (95%, v/v), Dimethyl sulfoxide (DMSO) (ACS reagent, ≥99.9% v/v), 2-Octen-1-yl succinic anhydride, mixture of *cis* and *trans*, triethylamine, purchased from Sigma Aldrich, UK and were used directly without further purification.

4.2.2. Methods

4.2.2.1. Isolation of Hemicelluloses

The extraction of hemicelluloses from dried sorghum stalk (HS) and bran (HB) was performed under alkaline conditions (Xiang, Watson, Tobimatsu, & Runge, 2014; Xiao, Sun, & Sun, 2001). Briefly, 5 g of dry-milled sorghum stalk and sorghum bran was mixed with 200 mL NaOH solution 1.0 M and 0.75 M, respectively at 50 ^{o}C, in a 1:20 solid to liquid ratio. The mixture was kept at 50 ^{o}C and stirred at 200 rpm for 3 h, followed by centrifugation at 17.105 x g for 20 min. The alkali-soluble supernatant was adjusted to pH 5.5 using 6 M HCl and concentrated to about one-third of its original volume in a rotary evaporator (Buchi R110, Switzerland). Subsequently, three volumes of ethanol (95%, v/v) were slowly poured into the solution under constant stirring. The precipitated solid was separated and washed with 95% ethanol (±30 ml) using a filter paper and was designated as alkali-soluble hemicellulose. Subsequently, the precipitate was freeze-dried (Virtis SP scientific model 2KBTES, USA) until further use.

4.2.2.2. Film Preparation

Films were prepared by casting method. Extracted hemicelluloses (400 mg) were dissolved in water (20 ml) at 70 ^{o}C for 30 min. During mixing, glycerol was added to the solution in different concentrations (10% and 30%, w/w from the amount of hemicelluloses weight), and air was removed from the mixture by sonication under vacuum. The solution was poured onto a teflon dish and allowed to dry in an oven at 40°C overnight. Film thickness was measured using a micrometer (Lorentzen & Wettre (Sweden), precision = 1 μm). Measurements were taken at 10 different locations on each film, and the mean value was calculated to determine the thickness. The thicknesses of the films ranged between 0.03 to 0.17 mm.

4.2.2.3. Preparation of 2-OSA Modified Xylan

Film modification was prepared as described by Zainal Abiddin, Yusoff, & Ahmad (2015) and Zhong et al.(2013). Extracted xylan from sorghum stalk (HS) and commercial xylan (XC) (1 g) were dissolved in DMSO (25 mL) at 90 °C for 60 min and then cooled down to room temperature to induce precipitation. The required amount of 2-OSA was added slowly over 2 h. The reaction was allowed to continue for 2 h at the same pH value of 8-8.5 using triethylamine (initial pH was 10) to precipitate the modified xylan. The reaction was stopped by quenching in ethanol, and the precipitate was centrifuged and rinsed with ethanol and then dried using a freeze dryer (Virtis SP scientific model 2KBTES, USA).

4.2.2.4. Thermal Analysis

Structural and thermal characterisation of sorghum by-products derived hemicellulosic fractions xylan fractions were carried out through differential scanning calorimetry (Perkin Elmer DSC 8000, USA). DSC scans were performed at a heating rate of 5◦C/min from -50 to 250°C under nitrogen environment.

4.2.2.5. Mechanical Properties

The tensile strength and breaking elongation of films were measured by texture analyser (model TexturePro CT V1.2 Build 9, Brookfield Engineering Labs, USA) using probe TA9 with target 50 mm, test speed 2 mm/s, trigger load 4.5 g and return speed 4.5 mm/s. It was used to determine tension of samples according to ASTM Standard Method D882-88. The films strips were stretched (each measuring 100 mm x 7.5 mm) at load cells 4500 g with an initial grip separation of 7.5 mm. Each film formulation was tested 3 times, and the reported values are the averages of each of the 3 samples tested. The tensile strength was calculated by:

Tensile strength = (load at break)/ (original width) (original thickness)

Percent elongation is calculated by dividing the elongation at the moment of rupture by the initial gauge length and multiplying by 100. The result is expressed in percent.

Percent elongation = (elongation at rupture) x 100 / (initial gauge length)

4.2.2.6. Water Absorbance

Exactly weighed blend films (±0.05 g) were immersed in 5 ml distilled water for 5 seconds. Then, the excess water was removed by tissue and swollen films were weighed. The water absorbency was calculated as suggested by Han, Chen, & Hu2009), which expressed as:

Water absorbance (%) = (swollen film) x 100 / (initial film)

4.2.2.7. Water Vapour Transfer Rate

The test was modified as described by Laboulfie, Hémati, Lamure, & Diguet (2013). Permeability cups were filled with dry silica particles to maintain 0% relative humidity. Films were clamped on top of the cups and the measurements were done at controlled conditions of 50 % RH in a desiccator at 20 °C in the presence of saturated $MgNO_{3}.6H_{2}O$ solution. After incubation for 24 h, the increase in the weight of the cup was monitored up to 3 days to assess the Water Vapour Transmission Rate (WVTR) of the films. Net weight (grams) versus time (hours) plots were obtained. The WVTR values of the films were calculated as follows, and the normalized values with respect to film thickness were reported to eliminate the possible effect of film thickness on permeability:

$$\text{WVTR= slope (g/h)} \times \text{(24 h /day)]/area of the film (m}^2\text{)}$$

$$\text{NWVTR= WVTR (g/day}\bullet\text{m}^2\text{)/film thickness (m)}$$

4.2.2.8. X-Ray Diffraction

All hemicellulose films were analysed on a Bruker D8 Advance using a copper X-ray source (wavelength 1.54 angstroms) with a Lynx eye detector. The collection parameters were: 2 theta 5.0-64 degrees, at a speed of 10°/min.

4.2.2.9. Scanning Electron Microscopy (SEM) Analysis

Differences in surface film morphology were investigated using SEM (Carl Zeiss, EVO MA 10, UK) with a detector SE (Secondary Electron), WD (Working Distance) 7 mm and EHT (Extra High Tension) with voltage 18 kV. The samples were sputter-coated with gold prior to examination.

4.2.2.10. Controlled release of hemicelluloses-based films containing fertiliser in soil

Sorghum bran hemicellulose (HB) and modified sorghum stalk hemicellulose films (HS) were prepared through cross-linking using 2-Octenyl Succinic Anhydride (2-OSA). As a comparison, commercial arabinoxylan-based films were also prepared. Commercial fertiliser containing nitrogen (N), phosphorus (P), and potassium (K) with ratio 5:3:8 was added at 2% (w/v) to the solution prior to film casting. N was measured by Kjeldahl analysis. P was measured by colorimetric analysis (Lu, 1999). The water soluble potassium content was measured by Atomic Absorption Spectroscopy (AAS) (Perkin Elmer Coorporation, 1996). The release ratios of nitrogen (N%), phosphorus, (P%) and potassium (K%) were calculated using the following equations:

$$N\% = m1/m0 \text{ X } 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

$$P\% = m3/m2 \text{ x } 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

$$K\% = m5/m4 \text{ x}100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

where, m0, m2, and m4 are the weights of the total nitrogen, phosphorus and total potassium, respectively. m1, m3, and m5 are the weights of water-soluble nitrogen, phosphorus and potassium in soil.

4.2.2.11. Biodegradability of Films

The biodegradability of the produced films was also determined. Films were cut into small squares (2 x 2 cm). Each specimen was weighed and placed in agricultural soil (15 g) in a pot. The pots were exposed to ambient conditions (25 ^{o}C) for 3 days. Variations in film morphology and the disintegration time were recorded.

4.3. Results and Discussion

4.3.1.1. Sugar Composition of Sorghum Hemicellulosic Fractions

The sugar composition of stalk-extracted hemicellulose (HS) and bran-extracted hemicellulose (HB) are shown in Table 4-1. Commercial arabinoxylan (AXC) and commercial xylan (XC) were also analysed for comparison purposes. The results revealed that the composition of HS, a hemicellulosic fraction of sorghum stalk, consisted primarily of xylose (~51%), glucose (~11%) and arabinose (~8%), whereas HB contained 75% of glucose and almost equal amounts of xylose and arabinose (5.95% and 5.54%, respectively). Based on previously conducted NMR and FTIR analysis in this study (Chapter 3), the type of hemicellulose in HB was mixed-linkage glucans, comprising mostly of glucose and are associated with arabinoxylan (AX) in the pericarp tissues. A previous study reported the isolation of low molecular weight of β-glucan in polished grain fractions of sorghum (Verbruggen, Beldman, Voragen, & Hollemans, 1993); while, hardly any information is available in the case of the bran. Xyloglucan (XG) is a hemicellulose that occurs in the primary cell wall plant of eudicots and non-graminaceous monocotyledonous plants (Hotchkiss et al., 2015).

Apart from sugar composition in the hemicelluloses fraction, the ratio between arabinose to xylose (A/X) also needs to be taken into account since it influences the characteristics of the obtained hemicellulose fraction and its subsequent polymerisation. HS had an A/X ratio of ~0.15, demonstrating low branching. This ratio provides insights on the substitution pattern of AX structure which has a function as a structural defect, preventing macromolecule crystallization (Sternemalm, Heikkinen, Tenkanen, & Gatenholm, 2008). The unsubstituted regions in the AX structure promote crystallization (Dervilly, Saulnier, Roger, & Thibault, 2000). Furthermore, the substitution of AX is a factor which influences its solubility in water and it is expected to affect the subsequent film morphology and functionality.

In HB, the xylose to glucose ratio was ~0.08, similar to the ratio of arabinose to glucose (0.07), whereas the A/X ratio was 0.93. It can be inferred that HB had a complex structure and was a high branching type of hemicellulose. Based on the data presented at Table 4-1, the commercial hemicellulose from wheat bran (AXC) has an A/X ratio of 0.61 which represents a quite high branching type of hemicellulose. The type and degree of substitution (DS) varies between species and between parts of the plant (Heikkinen et al., 2013). In wheat, hemicellulose from the straw is less substituted than hemicellulose from the endosperm and the bran. However, the birchwood hemicellulose had a very low ratio of A/X (0.01), representing a low branching type of hemicellulose.

Table 4-1 The content of sugars and uronic acid (% dry matter) of the arabinoxylan commercial, xylan commercial, stalk-extracted hemicellulose, and bran-extracted hemicellulose.

Sugars (%)	Sample			
	HS[a]	HB[b]	AXC[c]	XC[d]
xylose	51.83±0.11	5.95±0.48	55.85	87.62
arabinose	7.99±0.2	5.54±0.57	34.08	1.05
glucose	11.41±1.47	75.85±0.92	1.9	1.01
uronic acid	0.94±0.97	0.71±0.05	3.88	1.13
A/X	0.15	0.93	0.61	0.01
X/G	nc[e]	0.08	nc	nc
A/G	nc	0.07	nc	nc
Total carbohydrate	72.17	88.05	95.71	90.81

[a]stalk-extracted hemicellulose; [b]bran-extracted hemicellulose; [c]commercial arabinoxylan extracted from wheat bran; [d]commercial xylan extracted from birchwood; [e]nc=not calculated (Xyl is the backbone).

4.3.2. Development of hemicellulosic films

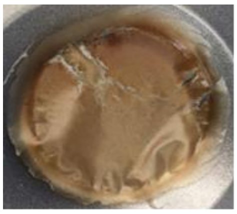
The structure of the hemicelluloses affects their subsequent film forming capacity (Egüés, Eceiza, & Labidi, 2013). In this study, HS film exhibited better film formation with the addition of 10% and 30% of glycerol, but brittleness and cracking still occurred on the film after drying. The film with 30% glycerol exhibited improved elongation at break and deformation at peak properties. Hemicellulose-based films have been previous studied by introducing long hydrophobic carbon chains into xylan which resulted in improved mechanical strength and less sensitivity to humidity (Zhong et al., 2013). The 2-OSA chains in the xylan backbone have a role in preventing the aggregation of xylan chains by disrupting the intramolecular and intermolecular hydrogen bonds during water evaporation as a result of the steric hindrance of long side chains. It also has a role as internal plasticizer and concomitantly reduces the tight packing of the polysaccharide chains, increases molecular mobility, and opens access routes for water to dissolve the polymer. Thus, the 2-OSA-HS film had better performance with no brittleness and form a continuous film as seen at Table 4-2.

In the case of HB films, the addition of 30% of glycerol in the solution lead into improved performance in term of deformation at peak and elongation at break compared to 10% of glycerol, which led into less brittle and more elastic films. The surface of HB films was uniform at the end of the drying process, and the produced films were easy to peel off the casting surface. However, in this study, they proved to be very sensitive to humidity at high RH (70%) as those reported by Alekhina et al. (2014) that plasticisation of films decreased the moisture uptake at low RH (<40%) and increased it at high RH (50%-90%) . This due to the presence of glycerol, which occupies the water sorption sites of films at low RH conditions; however, at high RH conditions, glycerol hydrophilicity increases and binds water from the environment. Thus, a suitable application of this type of film when considering potential packaging applications is as wrapping material for dried goods which are usually stored at low relative humidity conditions.

In terms of colour, HB and HS films had yellowish colour due to the natural yellow colour of hemicellulose and also due to the presence of lignin (HB and HS still contained 1.77% and 2.33% w/w of lignin, respectively). Films made from AXC were transparent whereas XC films had a yellow colour, and were transparent after the addition of 10% and 30% of glycerol. The films with 30% glycerol exhibited improved elasticity. Additionally, the thickness of the films varied between 0.02 and 0.17 mm. Moreover, the colour of 2-OSA-xylan film was brighter and more translucent compared to the one without modification (HS film).

In the case of XC films, the addition of 10% and 30% glycerol was not able to form a visually uniform and cohesive film. Therefore, XC film formulations were modified by adding 2, 5 and 10% of 2-Octenylsuccinic anhydride (2-OSA) was also conducted in order to improve the subsequent film development. Additionally, HS were also modified with 10% OSA for the same purpose. It was shown that the appearance of the films was greatly improved when the modification XC with 2-OSA occurred (Table 4-2).

Table 4-2 Visual appearance of developed films

Material	Appearance	
	10 % glycerol	30% glycerol
HS		
HB		
AXC		
XC		
XC- 10% OSA		
HS-10% OSA		

4.3.3. FT-IR Spectra

Both HS and HS-OSA films spectra demonstrated similar profiles. A broad peak at 3313 cm^{-1} mainly corresponded to O-H stretching of the abundant hydroxyl groups on hemicelluloses. The significant amount of hydroxyl groups may explain the hydrophilic nature of the films. The band at 2915 (cm^{-1}) indicated CH bond deformation of CH_2-CH_3 groups. The succinylation of xylan was verified by FTIR spectra of modified films (Figure 4-1), as the peak at 1721 (cm^{-1}) corresponded to a new ester bond in the xylan back bone, (Zhong, Peng, Yang, Cao, & Sun 2013). The prominent peak of hemicellulose was found at 1022 (cm^{-1}) and arabinose residues at 1151 (cm^{-1}), whilst the domain β-glycosidic bonds between sugars were represented by the peak at 894 (cm^{-1}).

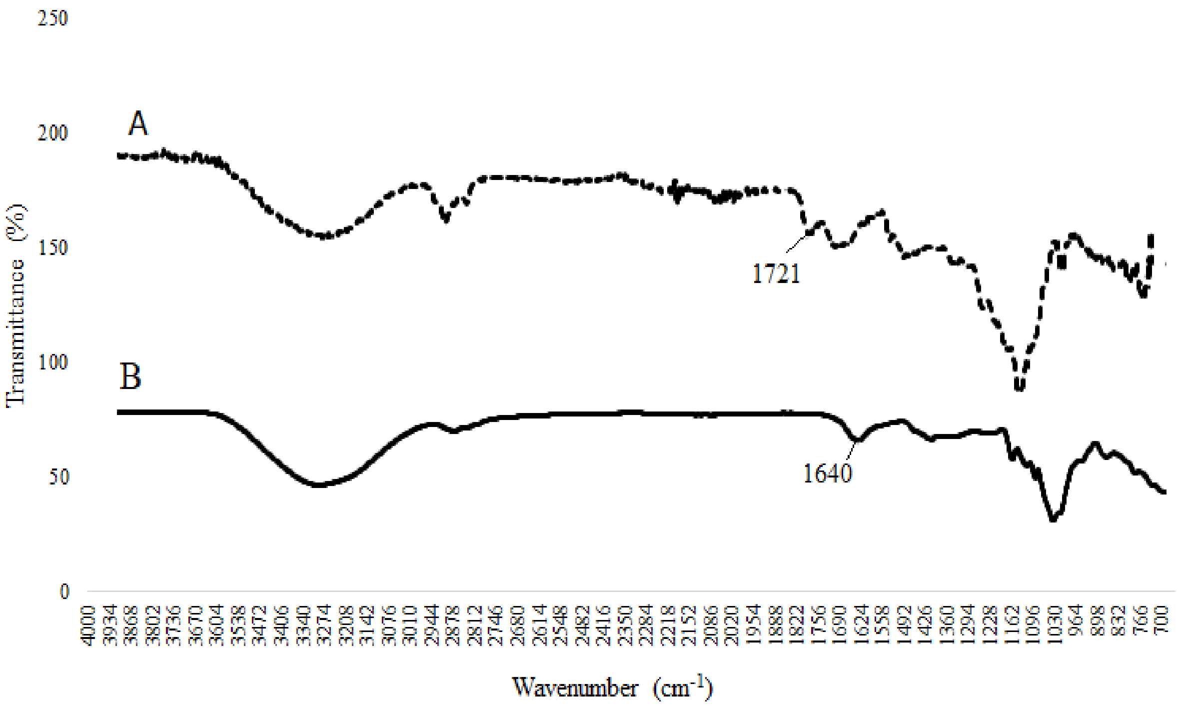


Figure 4-1 FT-IR spectra of xylan film 2-OSA-HS (A) and unmodified HS film (B)

4.3.4. Mechanical Properties

Mixed-linkage glucans originated from sorghum bran had similar A/X ratio to rye bran and oat bran (0.54 to 0.93); however, the structural conformation might differ. Films generated from mixed-linkage glucans have been reported to be cohesive but brittle without addition of plasticiser and prone to cracking (Sárossy, Tenkanen, Pitkänen, Bjerre, & Plackett,2013). Hence, glycerol imparts beneficial effects when incorporated into film formulations by increasing the elongation and deformation value of the films and at the same time decreasing their stiffness and strength (Blmfeldt, Hedenqvist, Koch, Ray, & Plackett, 2012). Mixed-linkage glucan-based films have been reported to exert good mechanical properties; however, they are less effective moisture barriers compared to arabinoxylan-based films (Mikkonen et al., 2009).

The mechanical properties of HB, HS, commercial arabinoxylan (AXC), and commercial xylan (XC) films were investigated. It can be observed from Figure 4-2, that HB films had better mechanical properties than HS films in terms of tensile strength (6.5 to 7.8 MPa), elongation at break (15.33 to 16.55%), deformation at peak (7.9 to 11.5 mm), and total work (10.5-13 mJ). This indicated that HB films were stronger and more elastic than HS, which is likely due to the presence of mixed-linkage glucans and a greater degree of branching compared to HS. Sárossy, Tenkanen, Pitkänen, Bjerre, & Plackett (2013) reported that the addition of mixed-linkage glucan into purified arabinoxylan resulted in an improvement in terms of the tensile strength and elongation at break of the films.

Overall, AXC films had superior mechanical properties with regards to tensile strength, deformation at peak, elongation, and work. AXC, a hemicellulose fraction from wheat bran, has a ratio A/X of 0.61 and its medium viscosity was ~30 cp. This highly branched AX from wheat bran has been reported to exert a beneficial influence on tensile strength and elongation at break of films (Heikkinen et al., 2013), a fact which was also confirmed in our study.

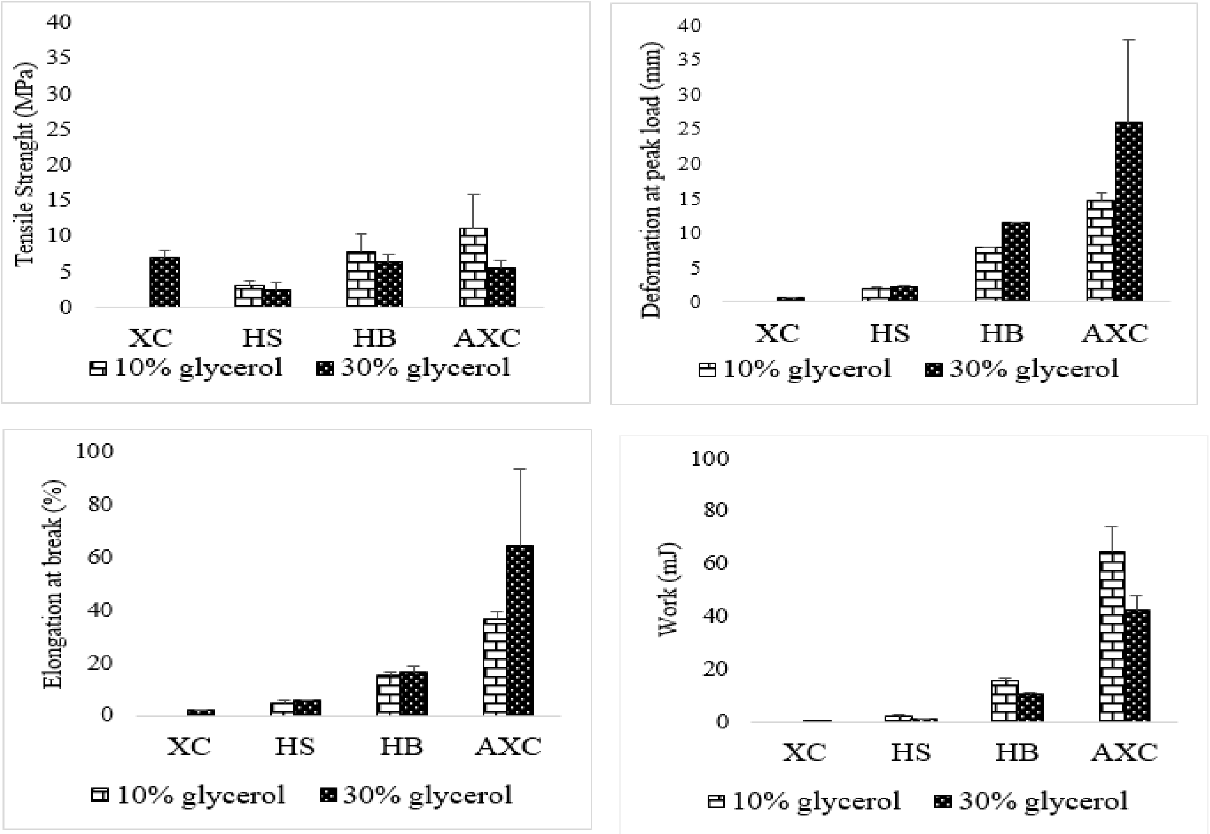


Figure 4-2 Mechanical properties of hemicellulosic films: commercial xylan from birch wood (XC), sorghum stalk-extracted hemicellulose (HS), sorghum bran-extracted hemicellulose (HB), commercial arabinoxylan from wheat bran (AXC). All films were added 10 and 30% of glycerol (w/w of hemicellulose). XC Film with addition 10% of glycerol could not form a continuous film.

The modification of XC films with 5% OSA improved their mechanical properties particularly in deformation at peak load and elongation at break for more than 45% compared to unmodified XC films (Figure 4-3). This demonstrated that the internal plasticisation of XC film was successful, leading to the derivatisation of the xylan backbone with long-chain succinic anhydrides even at low degrees of substitution (Hansen & Plackett, 2011). The addition of 2-OSA, which is a hydrophobic group, into XC and HS is one way to reduce the high moisture sensitivity and to generate hydrophobic esters. The substitution may occur at any hydroxyl group of the xylan back bone. The 10% 2-OSA-XC film gave the best performance in terms of thickness (0.15 mm) and total work. It can be seen that 10% 2-OSA-XC films had the highest work value (Fig. 4-3). Although OSA-XC 5% films were characterised by high tensile strength, they were relatively thin (0.04 mm) and not uniform. The 10% 2-OSA-HS demonstrated an improvement in terms of deformation at peak load and elongation at break. By taking all these into account, it was decided to modify HS fraction by adding 10% of OSA. The 10% OSA-HS film, as depicted in Table 4-2, demonstrated improved mechanical strength and barrier properties compared to the unmodified one.

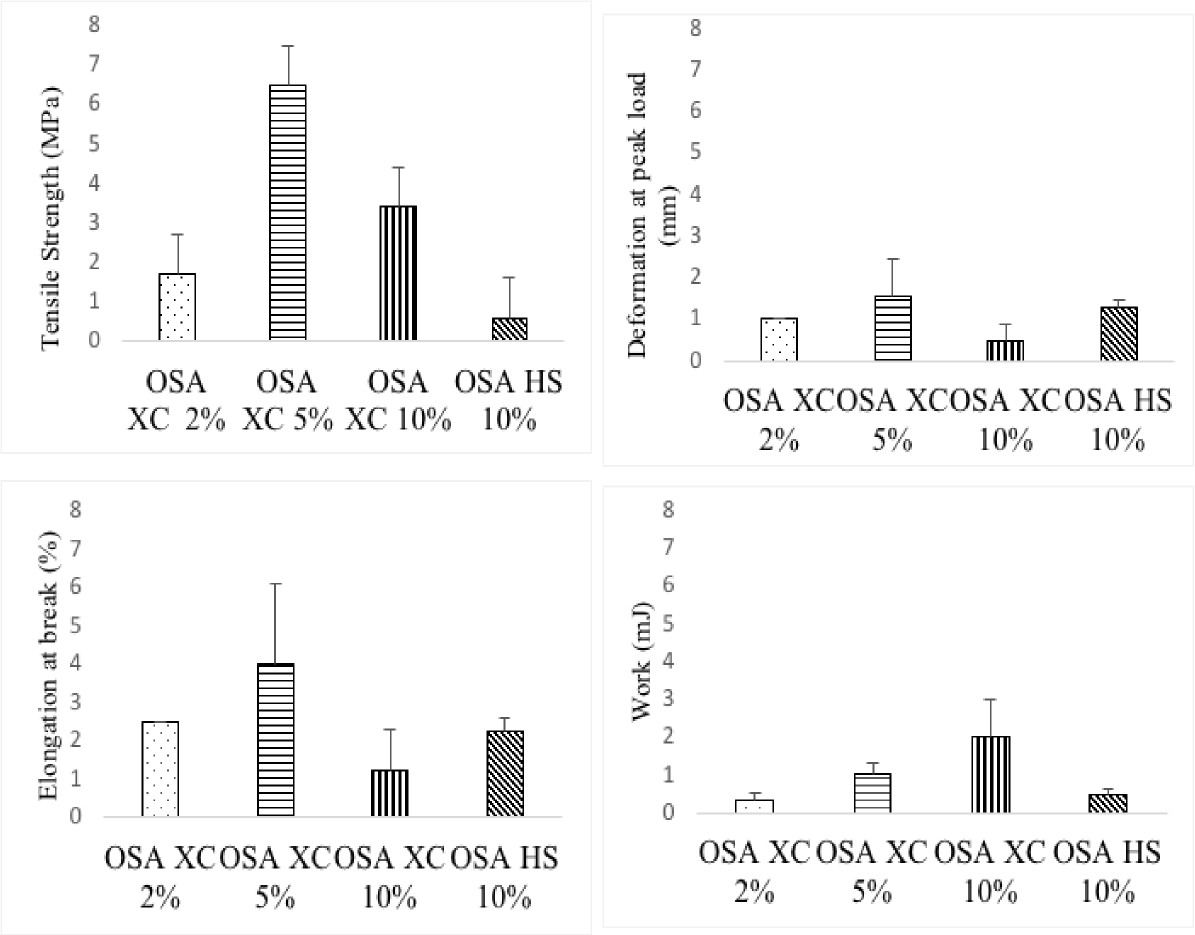


Figure 4-3 Mechanical Properties of commercial xylan (XC) and sorghum stalk-extracted hemicellulose films (HS) modified with 2-OSA.

4.3.5. Physical Properties of Hemicellulosic Films

The results obtained by differential scanning calorimetry (DSC) are shown at Table 4-3. All films demonstrated glass transition temperatures (Tg) below room temperature, indicating that at room temperature they are in the rubbery state. This property is similar to plastic packaging materials such as PP and PE. These plastics tend to be flexible and difficult to break at room temperature. However, below Tg values, the amorphous material is in a glassy state and as such the molecular motions are restricted and films are brittle and might fracture easily under mechanical stress (Heikkinen et al., 2013).

The Tg value also defines the amorphous state of the film material. It may be semi crystalline or completely amorphous. It can be seen from Table 4-3 that the use of higher concentration of glycerol (external plasticising) decreased the Tg value of HS, HB, XC, and AXC films. The presence of plasticiser increases the free volume in the polymer network by reducing the number of hydrogen bonds between the polymers, therefore increasing its flexibility and reducing the brittleness of the film. As a result, the physical and mechanical properties of the films change. On the other hand, the internal plasticising of HS revealed that the modification with 2-OSA decreased the Tg value compared to the unmodified film.

Furthermore, it can be observed that plasticising affects the melting point temperature of films and their water vapour permeability (WVP). The increment of glycerol inclusion from 10% to 30% slightly increased the melting point temperature (Tm) in AXC, HS, and XC films. Factors that affect WVP are the hydrophilicity or hydrophobicity of the materials within the film. Moisture barrier properties of films are important especially in the context of food packaging applications. It should be noted that the WVP values for HS and XC films were not obtained due to the poor structure of the films which did not allow their inclusion in the WVP tests. The increment of glycerol from 10% to 30% improved the WVP values of HB and AXC films. These results are in agreement with a previous study by Mikkonen et al. (2015), in which WVP values were improved in plasticized films. The WVP values of HB films were slightly higher than the AXC film. This can be explained by the fact that HB consisted of mixed-glucans which are more hydrophilic and have more binding sites to form hydrogen bonds in the presence of water compared to arabinoxylans (Sárossy et al., 2013).

However, OSA-HS exhibited comparable WVP values with HB and AXC films. All WVP values in this study were lower than hemicellulose of other cereal films in previous study such as WVP for the oat spelt AX with 10% glycerol was 3.3 [(g mm)/(kPa m2 d)], and with 10% sorbitol it was 1.1 [(g mm)/(kPa m2 d)] (Mikkonen et al., 2009). WVP of rye bran arabinoxylan film was 7.7 [(g mm)/(kPa m2 d)] (Sárossy, Tenkanen, Pitkänen, Bjerre, & Plackett, 2013). These results indicate that all films in this study had good moisture barrier properties at RH 50%.

Table 4-3 Physical properties of films

Films	Crystallinity (%)	Moisture content (%)	Tg (°C)	Tm (°C)	WVP [g mm/ (mm^2 K Pa d)]
HS 10% glycerol	0	nd	0	99.19	nd
HS 30% glycerol	0	nd	-20	134.72	nd
HB 10% glycerol	0	6.56%	-45	148.82	1.07
HB 30% glycerol	3.3	8.31%	-50	132.85	0.95
XC 10% glycerol	0	nd	-25	100	nd
XC 30% glycerol	0	nd	-50	110	nd
AXC10% glycerol	0	7.61%	5	99.21	0.56
AXC 30% glycerol	0	11.51%	-20	100.25	0.55
OSA-HS	27	8.42%	20	85.72	0.82

nd: not determined

4.3.6. X-ray Diffractogram of Hemicellulosic Films

Figure 4-4 shows the x-ray diffractograms of the films. All films except 2-OSA-HS exhibited an amorphous morphology. The distinctive crystalline peaks were observed in the case of 2-OSA-HS films and the crystallinity was 27% (Table 4-3). In addition, the major peaks of the XRD patterns for all films except 2-OSA-HS showed diffraction at point ~20o (in 2θ). The AXC film had higher peak intensity, indicating a higher level of molecular organisation compared to other films.

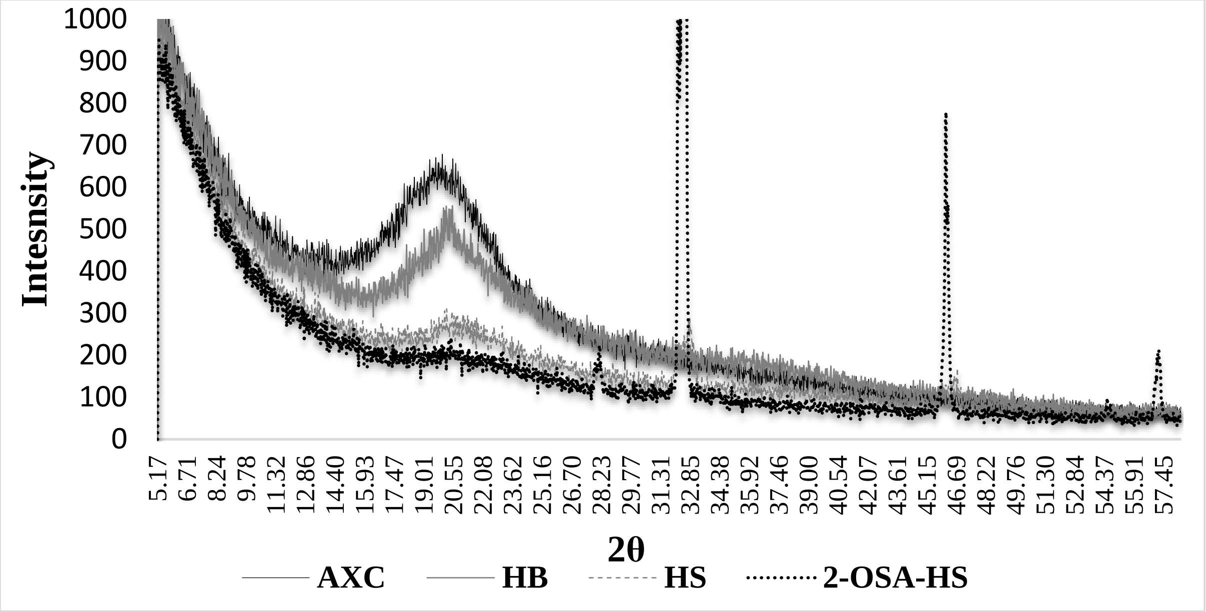


Figure 4-4 X-ray diffractograms of films

Only in the HB film, the increment of glycerol from 10% to 30% increased the crystallinity of the film to 3.3% which indicates that external plasticisation may increase the crystallinity of the hemicellulose film by increasing the polymer chain mobility. HB, a mixed-glucan hemicellulose, is more hydrophilic and may generate more hydrogen bonds in the presence of water (Sárossy et al., 2013).

4.3.7. Microstructure of Hemicellulosic Films

The microstructure of films was determined by scanning electron microscopy. Globule formation was observed on the surface of films that contained glycerol (Fig. 4-5A, 4-5B, 4-5C, 4-5D). The globules were larger in the aforementioned films than in the 2-OSA-HS film (Fig. 4-5E). This was related to the interaction of hemicellulose and glycerol which are connected by hydrogen bonds leading into a rough surface. However, 2-OSA-HS film exhibited a smooth surface with nodules dispersed on the surface. The size of nodules was approximately 559.6 nm (Fig. 4-5F). According to Zhong, Peng, Yang, Cao, & Sun (2013), the nodules on the surface of OSA-modified xylan films result from strong hydrogen bonds. The large nodules on the surface of modified films could potentially be the result of an incompact aggregation of xylan chains and could be regarded as an index of the degree of hydrogen bonds for hemicelluloses with and without modification.

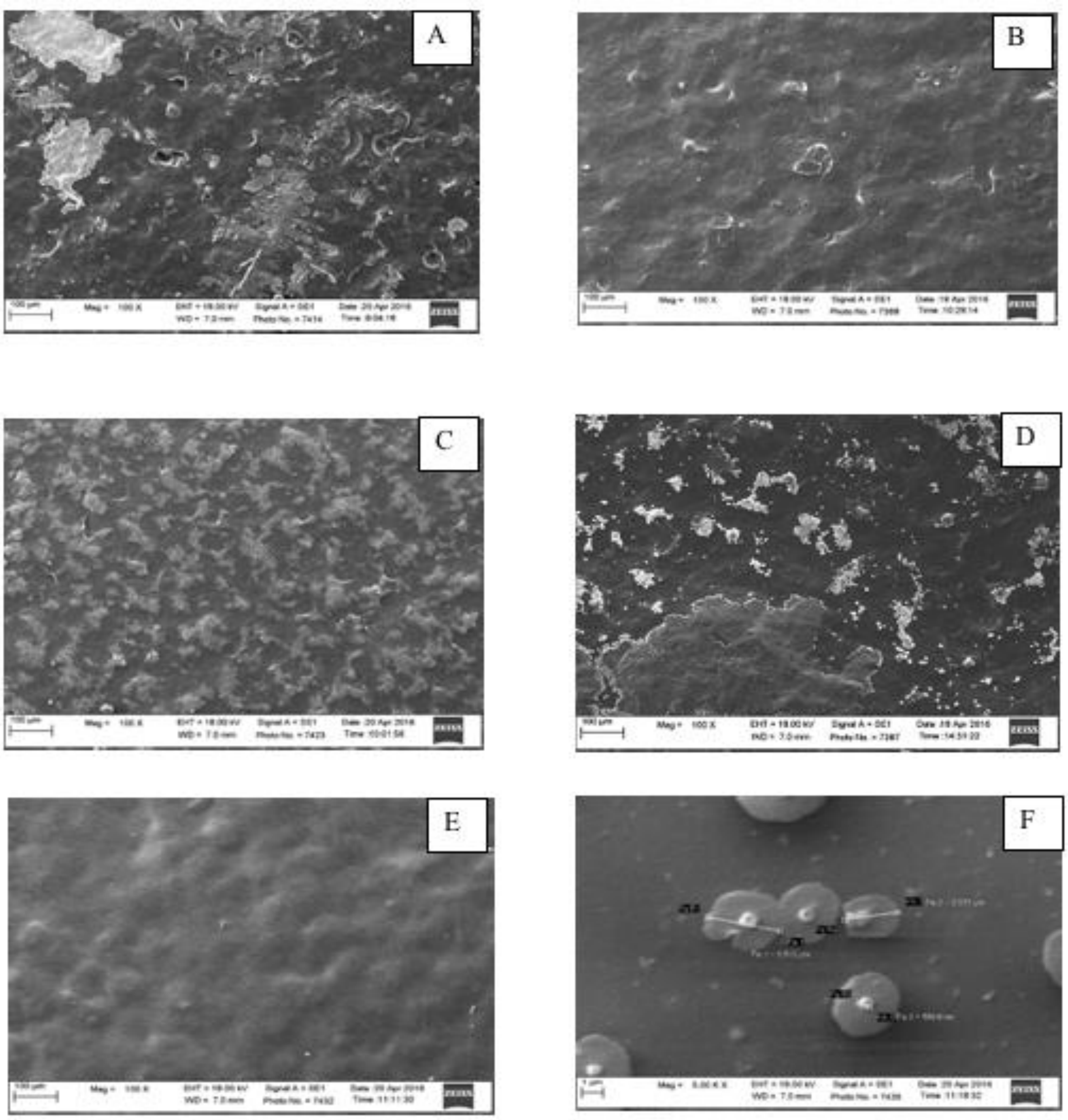


Figure 4-5 SEM images of films A. HB with 10% glycerol, B. AXC with 30% glycerol, C. XC with 10% glycerol, D. HS with 10% glycerol, E. 2-OSA-HS. (All Mag. 100x), and F. 2-OSA-HS (Mag. 5000x)

4.3.8. Food And Agricultural Applications Of Hemicellulosic Films

AXC, HB and 2-OSA-HS films were investigated for their potential as polymers containing fertilisers such as nitrogen, phosphate, and potassium (NPK) and also as wrapping material for perishable fruits such as grapes. On the first occasion, the goal was to entrap the fertiliser within the film polymer structure and subsequently monitor its release in the soil. This application may be relevant in grow bags in green house, hydroponic types of agriculture or even for domestic uses (plants growing in pots). The use of controlled-release fertilisers (CRFs) increases their efficiency, reduces nutrient loss and soil toxicity, minimizes the potential for negative effects associated with over dosage, and reduces the frequency of the applications in accordance with normal crop requirements (Corradini, de Moura, & Mattoso, 2010).

The release pattern of films containing fertiliser and their biodegradability were examined in soil. It is known that hemicellulose films are very sensitive to high humidity conditions (Zhong et al., 2013). Therefore, we examined the performance of the films at RH 80% and 20 ^{o}C while moisture content of the soil was 70%. The composition of commercial fertiliser included nitrogen (N), phosphorous (P), and potassium (K) in the soil and in the films are presented at Table 4-4.

Table 4-4 Concentration of N, P, K in soil and films (%w/w)

Material	**N (%)**	**P (%)**	**K (%)**
Soil	0.1058±0.04	0.026±0.003	0.01±0.00
OSA-HS	1.7505±0.08	0.003±0.001	0.04±0.001
HB	1.5087±0.05	0.006±0.001	0.03±0.001
AXC	0.3603±0.09	0.008±0.001	0.03±0.001

The soil used in this study had a pH value of 5.7 (slightly acidic) and low micronutrient content: N (0.1%), P (26.65%), and K (11.83%). The cation exchange capacity (CEC) as reported by Indonesian Agency for Agricultural Research and Development (IAARD) was also low (13.88%), comprised of Ca (7.2 cmol/kg), Mg (1.05 cmol/kg), K (0.12 cmol/kg), and Na (0.06 cmol/kg). According to the CEC data, it can be deduced that the soil was course- to very coarse-textured, formed from gravelly or sandy glacial outwash or glacial lake beach ridges or deltas. This type of soil needs to be essentially enriched with several actions, such as the application of acidifying nitrogen fertilisers and/or liming materials (ISRI, 2005). The lower the CEC, the faster the soil pH will decrease with time. So, sandy soils need to be limed more often than clay soils. The application of films with fertiliser in soil samples showed a quicker release of nitrogen to the soil compared phosphor and potassium (Fig. 4-6). All films degraded in one day. 2-OSA-HS film had the slowest release of fertiliser. The release of N was almost 100% after one day, a fact which suggested that 2-OSA-HS film constituted a weak carrier of fertiliser. Apparently, the N-H bonds which ascribed to hydrogen bonding to surface hydroxyls via the nitrogen atom of the amine group generated a weak interaction.

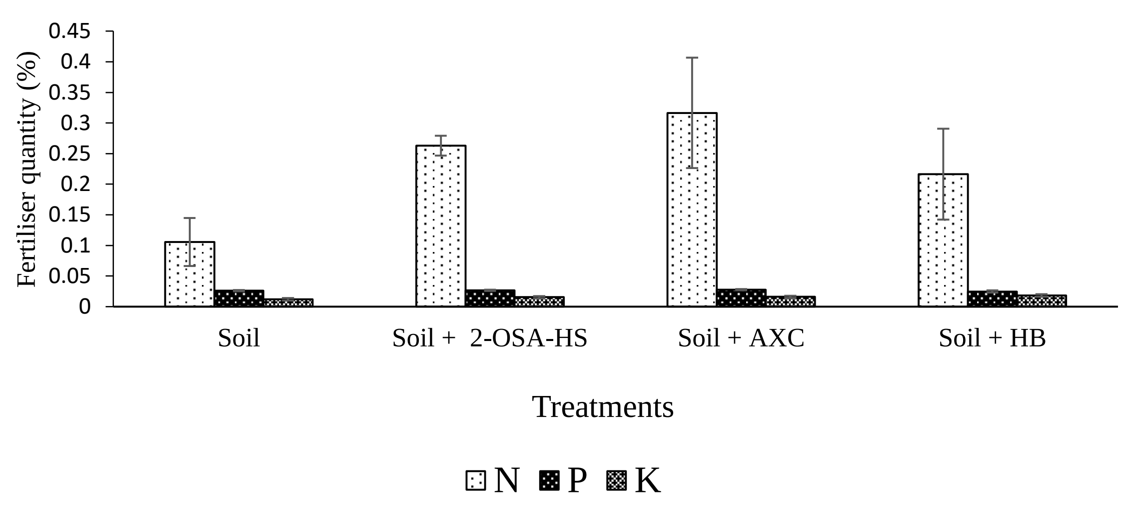


Figure 4-6 Fertiliser quantity released in the soil after 1 day.

According to the spectra from FTIR analysis in the 2-OSA-HS film containing fertiliser (Figure 4-7), there were no broad peaks at the region between 3400-2900 cm^{-1} which showed that there is a change in the structure of the 2-OSA-HS with the incorporation of N, P and K fertilisers. These peaks showed the absence of OH groups. Low & Rao (1969) reported that the broad peak at 3600 cm^{-1} was not produced in the absence of OH groups because of mild degassing of the film during production. In our study, degassing was part of film formation process in order to remove air bubbles from the film mixture. On the contrary, broad absorption peaks were shown the spectra of 2-OSA-HS films that did not contain NPK fertiliser (Figure 4-1Figure 4-1 FT-IR spectra of xylan film 2-OSA-HS (A) and unmodified HS film (B)). The prominent band of xylan at 1186 cm^{-1} is attributed to the C–O, C–C stretching or C–OH bending, while the sharp band at 985 cm^{-1}, corresponded to the characteristic β-glycosidic linkage between the sugar units. However, new bands at 1543 cm^{-1} and 1377 cm^{-1} were observed, suggesting that Schiff base (C-N double bond) was formed between reducing sugar of xylan and nitrogen from NPK fertiliser. The bond between xylan and K or P might be detected in the region of 1000-700 cm^{-1} as mentioned by Řeřicha et al. (2005), in which the bond between OH and K was predicted at the region of 1000-700 cm^{-1} .

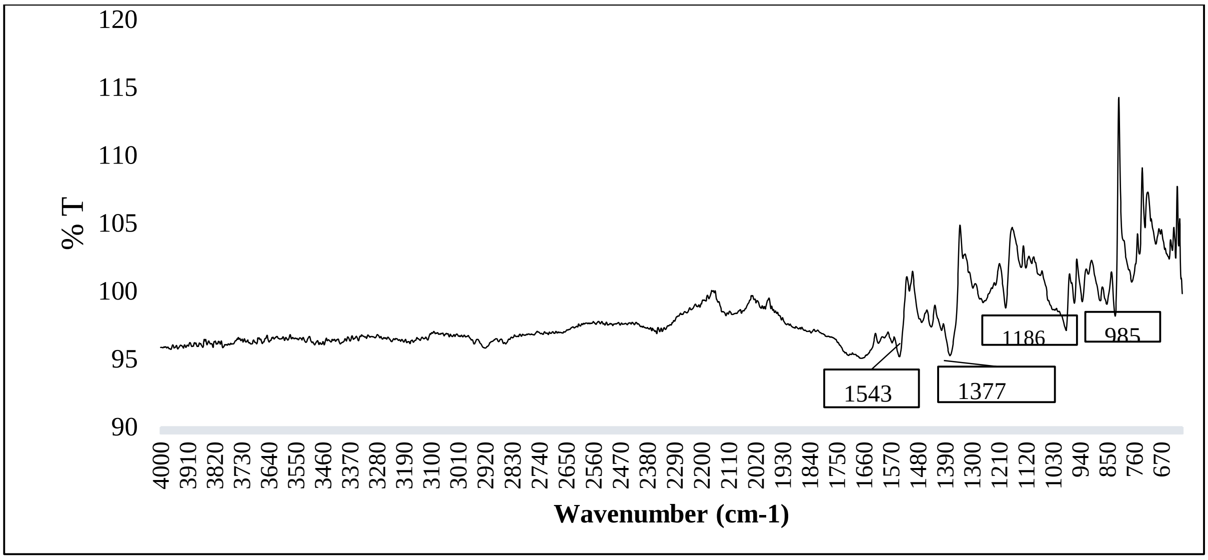


Figure 4-7 FTIR analysis of 2-OSA-HS film containing fertiliser.

Apart from agricultural applications, a selection of developed films, and more specifically AXC, HB and 2-OSA-HS films, were also evaluated as sealed-wrapping material, using perishable food products (grapes). Packaging materials that are considered suitable for such applications should be able to prevent moisture loss from the food product and potentially extend their shelf-life. Figure 4-8 shows the weight loss of the grapes over the course of 7 days of storage at room temperature (A) and at chilled conditions (4°C) (B). The weight loss of grapes decreased after sealed-wrapping storage, especially for grapes wrapped with 2-OSA-HS films. 2-OSA films resulted in a fruit weight loss of 14% (w/w) in room temperature and 7% (w/w) in the fridge after 7 days of storage (Figure 4-9). AXC and HB films had almost comparable weight losses at the end of the 7-day storage (23 and 24% w/w in room temperature and 11% and 18% w/w in the fridge, respectively) (Figure 4-9). These findings demonstrate the good moisture barrier ability of all films and in particular of the 2-OSA-HS films. It has been previously reported that non-film coated grapes may lose more than 80% of their initial weight after 7 days storage at ambient conditions (22°C and RH 40 ± 10%) (Zhang & Whistler, 2004).

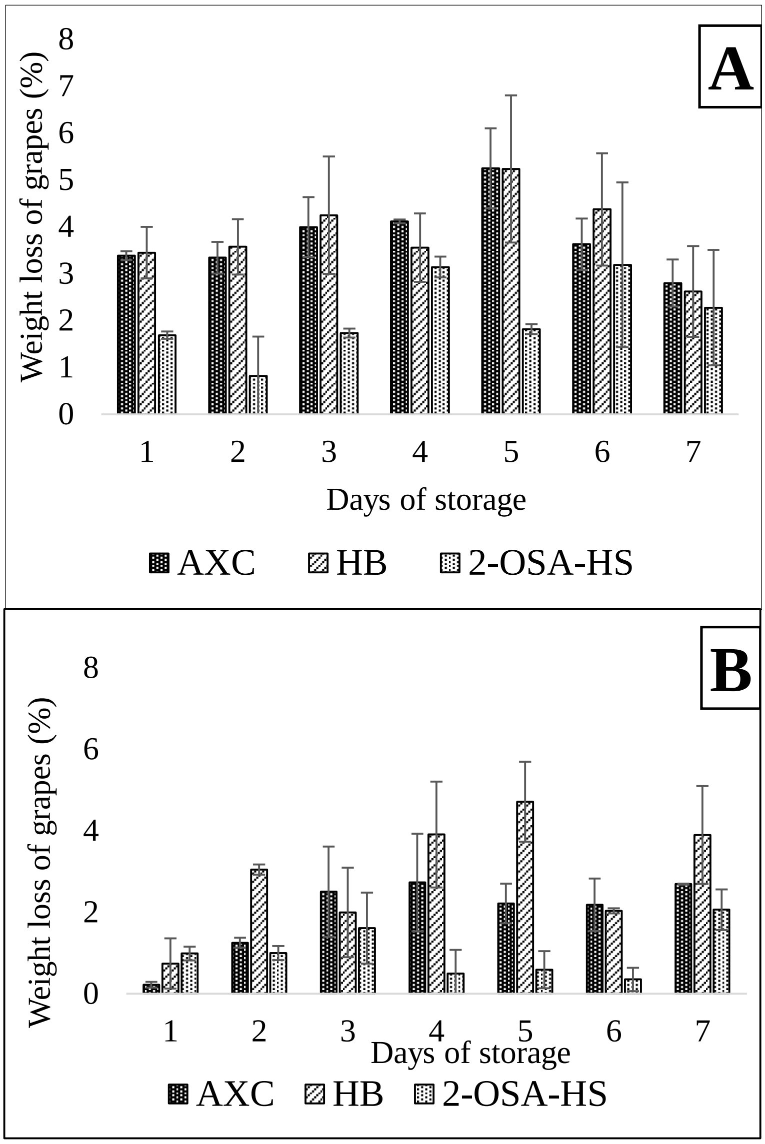


Figure 4-8 Weight loss of grapes sealed with films of arabinoxylan (AXC) (▦), hemicellulose bran (HB) (■) and modified xylan with succinyl anhydride (2-OSA-HS) (⊞) at room temperature (A) and fridge (4°C) for a 7-day period (B).

All three films, and particularly 2-OSA-HS ones, were shown to significantly delay moisture loss from grapes and especially during storage at 4°C. This could be directly related with the fact that 2-OSA-HS films exhibited high moisture barrier properties (0.82 g mm/(mm^2 K Pa d) and had a smooth microstructure based on the outcomes of the SEM images (Fig. 4-5). Films with adequate moisture barrier properties can prevent deterioration and extend the shelf life of food products (Zhang & Whistler, 2004).

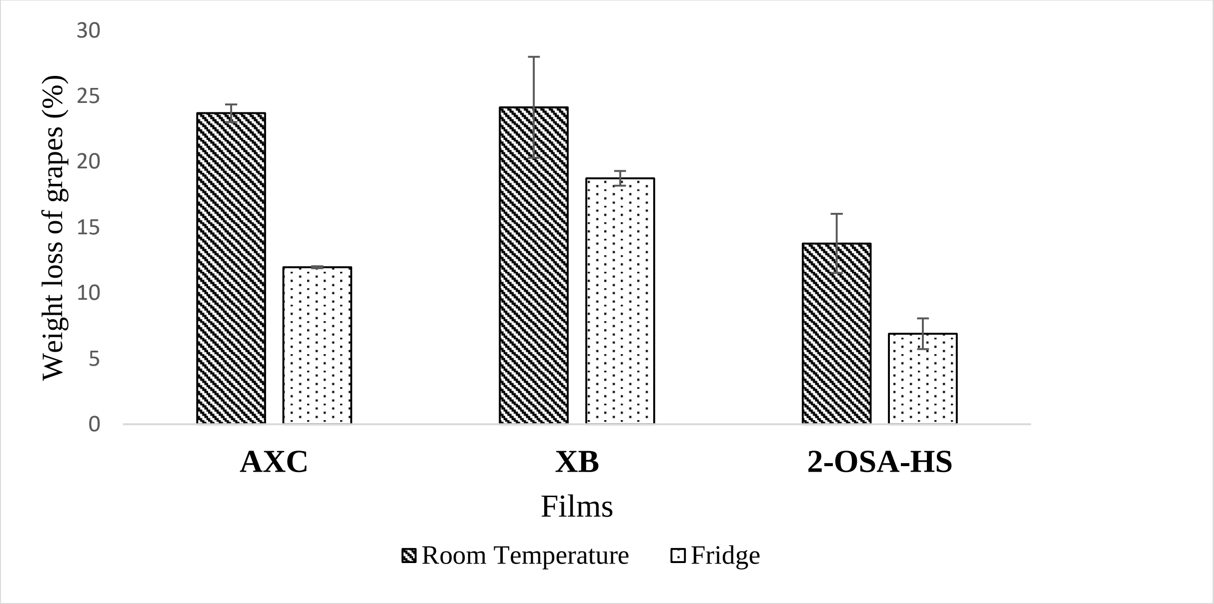


Figure 4-9 Total weight loss of grapes (%) during storage at room temperature (RT) (▧) and fridge (⬚)

4.4. Conclusion

The application of bran- and stalk-derived hemicellulose fractions as starting materials for the development of films represents an alternative valorisation route for sorghum by-products. With regards to the key characteristics of the developed films, chemical plasticisation of stalk-derived hemicellulose with 2-OSA was shown to improve the mechanical properties of the films. However, the internal plasticisation of bran-derived hemicellulose with glycerol aided into the development of films with better mechanical and barrier properties. The degree of substitution (A/X ratio) as well as the type of hemicelluloses was found to affect the internal plasticisation and the moisture barrier properties of the films. Based on the findings of the study, sorghum-derived hemicellulosic films could be applicable as polymers for controlled-release fertilisers, as well as in the food industry as packaging material.

Acknowledgements

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Chapter 5: Starch-based Biodegradable Foams with Fractions Extracted From Sorghum By-Products

Abstract

Agricultural by-products comprise abundant, renewable sources of value-added compounds for the food and packaging industries. The production of biodegradable food trays could be focused on the replacement of polystyrene-based foams with biodegradable materials, which are regarded as environmentally friendly. In this study, biodegradable food trays were developed via thermopressing, using modified and unmodified corn starches with the addition of extracted fractions (cellulose, hemicellulose and lignin) from sorghum by-products (stalk and bran). All trays presented thicknesses between 3 and 4.17 mm, density between 0.16 and 0.64 g cm^{-3}, and moisture content between 5 and 12% (dry basis). The foam trays made from chemically modified starch had lower density, higher tensile strength and elongation at break than those with unmodified starch. The inclusion increment of cellulose fraction from 2% to 5% and hemicellulose fraction from 1% to 10% had a significant impact on the trays' colour and mechanical properties, water absorption capacity, microstructure and crystallinity. All trays exhibited no colour and structural changes during storage at 25 ^{o}C and 4 ^{o}C for 7 days. The food tray formulation that exhibited the best properties contained 2% of cellulose and 1% of hemicellulose, had a maximal resistance of 0.77 MPa and 9.46 mJ of total work, attributes which corresponded to a compact, homogenous, and dense microstructure. The half-life time of the particular food tray according to Arrhenius equation was predicted to be 3 months.

Keywords: biodegradable foam, sorghum, thermopressing, corn starch, cellulose, hemicellulose, lignin.

5.1. Introduction

The valorisation of agricultural by-products comprises an ongoing field of research, aiming to identify novel pathways for their exploitation as abundant renewable and cheap sources. One such pathway represents their utilisation as food packaging material, targeting the replacement of petroleum-based materials with environmentally friendly and biodegradable ones. Sorghum (Sorghum bicolor L. Moench) by-products represent potentially suitable materials for the development of biofoams as they have high cellulose content, which is needed to impart strong mechanical properties and water resistance. Previous research has investigated pure cellulose and xylan as raw materials by using extrusion or hot mould baking as methods to produce biodegradable packaging products (Xu, Kim, Hanna, & Nag, 2005). Biodegradable foams can be used in combination with biodegradable fillers, such as lignin, for the development of biocomposites, which have applications in a variety of industries, including food, agricultural, automotive and chemical.

In biodegradable foam production, starch is the main material due to its swelling properties. The type of starch may influence the characteristics of biofoam, due to the ratio of amylose to amylopectin. The foam developing process includes the gelatinisation of starch, the evaporation of water and finally the molding stage (Kaisangsri, Kerdchoechuen, & Laohakunjit, 2014; Lawton, Shogren, & Tiefenbacher, 2004; R. L. Shogren, Lawton, & Tiefenbacher, 2002). However, biodegradable foams are susceptible to water absorption and relative humidity which ultimately affect their mechanical properties (Kaisangsri et al., 2014; Polat, Uslu, Aygün, & Certel, 2013; Soykeabkaew, Supaphol, & Rujiravanit, 2004). Therefore, several studies have focused on the improvement of water resistance properties of foams by adding fibre (Kaisangsri et al., 2014; Lawton et al., 2004), oil (Kaisangsri et al., 2014), protein (Salgado, Schmidt, Molina Ortiz, Mauri, & Laurindo, 2008), chemical compounds such as PVA (Shogren et al., 2002), chitosan (Kaisangsri et al., 2014), kaolin and beeswax (Polat et al., 2013b).

In this study, the focus was on the development of packaging materials, and in particular food trays. Starch-based foams were developed in order to replace the expanded polystyrene foam packaging currently in use. Key aspects of investigation were the compatibility between sorghum-derived cellulose fractions and xylan (often used for biocomposites) with starch and other additives, as well as the mechanical and physical properties and their shelf life of the subsequently developed food trays. Additionally, sorghum-derived lignin fraction was used as a filler for food trays' formulations, with a view to increase the water resistance and shelf life of the developed biofoams.

5.2. Materials and methods

5.2.1. Materials

Commercial cassava and corn starch were used in this study, and had a moisture content of 12% (w/w, dry basis). The amylose content of cassava and corn starch was 28% (w/w) and 17% (w/w), respectively. Cellulose, hemicellulose and lignin fractions extracted from sorghum by-products, as described in Chapter 3, were also used in this study. Commercial poly vinyl alcohol (Mw 146,000-186,000) and magnesium stearate (technical grade) were used as additives and purchased from Sigma Aldrich as well as 2-Octenyl succinic anhydride (OSA).

5.2.2. Methods

5.2.2.1. Preparation of cross-linked corn starch with 2-OSA

Corn starch modification was prepared as described by Zainal Abiddin, Yusoff, & Ahmad (2015) with some modifications. Briefly, 150 g of corn starch were dispersed in 400 ml distilled water with agitation. To this mixture, OSA at 2 % (based on dry starch basis) was added dropwise for 2 hours. The reaction was allowed to continue for 24 hours. The 2-OSA corn starch was washed three times with distilled water, collected by filtration through Whatman filter paper No. 1 and dried in an oven at 45°C for 24 hours.

5.2.2.2. Tray Processing by Thermopressing

The trays were prepared with two types of starch (cassava and corn starch) and several formulations were tested. Table 5-1 lists the formulations that were applied, using corn and cross-linked corn starch, whereas the formulations using cassava starch are shown in Table 5-2. Additives (PVA, magnesium stearate, xylan, and lignin) were mixed with starch (60-80% w/w). The method of tray processing that was followed, was based on a previous study (Schmidt & Laurindo, 2010). Cellulose (2, 3, 4, 5% w/w) and water (45-50 g) were also added into the dough and mixed using a mixer. 70-80 g of dough for each formulation were layered into a thermopressing plate. A Teflon lid was placed over the plate and the system was set at 170 ^{o}C for 3-4 minutes. Then, the trays were removed from the equipment, cooled down to room temperature and cut into square molds. The trays were stored at room temperature and RH 50% before further characterisation analysis.

Table 5-1 Food tray formulation from corn-starch, cross-linked corn starch, cellulose, polyvinylalcohol (PVA), hemicellulose of bran, lignin and water (w/v of solid proportion).

Sample	Batter solid mass (60 g)**					
	S	PVA	MgS	C	H	L
	(%)					
A	80%	10	5	5	-	-
B	80%	10	5	5*	-	
C	50%	15	5	2		
D	CL 80%	10	5	2	1	-
D-1	CL 80%	10	5	3	1	-
D-2	CL 80%	10	5	4	1	-
D-3	CL 80%	10	5	2	5	-
D-4	CL 80%	10	5	2	10	-
E	80%	10	5	2	1	
F	60%	20	5	2	1	
F-1	CL 60%	20	5	2	1	
G	60%	20	5	2	1	10

*cellulose was soaked in water for 30 min to increase the moisture content, PVA: polyvinilalcohol, MgS: magnesium stearate, CL: cross-linked.
**40-50 mL water was added to the batter and baking time was 240 s.

Table 5-2 Food tray formulation from cassava-starch, cellulose, polyvinylalcohol (PVA), hemicellulose of bran, lignin and water (w/v of solid proportion).

Sample	Batter solid mass (60 g)*						
	S	PVA	MgS	C	H	L	OSA
	(%)						
H	83%	10	5	2			
I	73%	-	5	4	8	10	
J	71%	-	5	4	10	10	5% (w/v of water)

PVA: polyvinilalcohol, MgS: magnesium stearate,.
*40-50 mL water was added to the batter and baking time was 240 s.

Density was calculated as the ratio between weight and volume (Shogren et al., 1998). Reported values are the mean of five independent measurements for each formulation.

5.2.2.3. Mechanical Properties of Food Trays

Before testing, the thickness was measured by a digital micrometer. Measurements were conducted in triplicate. A texture analyser model Texturepro CT V1.2 Build 9 (Brookfield Engineers Lab. Inc) using probe TA 18 was used to measure tensile strength (according to

ASTM Standard Method D882-88). The condition set up was trigger load 50 g, test speed 1 mm/s, load cell 4.5 g and return speed 1 mm/s. The trays were compressed (3 cm x 3 cm) at load cells 4500 g. Each tray formulation was tested 3 times, and the reported values are the averages of each of the 3 samples tested. Tensile strength was calculated by dividing the load at break by the original width. The results were expressed in megapascals (MPa).

Tensile strength = (load at break)/ (original width) x (original thickness)

5.2.2.4. Water Absorbency

Trays (2 cm x 1 cm) were immersed in 5 ml distilled water for 1 minute until sufficiently swollen. Then, the excess water was removed by tissue and the trays were weighed in an analytical balance to calculate the absorbency. The water absorbency was calculated as suggested by Han, Chen, & Hu (2009), which expressed as:

Water absorbance (%) = (swollen tray) x 100 / (initial tray)

5.2.2.5. Thermal Analysis

Structural and thermal characterisation of cellulose and hemicellulose fractions was carried out by differential scanning calorimetry (DSC) (Perkin Elmer DSC 8000, USA). Approximately 5-10 mg of sample was measured for each pan. An empty pan was also used as a reference. Both the samples and the reference were heated at a heating rate of 10oC/min from -50°C to 250°C using nitrogen flushing.

5.2.2.6. X-Ray Diffraction

A X-ray powder diffractometer (Bruker D8 Advance, Germany) was used to record the diffractogram of biodegradable foams at 25 °C. The X-ray source was Cu Kα radiation at 40 kV and 35 mA (λ = 1.54 Å). The samples were mounted on a sample holder, and the pattern was recorded in the reflection mode at an angle of 2θ over a range of 5.000° to 80.030° at a speed of 10°/min. The crystallinity index (Cr.I) was calculated as suggested by Ciolacu, Ciolacu, & Popa (2011) as follows:

$$Cr.I. (\%) = (Sc / St) \times 100,$$

where: Sc – area of the crystalline domain, St – area of the total domain.

5.2.2.7. Colour Analysis

Foam colour was determined using a colorimeter (CR 300, Minolta Chroma Co., Osaka, Japan). A CIE Lab color scale was used to measure the degree of lightness (L), redness (+a) or greenness (a), and yellowness (+b) or blueness (b) of the foams. The instrument was calibrated using a set of three Minolta calibration plates. Foams were measured on the surface of the white standard Expanded Polysterene Styrofoam (EPS) with color coordinates of L = 97.3, a = 0.14 and b = 1.71. Total color difference (ΔE) was calculated as follows:

$$\Delta E = [L_{foam} - L_{standard})^2 + (a_{foam} - a_{standard})^2 + (b_{foam} - b_{standard})^2]^{0.5}$$

Values were expressed as the mean of three measurements for each sample.

5.2.2.8. Pasting Characteristics

The pasting properties of 5% starch pastes were measured using a Rapid Visco Analyser (Perten Instruments, Australia) with Thermocline for Windows (TCW) software for viscometric data acquisition and analysis. The starch suspensions were stabilised for 2 min at 50 ˚C, and then heated to 95 ◦C for 10 min, kept at that temperature for 5 min, then cooled to 50 ˚C within 10 min, and maintained at 50 ˚C for 5 min (duplicate). Pasting parameters were measured over time under constant shear, and included the onset of pasting to peak viscosity (Ptime); temperature at which peak viscosity was reached (Ptemp); peak viscosity (PV); viscosity at the end of the holding time at 95°C or hot paste viscosity (HPV) and viscosity at the end of the holding time at 50°C or cold paste viscosity (CPV). Based on these parameters, breakdown, final viscosity and setback were calculated. Typical RVA pasting curves are presented in Figure 5-1.

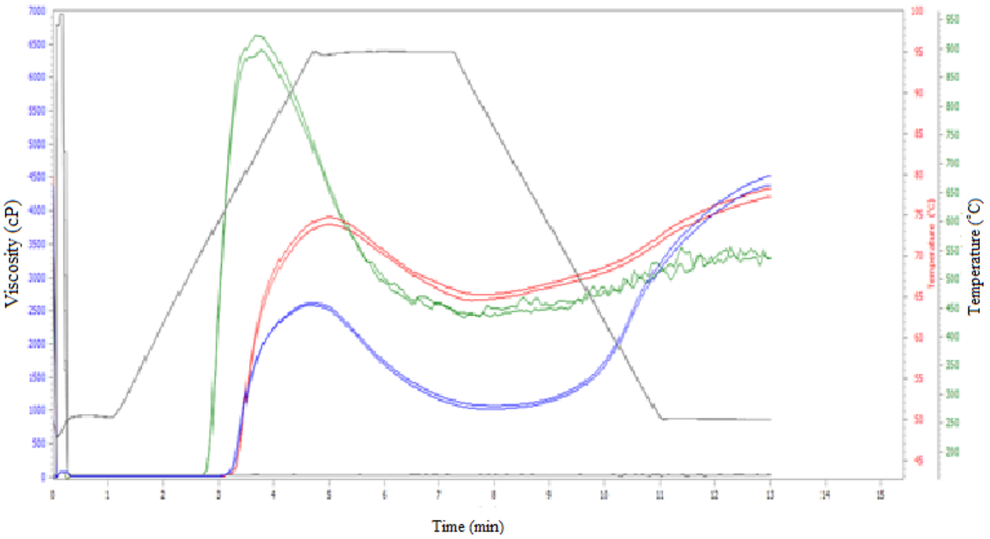


Figure 5-1 Typical RVA pasting curves for the corn starch (—); cross –linked starch (—), and cassava starch showing (—).

5.2.2.9. Biofoam Storage Test

The shelf life of biofoams was determined in food storage trials. Selected biofoams were used as a tray for fruits with high moisture content (grapes), wrapped in thin polyethylene film (cling film). The samples were stored at room temperature (25 °C) and in the fridge (4 °C) for 9 days. The measurements conducted included a visual observation of the biofoams each day in terms of discoloration, moisture migration and condensation, as well as weight recording.

5.2.2.10. Accelerated Shelf Life Test of Biodegradable Foams

To predict the shelf-life of the biofoams, the Arrhenius equation was applied through linear regression analysis of moisture content at different relative humidity conditions (53%, 75%, and 83%) at 25 oC. In most cases, deterioration of product quality follows a first-order reaction formula, indicated by the following numerical expression:

$$(dA/dt) = k (A) n (n = 1),$$

Arrhenius equation expressed adequately the relative humidity (RH) dependence of reaction rate of moisture content (dry basis).

$$A = Ao \exp(-kt) \text{ or } \ln At = \ln Ao - kt \qquad \text{(Eq. 1)},$$

where At and Ao are the moisture content (dry basis) at time t and zero respectively and k is the apparent reaction of moisture content, estimated by the slope of the linearized plot of ln (A) vs time.

$$t_{1/2} = \ln 2/k_{eff} = 0.693/k_{eff} \qquad \text{(Eq. 2)},$$

where t ½ is the half life time of the biofoam.

5.3. Results and Discussion

5.3.1. Pasting Behavior

Commercial cassava, corn and modified corn starch with 2-OSA were used in this study as the main components of the developed food trays. The starches' pasting behaviour is presented in Table 5-3. The most important qualities that determine starch functionality are the amylose to amylopectin (AM/AP) ratio, the viscosity development characteristics, and some other minor constituents of the abovementioned starches such as protein, phosphorus and lipid.

Table 5-3 Parameters of pasting characteristics of native corn, cross-linked corn, and native cassava starches.

Samples	AM/AP	PT (^oC)	PV (cP)	HPV (cP)	BD (cP)	FV (cP)	SB (cP)
Native corn	0.66±0.01^a	76.7±0.01^a	3838±76^a	2684±62^a	1154±14^a	2776±71^a	417±14^a
CL corn	0.65±0.02^b	75.8±0.21^b	2584±22^b	1034±37^b	1125±38^b	1150±16^b	1855±69^b
Native cassava	0.53±0.03^c	71.1±0.14^c	6509±37^c	2383±4.59^c	2522±2.12^c	4126±16^c	-3240±69^c

Values followed by the same letters in columns are not statistically different at α= 0.05; n=2. AM/AP-ratio amylose to amylopectin, PT-pasting temperature, PV-peak viscosity, HPV-hot paste viscosity, BD-breakdown (PV-HPV), FV-final viscosity, SB-setback (FV-HPV).

It can be observed that the ratios of AM/AP in corn and CL-corn starch were not significantly different ($p>0.05$); however, they were significantly different with the native cassava starch ($p<0.05$). The different ratio of AM/AP between cassava and corn starch is due to the fact that cassava starch has a larger proportion of short branch chains of amylopectin and also due to the presence of phosphate monoesters in root starches (Jane et al., 1999). The results suggested that cassava starch was more stable than corn starch under storage. A sudden drop of cassava starch viscosity (-3240 cP at setback) after the maximum indicates the breakdown on cooking as well as a great ability to swell. Due to this property, cassava starch is suitable for food products which are stored for long periods and require to retain their soft texture, such as in the case of desserts (Raphael et al., 2011).

Additionally, the modification of corn starch using 2-OSA affected the pasting behaviour of the sample (Table 5-3). The pasting temperature or gelatinisation of native corn (76.7 ^{o}C) and CL-corn starches (75.8 ^{o}C) in this study were higher than the cassava starch (71.1 ^{o}C) (Table 5-3). This result is in agreement with a previous study conducted by Jane et al (1999), in which the cassava starch had lower pasting temperature than normal and waxy corn starches. This difference could be attributed to differences in starch granule sizes and the starch components (amylose, amylopectin etc). It has been reported that starches with large granules undergo gelatinisation relatively faster than smaller fractions because of less molecular bonding in their structure that causes them to swell faster as well as break down faster (Jay lin Jane, 1992). The structure of starch consists of α-(1,4)-D-glucose units (amylose) and α-(1,6)-D-glucose units (amylopectin). Amylose and amylopectin are linked through hydrogen bonding and arrange themselves radially in layers to form granules. Larger starch granules have higher viscosity than smaller sized ones, as the latter have been shown to be more sensitive to shear forces (Tsakama, Mwangwela, Manani, & Mahungu, 2010).

Corn starch granules absorb less water, are resistant to swelling and could not freely leach amylose during heating cycles in pasting analysis. A modification is essentially needed to improve specific starch functionalities such as low pasting temperature and low final viscosity which render the starch suitable for biodegradable foam applications. In this study, the modification with 2-OSA led into a less hygroscopic corn-based starch. It is suggested that the hydroxyl groups in the modified starch molecules, which are responsible for forming hydrogen bonds with the surrounding moisture, could increase the strength of subsequently developed foams (Soykeabkaew, Thanomsilp, & Suwantong, 2015).

During the cooling stage of the pasting process, the viscosity of starches decreased due to the disintegration of starch granules and polymer realignment. It can be observed that CL-corn starch could maintain the paste viscosity as opposed to other starches. The 2-OSA as a crosslinking agent generates linear or branched chains which are interconnected covalently to starch chains. Therefore, this modified starch was more stable, more transparent, and less prone to retrogradation or recrystallization of the starch molecules. The short-term and long-term developments of crystallinity in starch have been attributed to the gelation and retrogradation of the amylose and amylopectin fractions, respectively. This processes take place within the temperature range of glass transition temperature (T_g) < T < melting point temperature (T_m) (Alcázar-Alay & Meireles, 2015; Soykeabkaew et al., 2015). This pasting behaviour is essential in order to determine the suitability of starch in biodegradable foams and it can affect the tray's characteristics as well as the interaction of starch with other components within the trays' batter.

5.3.2. Development of Biodegradable Foams

Defined concentrations of each component (starch, cellulose, hemicellulose, PVA, and lignin) (Table 5-1 and Table 5-2) were chosen for the preparation of the tray batters, based on preliminary tests and previous studies. PVA was added to the batter to increase the foam strength and water resistance (Shogren, Hashimoto, & Pomeranz, 1988). Magnesium stearate has a role as a mold release agent and prevents starch from sticking to the walls of the mold (Shogren et al., 2002). The addition of water was kept minimum (45- 50 mL per 60 g of batter), in order to assist the solubilisation of cellulose in the batter and allow the formation of homogeneous dispersions. In order to investigate any improvement in the mechanical properties of the developed trays, up to 5% (w/w) of cellulose was added in the formulation.

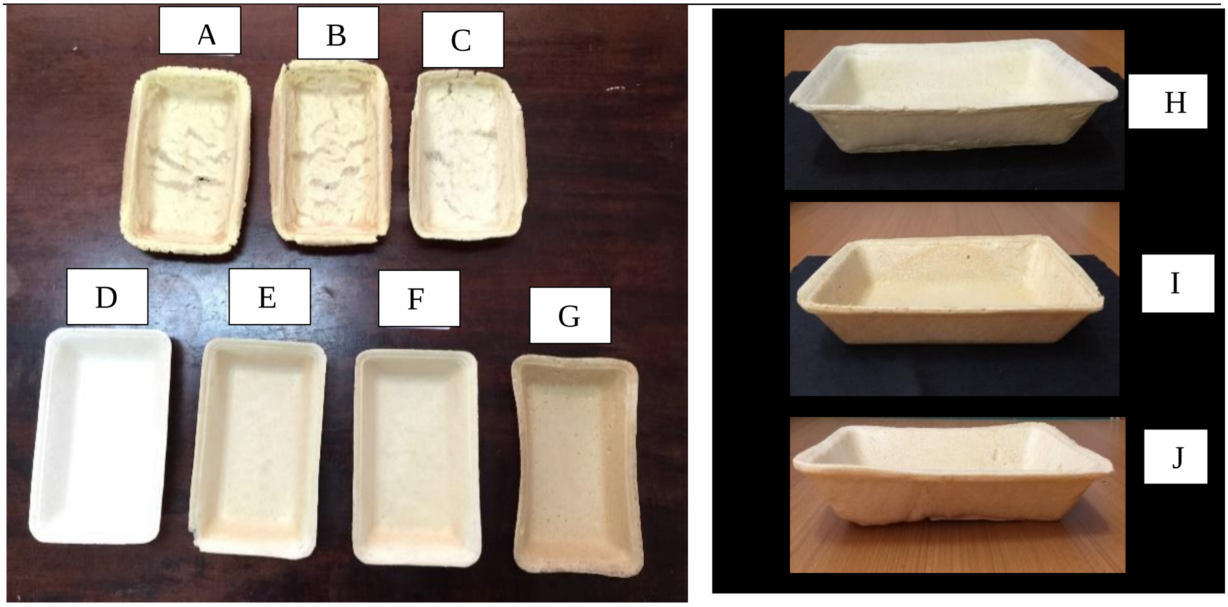


Figure 5-2 Biodegradable Foams based on corn, cross-linked corn and cassava starch. A (80% corn starch, 10% PVA, 5% MgS, 5% cellulose); B (80% corn starch, 10% PVA, 5% MgS, 5% soaked cellulose); C (50% corn starch, 15% PVA, 5% MgS, 2% cellulose); D (80% CL-corn starch, 10% PVA, 5% MgS, 2% cellulose, 1% hemicellulose); G (60% corn starch, 20% PVA, 5% MgS, 2% cellulose, 1% hemicellulose, 10% lignin); H (83% cassava starch, 10% PVA, 5% MgS, 2% cellulose); I (73% cassava starch, 5% MgS, 4% cellulose, 8% hemicellulose, 10% lignin); J (71% cassava starch, 5% MgS, 4% cellulose, 10% hemicellulose, 10% lignin, 5% Octenyl succinic anhydride (OSA) (w/v)).

A 5% (w/w) cellulose inclusion in the formulations led to agglomeration of cellulose in the biofoams, as seen in Figure 5-2A and Figure 5-2B. This finding suggested that cellulose could not disperse well in the batter matrix. However, formulations with 50% corn starch, 15% PVA, 5% MgS and 2% cellulose (Figure 5-2C) exhibited agglomeration of cellulose and led into not fully well-shaped trays. The rest of formulations which included 60-80% (w/w) of starch and 2-4% (w/w) of cellulose, led into successfully formed, well-shaped trays, lacking of evident cracks (Figure 5-2).

5.3.3. Physical Properties of Developed Trays

The thickness of the foams ranged from 3.00 to 4.43 mm (Table 5-4). The addition of lignin to the batter was correlated with a higher thickness value (Table 5-4G and J). Furthermore, lignin resulted in trays that were darker in colour and very susceptible in high relative humidity (RH > 70%).

In terms of moisture content, it can be observed that trays based on cassava starch tended to have higher moisture content (16.54-22%, w/w dry basis) compared to corn starch and CL-corn starch trays (10-12%, w/w dry basis) (Table 5-4). Similar findings on moisture content values of corn and CL- corn starch biofoams have been reported in a previous study (8.65 to 11.37%, w/w) (Polat, Uslu, Aygün, & Certel, 2013a). It is likely that different types of starch, fibre and additives affect significantly the water content of the final trays.

However, the density of trays with cassava starch was lower (0.15-0.19 g/cm^3) compared to corn and CL-corn starch trays (0.25-0.64 g/cm^3). All density values of the developed biofoams (approximately 0.15-0.64 g/cm^3) were higher than those of expanded polystyrene (EPS) (0.05–0.09 g/cm^3) (Glenn, Orts, Nobes, & Gray, 2001). Low density biofoams are preferable as they are light and can be stored efficiently, by occupying minimum space (Kaisangsri et al., 2014). Additionally, the addition of 4% of cellulose in cassava starch trays reduced the foam density, similar to the findings reported by Salgado, Schmidt, Molina Ortiz, Mauri, & Laurindo (2008), in which a reduction of foam density was influenced by the addition of corn fibre, where the fibre adhered well to the starch matrix, and led ultimately into reinforcing the produced foam.

In terms of colour parameters of the generated biofoams, the additives influenced the colour of starch foams (Table 5-4 and Table 5-5). Originally, cellulose fractions had yellowish colour and hemicellulose fractions had white colour. The corn and cassava starch trays had yellowish colour as depicted in Figure 5-2. It can be seen that CL-corn starch trays had higher value (L) compared to the other trays. The colour difference (ΔE) of CL-corn starch trays was lower than that of other starch trays. These colour difference (ΔE) values are comparison between the colour of trays and commercial EPS. The colour difference (ΔE) values decrease by addition of CL-corn starch in the formulation. It is suggested that the addition of cellulose and hemicellulose fractions affected the final colour of the trays. Specifically, cellulose increased the ΔE values compared to formulation D (80% CL-corn starch, 10% PVA, 5% MgS, 2% cellulose, 1% hemicellulose), while the ΔE values remained relatively stable regardless of the increment in the concentration of hemicellulose.

Starch foams are usually susceptible to moisture, particularly in conditions of high relative humidity (Mali, Debiagi, Grossmann, & Yamashita, 2010). Water Absorbance Capacity (WAC) was determined to evaluate the resistance of biofoams to water. Overall, the highest WAC was observed in cassava starch biofoams (H, I, and J) as depicted in Table 5-4, at approximately 16-22%, regardless of any additive inclusion. This result is in agreement with previous study by Kaisangsri et al. (2014), in which cassava starch foams had high water absorption index (WAI), since the dissociation of water molecules to H-bonds with OH groups of glucose units along the polymer chains could have easily occurred. In addition, the water holding capacity of cellulose rich-fraction from sorghum stalk could hold 22.76 to 35.27 times water per dry weight at room temperature (Qiu, Yadav, & Yin, 2017). The WAC of trays depends on the porosity and surface area of trays. It has been suggested that water resides inside the pores of trays and binds to cellulose fibrils through hydrogen bonding(Qiu, Yadav, & Yin,2017) .

Taking all these parameters into account, the CL-corn starch (formulation D) was selected to be further studied due to its improved WAC, density, low moisture content, thickness and colour properties compared to other foams. To this end, various cellulose and hemicellulose inclusions were applied, in order to understand the effect of the sorghum by-product fractions on the functionality of the developed biofoams. The addition of cellulose (D-1 and D-2) and hemicellulose (D-3 and D-4) to the CL-corn starch formulation (D) increased the thickness of the developed trays, indicating that the addition of cellulose and hemicellulose changed the ability of the starch paste to foam. The lowest foam density (0.25 g/cm^{-3}) was noted in CL-corn starch batter formulations mixed with 1 % (w/w) of hemicellulose and 2% (w/w) of cellulose. The addition of cellulose increased the thickness and WAC of the foams, whereas at the same time reduced their final moisture content. Cellulose adheres well to the starch matrix, and thus acts as reinforcement. Starch becomes very brittle at low humidity and additional fibre can bridge cracks in the starch. On the contrary, at high humidity conditions, the amorphous starch foam starts to become soft, and as such, cellulose builds an intermolecular network that provides additional strength (Shogren, Lawton, & Tiefenbacher, 2002).

However, the addition of hemicelluloses (i.e sorghum-derived xyloglucans), decreased the density of the trays. This means that glucans decreased the foam cell size due to their role as a thickener, and concomitantly increased the adhesiveness with other compounds in the batter. On the other hand, the addition of hemicellulose increased the WAC due to the high water holding capacity property of xyloglucans (Mishra & Malhotra, 2009).

Table 5-4 Physical properties of biodegradable foams based on corn and cassava starches

Formulations	**Thickness (mm)**	**Moisture content (%)**	**Density (g cm^{-3})**	**WAC* (%)**	**L**	**a**	**b**	**ΔE****
A	3.03±0.11	16.54±4.49	0.64±0.02	9.07±1.25	85.21±0.43	-0.18±0.46	29.85±0.24	30.63±0.13
B	3.00±0.08	9.06±01.25	0.60±0.03	9.43±2.67	84.63±6.13	0.01±1.65	29.73±3.99	31.03±5.55
C	3.01±0.04	14.63±1.56	0.56±0.01	14.63±1.56	90.74±1.86	-2.25±0.59	24.22±6.06	23.59±6.22
D	3.66±0.30	17.91±4.1	0.25±0.02	17.91±4.1	92.54±1.76	-1.28±0.18	11.03±1.09	10.61±1.61
E	3.61±0.13	10.44±2.7	0.25±0.01	10.44±2.7	88.77±0.63	-1.85±0.92	18.93±1.46	19.34±1.43
F	3.46±0.01	8.94±0.02	0.26±0.00	8.94±0.018	91.19±0.80	-2.04±0.79	19.05±0.76	18.53±0.89
F-1	3.16±0.01	11.41±0.06	0.17±0.01	33.0±2.99	87.57±0.44	-0.91±0.07	15.89±0.18	17.23±0.07
G	4.17±0.19	11.21±3.79	0.31±0.00	11.21±3.79	78.94±0.66	1.37±0.48	26.38±2.85	30.82±2.20
H	3.66±0.27	16.54±0.87	0.15±0.00	16.54±0.87	89.17±1.45	-1.14±0.20	11.17±1.22	13.01±1.20
I	3.72±0.09	22.54±2.51	0.17±0.00	22.56±2.51	76.95±1.17	1.6±0.30	25.66±2.45	31.53±1.07
J	4.31±0.28	19.47±0.89	0.19±0.00	19.47±4.49	85.64±0.29	0.74±0.12	20.51±0.49	22.13±0.41

*water absorbance capacity, **compared to Styrofoam (L=97.3, a=0.14, b=1.71). Formulations A (80% corn starch, 10% PVA, 5% MgS, 5% cellulose); B (80% corn starch, 10% PVA, 5% MgS, 5% soaked cellulose); C (50% corn starch, 15% PVA, 5% MgS, 2% cellulose); D (80% CL-corn starch, 10% PVA, 5% MgS, 2% cellulose, 1% hemicellulose); E (80% corn starch, 10% PVA, 5% MgS, 2% cellulose, 1% hemicellulose), F (60% corn starch, 20% PVA, 5% MgS, 2% cellulose, 1% hemicellulose); F-1 (60% CL-corn starch, 20% PVA, 5% MgS, 2% cellulose, 1% hemicellulose); G (60% corn starch, 20% PVA, 5% MgS, 2% cellulose, 1% hemicellulose, 10% lignin); H (83% cassava starch, 10% PVA, 5% MgS, 2% cellulose); I (73% cassava starch, 5% MgS, 4% cellulose, 8% hemicellulose, 10% lignin); J (71% cassava starch, 5% MgS, 4% cellulose, 10% hemicellulose, 10% lignin, 5% Octenyl succinic anhydride (OSA) (w/v)).

Table 5-5 Physical properties of biodegradable foams based on cross-linked (CL) corn starch

Formulations	**Thickness (mm)**	**Moisture content (%)**	**Density (g cm^{-3})**	**WAC* (%)**	**L**	**a**	**b**	**∆E****
D-1	4.08±0.06	10.2±0.07	0.25±0.02	29.17±1.25	92.81±0.27	-1.41±0.03	8.12±0.12	13.05±1.62
D-2	4.43±0.13	10.67±0.01	0.29±0.01	51.07±0.9	87.72±0.14	-0.84±0.06	10.85±0.12	16.38±0.02
D-3	4.09±0.01	12.21±0.11	0.16±0.01	57.28±1.98	88.80±1.36	-0.38±0.05	6.74±0.86	9.96±0.77
D-4	4.14±0.03	10.2±0.07	0.17±0.00	45.59±0.99	87.39±0.19	-0.28±0.02	8.12±0.18	11.81±0.07

*water absorbance capacity, **compared to Styrofoam (L=97.3, a=0.14, b=1.71). Formulations D-1(80% CL-corn starch, 10% PVA, 5% MgS, 3% cellulose, 1% hemicellulose); D-2 (80% CL-corn starch, 10% PVA, 5% MgS, 3% cellulose, 1% hemicellulose); D-3 (80% CL-corn starch, 10% PVA, 5% MgS, 2% cellulose, 5% hemicellulose); D-4 (80% CL-corn starch, 10% PVA, 5% MgS, 2% cellulose, 10% hemicellulose;

5.3.4. Morphology of Biodegradable Foams by Scanning Electron Microscopy (SEM)

Figure 5-3 depicts (a) the surface and (b) the cross-section microstructures of EPS (S) and CL-corn starch biofoams (assigned the letter D). It can be observed that the surface of CL-corn starch biofoams (Figure 5-3 D-b: 80% CL-corn starch, 2% cellulose, 1% hemicellulose) were smoother than all other biofoams (Figure 5-3 D-1b, D-2b, D-3b, and D-4b). This is likely due to vapour bubbles that were trapped during hot molding process. Specifically, during hot molding process, the batter becomes denser due to the rapid gelling and drying, then the foam structure is stabilized and in the molding stage the foam does not collapse, as residual water further evaporates. The polystyrene foam had a close-packing arrangement of air bubbles whereas the starch-based biofoams had large cells in their interior due to water emitting outside the mold and ultimately leading into cells cracks.

The addition of cellulose into the batter created larger foams in the interior, a denser outer skin, and rougher structure in the surface (Figure 5-3 D-1 and Figure 5-3 D-2). The addition 3% and 4% of cellulose led into foams with more fibre in the outer skin, resulting in homogenous and expandable structure, as confirmed by the increased foam thickness on this occasion. However, the addition 4% of cellulose into the batter decreased the density of the tray, indicating that cellulose at this level of inclusion could not mix well, leading into non-homogenous batters.

The microstructure of foams with 5% of hemicellulose showed homogenous structure; however, this did not occur with the addition 10% of hemicellulose as the outer skin of the trays on this occasion was less dense. The synergistic action of the individual components (starch, cellulose, PVA, and hemicellulose) in the matrix could have possibly trapped more gas cells during their expansion. An increase in the hemicellulose content in the batter led to increased viscosity, resulting in less expandable foams. The size of' foams in the trays represented the density of the biofoams. The small size of foams of the trays was directly related to a low density and this result was also further confirmed according to the morphology structures of the trays (showed as cross-sections trays in Figure 5-3).

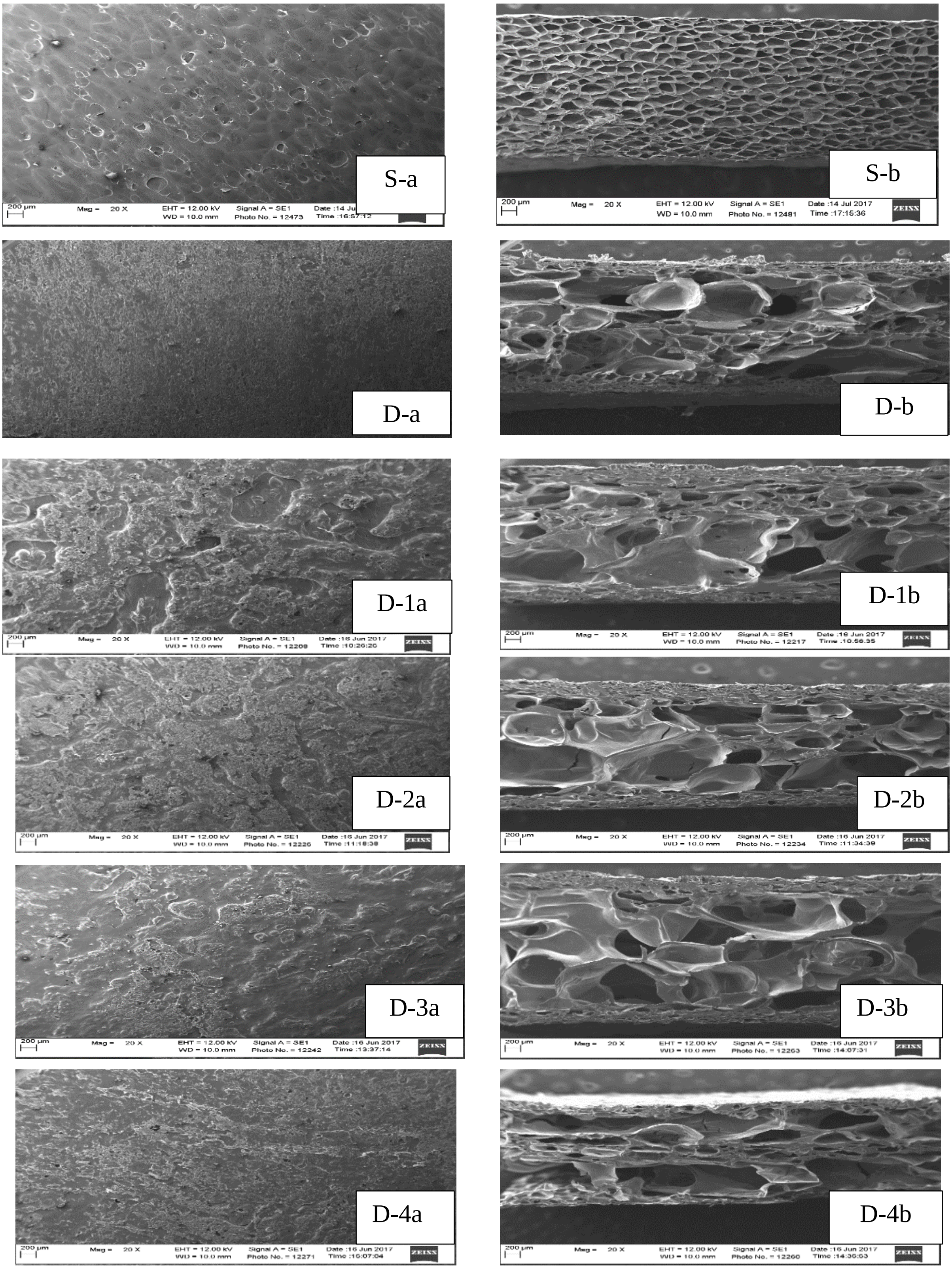


Figure 5-3 SEM images of the CL-corn starch biofoams showing (a) surface at 20 x magnification and (b) cross-sections at 20 x magnification. D (80%CL-corn starch,10% PVA, 5% MgS, 2% extracted cellulose, 1% extracted hemicellulose); D-1 (80%CL-corn starch,10% PVA, 5% MgS, 3% extracted cellulose, 1% extracted hemicellulose); D-2 (80%CL-corn starch,10% PVA, 5% MgS, 4% extracted cellulose, 1% extracted hemicellulose); D-3 (80%CL-corn starch,10% PVA, 5% MgS, 2% extracted cellulose, 5% extracted hemicellulose); D-4 (80%CL-corn starch,10% PVA, 5% MgS, 2% extracted cellulose, 10% extracted hemicellulose).

5.3.5. Crystallinity of biodegradable foams

Figure 5-4 shows the XRD patterns of formulation D (80%CL-corn starch, 2% cellulose, 1% hemicellulose) and its subsequent versions with varying cellulose and hemicellulose additions (2-4% and 1-10% respectively). It can be observed that all biofoams were diffracted at $2\theta = 19.6^{o}$. This peak is correlated with an amorphous region of the sample that most likely resulted from the molding process, in which the rigid structure of biofoams was completely or partially destroyed. The incremental addition of cellulose in formulation D (up to 4%) led into a parallel increase in the intensity and crystallinity of the samples, while the addition of hemicellulose (up to 10%) decreased the crystallinity of biofoams. This indicated that cellulose, when mixed with starch, generates strong hydrogen bonds which contribute towards a structural integrity and an increased strength. Meanwhile, the hemicellulose fraction from sorghum bran (which was previously characterised in Chapter 1 as xyloglucan) is a semi crystalline material. Based on the findings of this study, it is suggested that hemicelluloses increase the viscosity of batter and hinder hydrogen bonds, leading to subsequent loss of the structural rigidity in biofoams. Moreover, the interaction between starch and hemicellulose enhances or modifies the gelatinisation and the rheological properties of starch pastes. In addition, the interaction between hemicellulose and starch resulted in higher paste viscosity, less expandable foams and in a lower degree of pseudoplasticity.

The percentage of crystallinity for biofoams D, D-1, D-2, D-3, and D-4 were 35.1%; 35.5%; 40.5%; 29.5%; and 34.3%, respectively. Apart from biofoam D-2, all biofoams in this study were more amorphous than the expanded polystyrene (EPS), which had a crystallinity of 38.8%. EPS and biodegradable foams are in solid (glassy) state at room temperature. This characterisation is needed to determine the mechanical and thermal properties of products. For food tray applications, a crystalline structure is directly correlated with the strength of the final product, while amorphous regions are correlated with the flexibility of the trays.

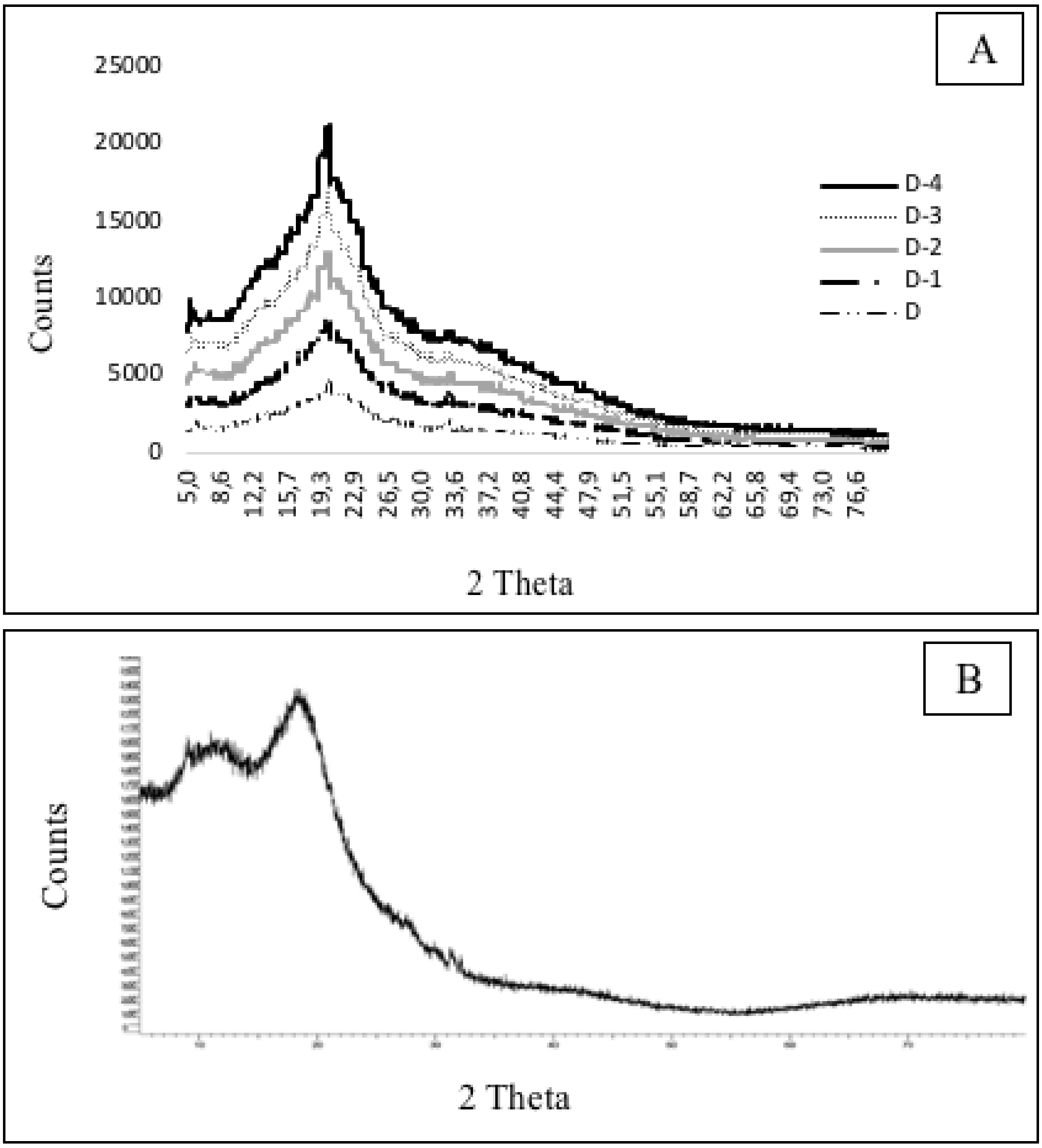


Figure 5-4 X-ray diffraction patterns of CL-corn starch starches composite trays (A) and polystyrene foam (B)

5.3.6. Thermal Properties of Biodegradable Foams

The thermal properties of biofoams were measured by diffraction scanning calorimeter (DSC), in order to evaluate their thermal stability, based on their glass transition (Tg) and melting point (Tm) temperature values. Biofoams had a Tg range between 40 to 60 °C (Table 5-5). This indicated that above the Tg and below Tm (Table 5-5), the biofoams will be at rubbery state. At this state, they are soft and flexible. However, in this study, the Tg of all biofoams was lower than EPS (~90 ^{o}C). This high Tg of EPS is due to the addition of additives such as phenyl groups.

The highest Tg of biodegradable foams was obtained by formulation D. It can be observed that the addition of cellulose and hemicellulose decreased the Tg values. This result was in contrast with the hypothesis that the increment of crystallinity (D1 and D2) leads into higher Tg and vice versa (D3 and D4), which indicated that not only one component may affect the Tg and it is more likely that the synergistic effect of the main components in the batter (starch, PVA, cellulose, and hemicellulose) could influence the Tg value.

In terms of melting point, it can be observed that the biofoam D also had the highest Tm (95.72 oC) as opposed to other biofoams (Table 5-6). These Tm values were lower than those reported in a previous study for cassava starch foams with addition kaolin and beeswax (114-161°C) (Polat et al., 2013b), which suggests that the Tm of biofoams depends on crystallinity and the presence of cross-linking bonds from the additive components. However, the DCS results of the current study suggested that the incorporation of starch, cellulose, hemicellulose, and other minor components, caused a shift of the endothermic peaks to lower temperatures, resulting in lower Tm values. One possible explanation is that xyloglucan acts as a thickener or as a catalyst to increase the viscosity of batter and interacts with starch and cellulose crystalline regions, increasing the mobility of polymer molecules and thus decreasing the transition temperature of melting. Meanwhile, all Tm values of biofoams were still below that of EPS (240 oC).

Table 5-6 Percentage of crystallinity, transition glass temperature, and melting point of CL-corn starch biodegradable foams.

Samples	**Crystallinity (%)**	**Tg (^{o}C)**	**Tm (^{o}C)**
D	35.1	60	95.72
D-1	35.5	50	81.17
D-2	40.5	45	78.45
D-3	29.5	42.5	84.36
D-4	34.3	40	81.17

Formulations: D (80%CL-corn starch, 2% extracted cellulose, 1% extracted hemicellulose); D-1 (80%CL-corn starch, 3% extracted cellulose, 1% extracted hemicellulose); D-2 (80%CL-corn starch, 4% extracted cellulose, 1% extracted hemicellulose); D-3 (80%CL-corn starch, 2% extracted cellulose, 5% extracted hemicellulose); D-4 (80%CL-corn starch, 2% extracted cellulose, 10% extracted hemicellulose). All formulations contained 10% PVA and 5% MgS.

5.3.7. Mechanical Properties of Food Trays

The tensile, elongation at break, and total work were determined using foam plate specimens, which derived from the same formulation as the trays. The mechanical properties of biofoams are presented in **Error! Reference source not found.**. It can be observed that the addition of cellulose (2% and 3%) to the tray batter formulations decreased the tensile strength and strain of the CL-corn starch biofoams. Meanwhile, the total work increased. However, the increment of cellulose to 4% into the batter formulations increased the tensile strain and total work, but decreased the tensile strength. The results demonstrated that the addition of cellulose did not affect the tensile properties to a great extent, a fact which is in agreement with previous studies which stated that the addition of corn fibre to tray formulations did not significantly affect the tensile and flexural properties of the cross-linked starch foams (Polat et al., 2013b).

The addition of hemicellulose 1% (D), 5% (D-3) and 10% (D-4) to batter formulations decreased the tensile properties and elongation at break, whereas increased the total work. The addition of hemicellulose as hydrocolloid may increase the viscosity of the batter (Sullo & Foster, 2010), leading to decrease in the density of trays, as was seen in the morphology structure of foams (table 5-7). As previously mentioned, the mechanical properties depend on the synergistic effect and compatibility of all components in the batter (Shogren, Lawton, & Tiefenbacher (2002). Previous studies have reported that trays prepared with different proportions of additives had varying homogeneity and density, and that trays which had the smallest cell size were the most homogenous and exhibited the best mechanical properties (lower water absorption, lower thickness and a higher density) (Polat et al., 2013a). We found that the addition of hemicelllulose and PVA increased the cell size in the centre of the foams and decreased tensile and flexural strength; however, these additives also reduced the water absorption of the foam trays. Overall, the formulation D was the best formulation in terms of mechanical properties.

Table 5-7 Mechanical properties of CL-corn starch biodegradable foams.

Samples	S_t^a (MPa)	e_t^b(%)	Lm^c (mm)	Fm^d (mJ)
D	0.77±0.07	14.35±2.62	2.94±0.50	9.46±2.29
D-1	0.61±0.14	8.55±0.35	1.71±0.09	18.55±4.82
D-2	0.48±0.09	9.1±1.56	1.79±0.29	20.29±2.11
D-3	0.45±0.22	7.85±0.21	1.57±0.01	14.15±2.05
D-4	0.43±0.21	10.75±1.77	2.12±0.35	16.98±4.01

Values are mean±standard deviation.

[a]Tensile strength; [b]Tensile strain at the breaking point; [c]Elongation at break; [d]Total work.

Formulations: D (80%CL-corn starch, 2% extracted cellulose, 1% extracted hemicellulose); D-1 (80%CL-corn starch, , 3% extracted cellulose, 1% extracted hemicellulose); D-2 (80%CL-corn starch, 4% extracted cellulose, 1% extracted hemicellulose); D-3 (80%CL-corn starch, 2% extracted cellulose, 5% extracted hemicellulose); D-4 (80%CL-corn starch, 2% extracted cellulose, 10% extracted hemicellulose). All formulations contained 10% PVA and 5% MgS.

5.3.8. Biofoam Applications and Storage

Biofoam trays (D, D-1, D-2, D-3, and D-4) and commercial styrofoam (EPS) were tested in storage trials at room temperature and at chilled conditions. This test aimed to determine the stability of the trays in terms of mass changes during fruit storage simulation trial. The total duration of the storage trial was 9 days.

All trays exhibited no visual structural changes during storage on both conditions (Figure 5-5 and Figure 5-6). The weight of trays and EPS were stable during storage at both conditions. On the other hand, the weight of fruits declined due to loss of water that occurred gradually after the first day storage. Particularly, the weight of fruits significantly dropped at room temperature (~26-42%). Thus, it is suggested that the developed biofoams can be used as food trays for fruits and vegetables for at least one of week storage and under chilled conditions.

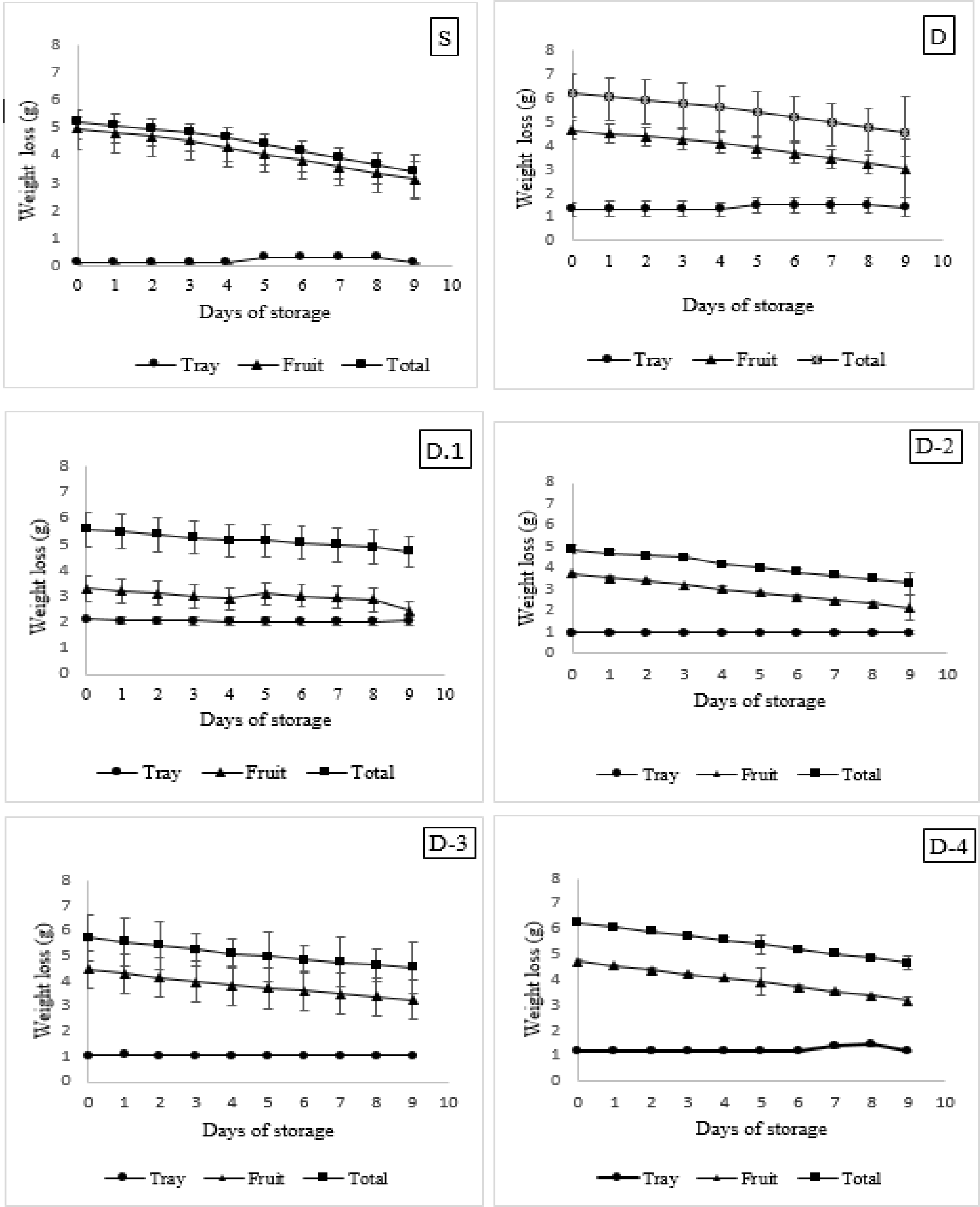


Figure 5-5 Weight of styrofoam (S) and CL-corn starch trays, fruits, and total during storage at room temperature.

Treatment condition: D (80%CL-corn starch,10% PVA, 5% MgS, 2% extracted cellulose, 1% extracted hemicellulose); D-1 (80%CL-corn starch,10% PVA, 5% MgS, 3% extracted cellulose, 1% extracted hemicellulose); D-2 (80%CL-corn starch,10% PVA, 5% MgS, 4% extracted cellulose, 1% extracted hemicellulose); D-3 (80%CL-corn starch,10% PVA, 5% MgS, 2% extracted cellulose, 5% extracted hemicellulose); D-4 (80%CL-corn starch,10% PVA, 5% MgS, 2% extracted cellulose, 10% extracted hemicellulose). All formulations contained 10% PVA and 5% MgS.

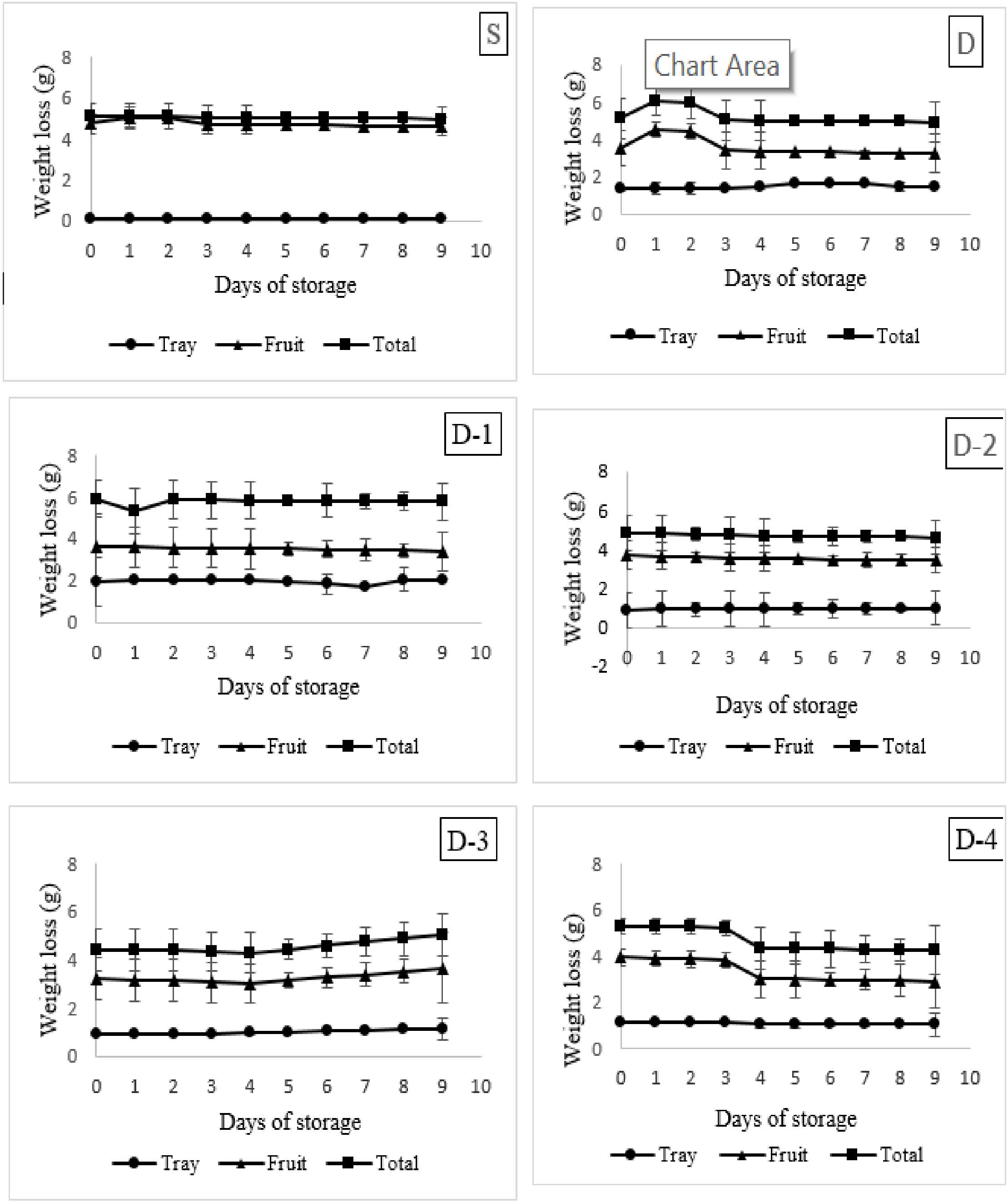


Figure 5-6 Weight of styrofoam (S) and CL-corn starch trays, fruits, and total during storage at refrigerator.

Formulations: D (80%CL-corn starch, 2% extracted cellulose, 1% extracted hemicellulose); D-1 (80%CL-corn starch, , 3% extracted cellulose, 1% extracted hemicellulose); D-2 (80%CL-corn starch, 4% extracted cellulose, 1% extracted hemicellulose); D-3 (80%CL-corn starch, 2% extracted cellulose, 5% extracted hemicellulose); D-4 (80%CL-corn starch, 2% extracted cellulose, 10% extracted hemicellulose). All formulations contained 10% PVA and 5% MgS.

5.3.9. Shelf Life Prediction of Biodegradable Foams

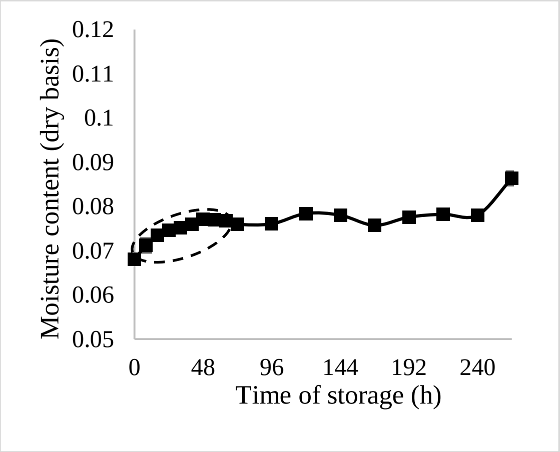
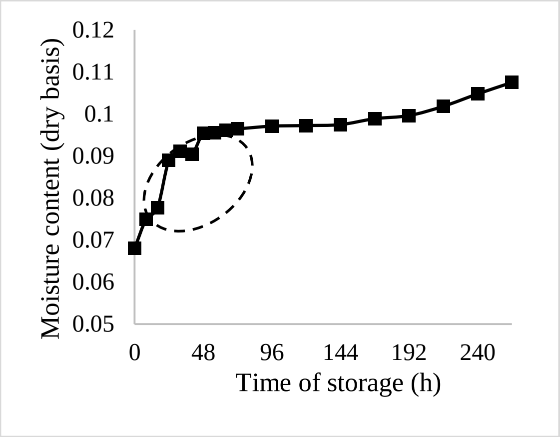
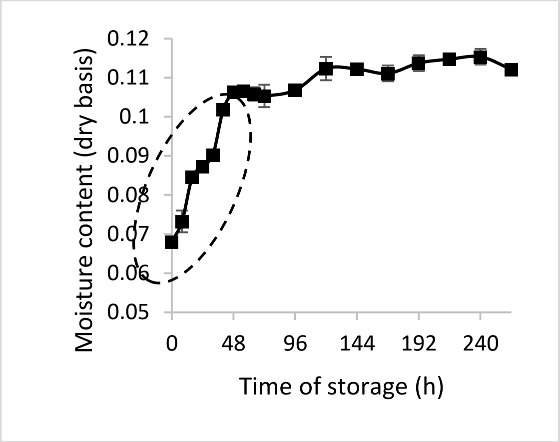
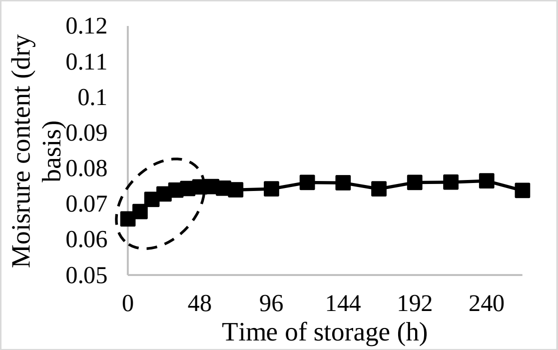
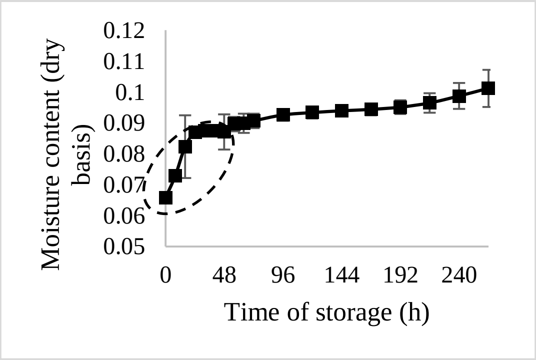
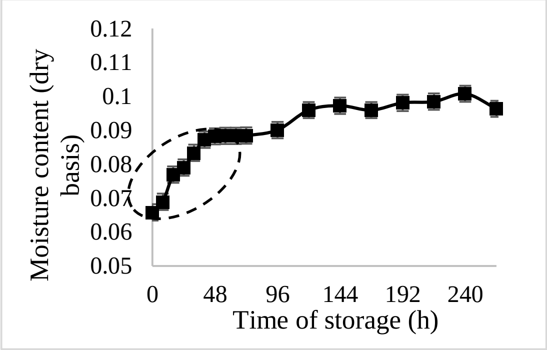
The prediction of the shelf life of the developed biofoams was conducted by accelerated shelf life testing. According to Biodegradable Products Institute (BPI), biodegradable products are naturally decomposed or degraded by microorganisms such as bacteria, fungi, or algae. This phenomenon is initiated by the presence of moisture, heat or microbial enzymes. Also, relative humidity is a critical factor for biodegradable foams due to their hygroscopic characteristics, since storage under conditions of high humidity promotes faster degradation kinetics (Nielsen, 2010). The developed foams in this study were made from bio-based materials (starch, cellulose, hemicelluloses). Therefore, it is likely that they are more easily degraded as opposed to petroleum-based trays such as EPS which require hundreds of years. To our knowledge, this study is the first one to report on the shelf life prediction of biofoams.

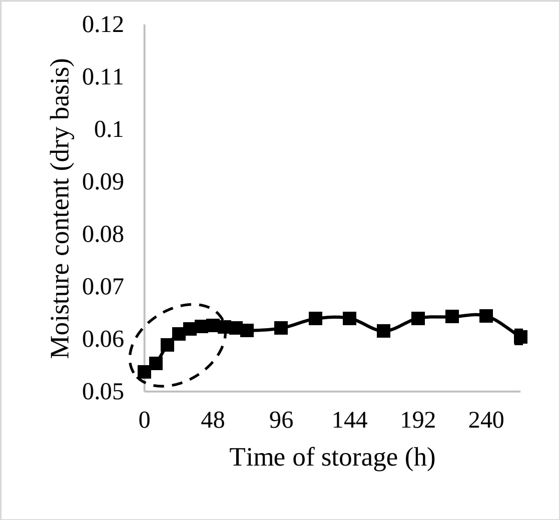
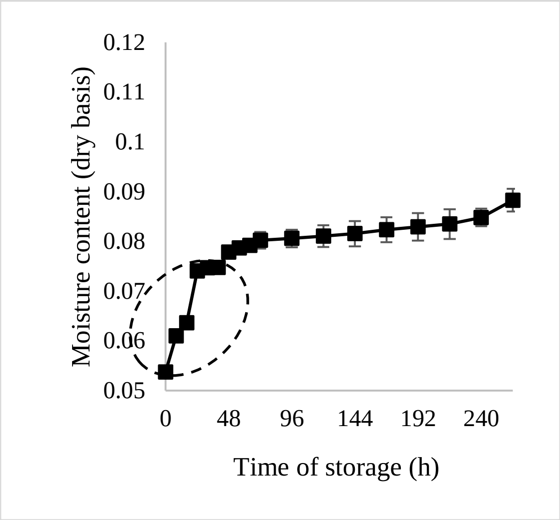
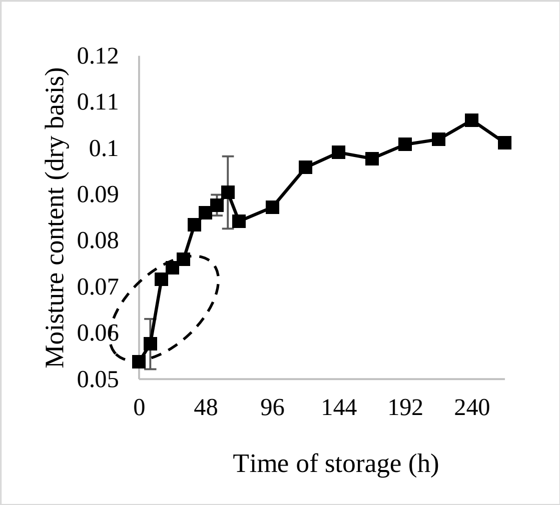
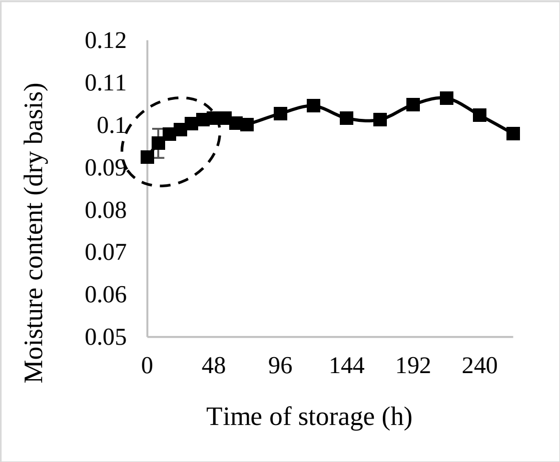
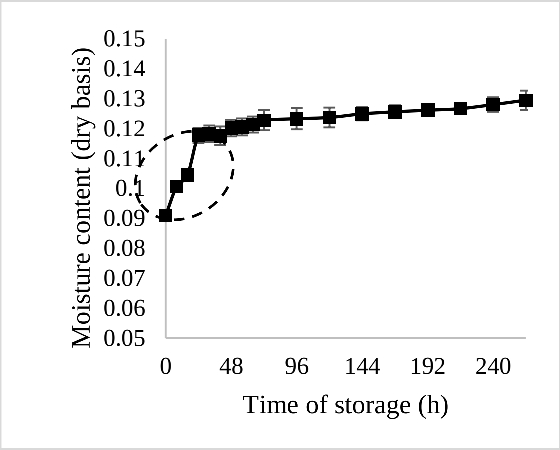
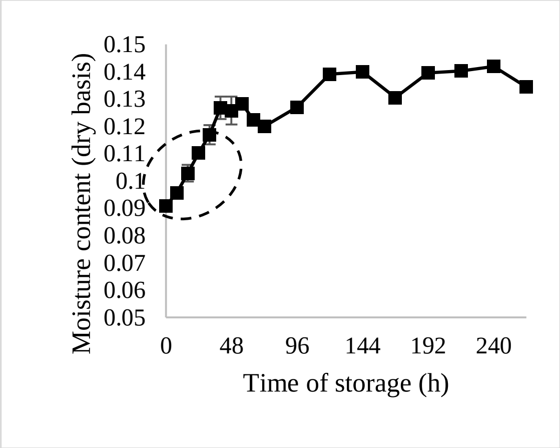
Kinetic models were developed to determine the food trays quality loss and calculate their shelf life. The dependence of foam biodegradability on the relative humidity under different storage conditions was predicted based on the Arrhenius equation (Dermesonluoglu, Katsaros, Tsevdou, Giannakourou, & Taoukis, 2015). The moisture content of trays was regarded as the parameter of shelf life prediction and was measured every 8 hours for the first three days of storage and after that for a total of 11 days at RH conditions of 53%, 75%, and 83% (Table 5-7). The lines representing Eq. 1 were defined based on the exponential curve of moisture content which is pointed out in Table 5-8. It can be observed that the water uptake in all biofoams increased together with the increment of RH, particularly during the first two days, whereas, the water uptake tended to be more stable or at an equilibrium state after approximately 48 hours of storage (Table 5-8).

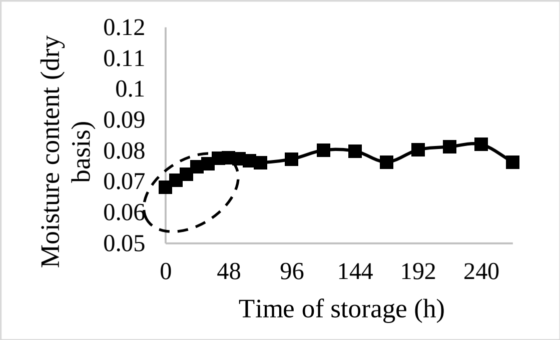
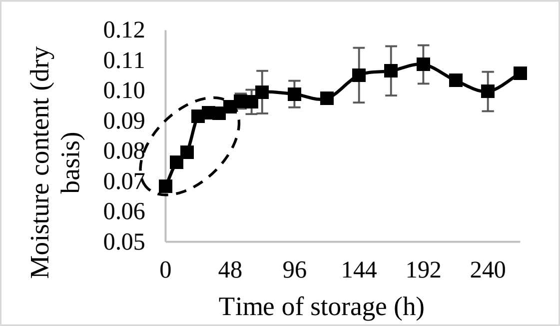
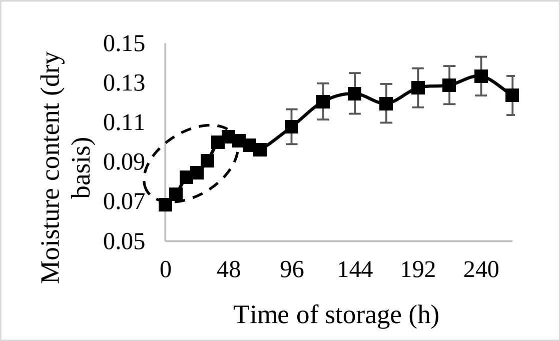
Table 5-9 shows the Arrhenius equation between the ln X (linearised Arrhenius plot) vs time at different RH values. $k_{efficient}$, represents the moisture uptake rate at a given relative humidity. The increment of RH increased the k_{eff} values, meaning that the moisture uptake of biofoams is directly correlated with the relative humidity. It is suggested that at higher RH, biofoams will absorb more water which will eventually lead to degradation. The actual appearance of trays got softer at high RH (83%) after 4 days storage.

The goal of this shelf life test was to evaluate whether the shelf-life of the developed trays is longer than the time needed for a given product (e.g. fresh produce) to be degraded. The knowledge of kinetics and the modelling of moisture sorption will help to determine the shelf-life of a product at a targeted storage temperature and to predict the shelf-life of the food under fluctuating environmental conditions. Table 5-10 shows that the generated Arhenius equations have R^2 of almost 1, meaning an adequate fitting of the models. Therefore, the generated k_{eff} values can be used to calculate the half-life time of the foams. It can be observed that the half-life prediction of all biodegradable foams was less than one year. The longest half-life time was for biofoam D-1 (9 months) whereas the shortest half-life time was for D-4 (2 months). However, these results indicated that the addition of cellulose and hemicellulose to the batter gave no effect significant on the half-life of biofoams (Table 5-10). Thus, the shelf life of biofoams depends mainly on the composition of the trays and also on external factors such as the conditions of RH and the presence of soil microorganisms that eventually contribute to polymer degradation.

Table 5-8 Plot moisture content (dry basis) vs time of CL-corn starch biodegradable foams at three different relative humidity (%).

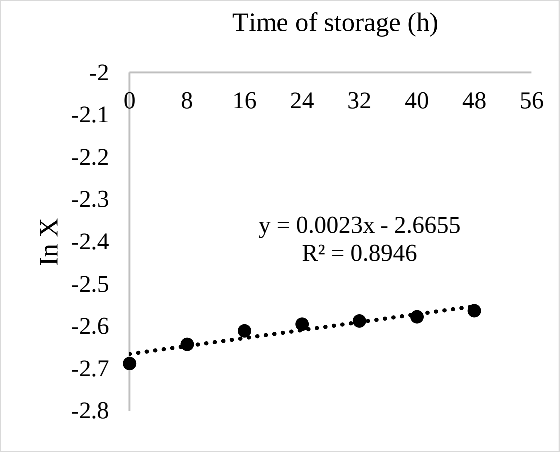
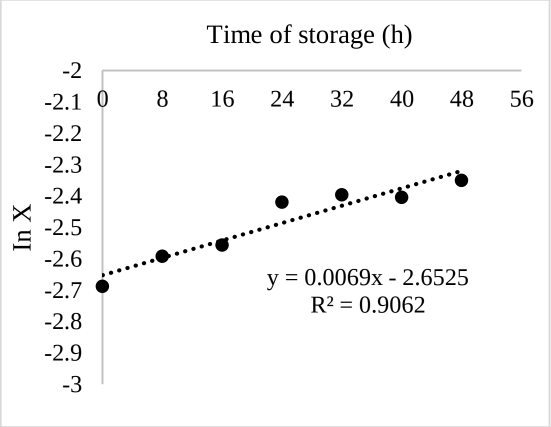
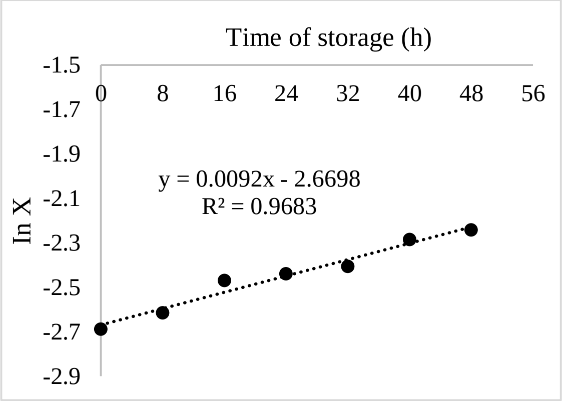
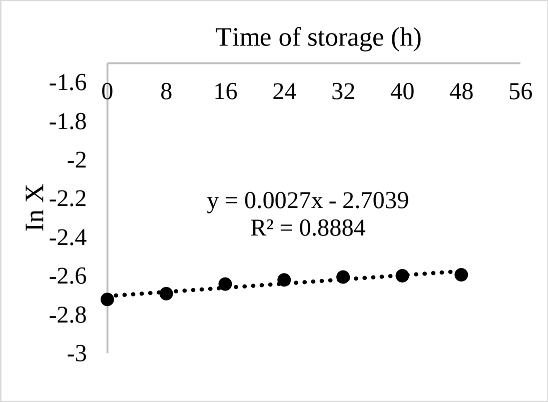
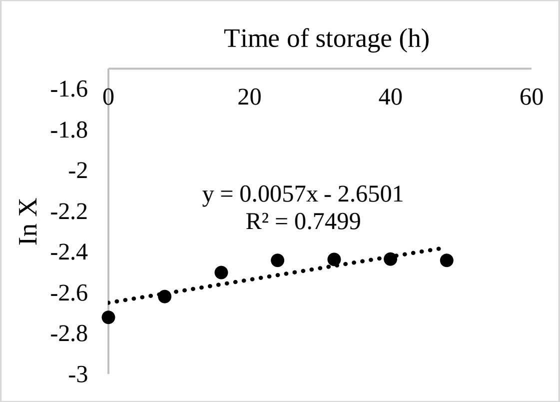
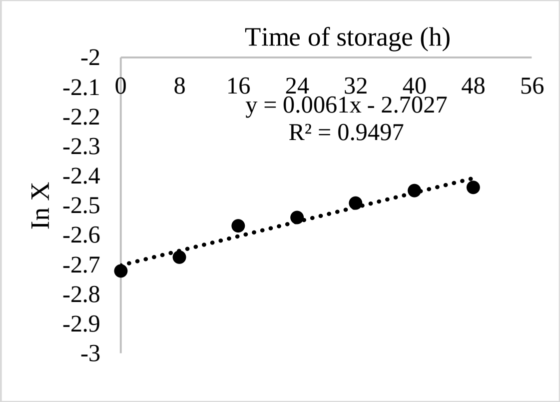
Trays	RH 53 %	RH 75%	RH 83%
D			
D-1			

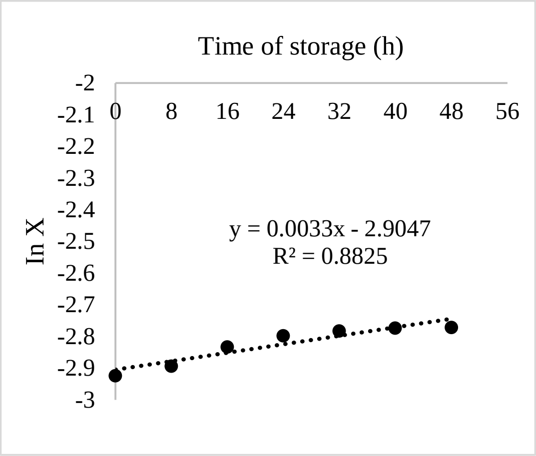
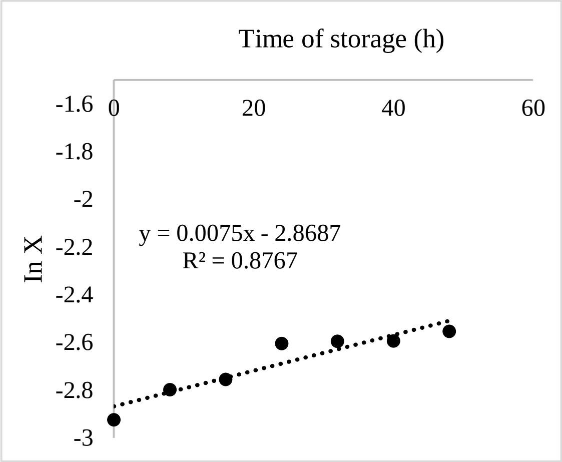
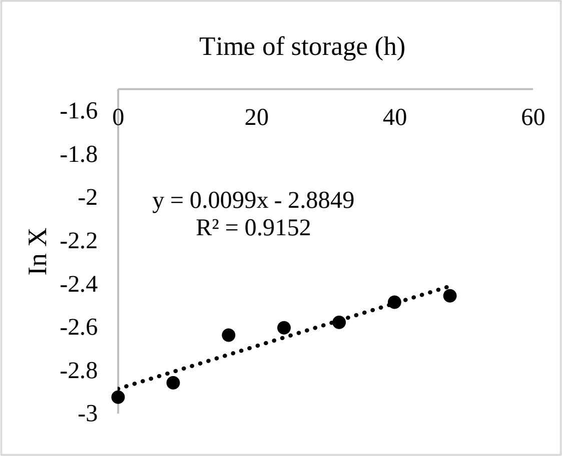
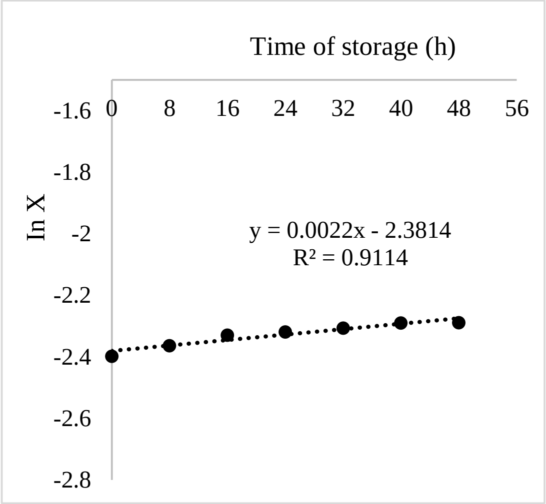
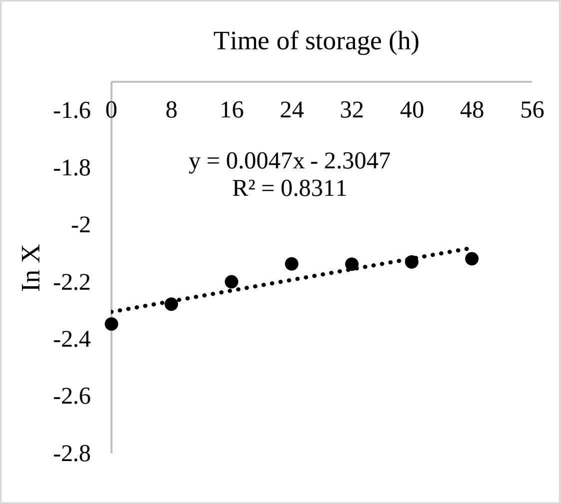
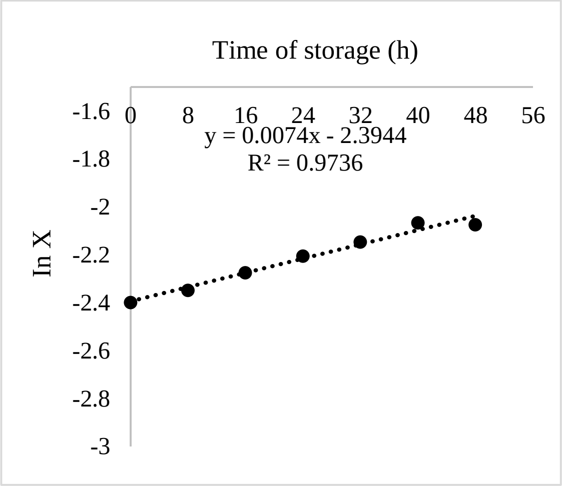
D-2	Moisture content (dry basis) 0.12 0.11 0.1 0.09 0.08 0.07 0.06 0.05 0 48 96 144 192 240 Time of storage (h)	Moisture content (dry basis) 0.12 0.11 0.1 0.09 0.08 0.07 0.06 0.05 0 48 96 144 192 240 Time of storage (h)	Moisture content (dry basis) 0.12 0.11 0.1 0.09 0.08 0.07 0.06 0.05 0 48 96 144 192 240 Time of storage (h)
D-3	Moisture content (dry basis) 0.12 0.11 0.1 0.09 0.08 0.07 0.06 0.05 0 48 96 144 192 240 Time of storage (h)	Moisture content (dry basis) 0.15 0.14 0.13 0.12 0.11 0.1 0.09 0.08 0.07 0.06 0.05 0 48 96 144 192 240 Time of storage (h)	Moisture content (dry basis) 0.15 0.14 0.13 0.12 0.11 0.1 0.09 0.08 0.07 0.06 0.05 0 48 96 144 192 240 Time of storage (h)

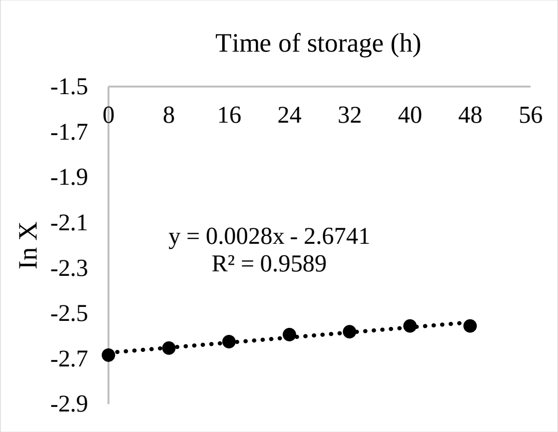
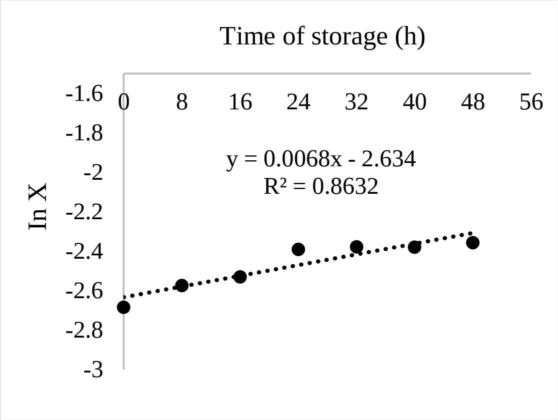
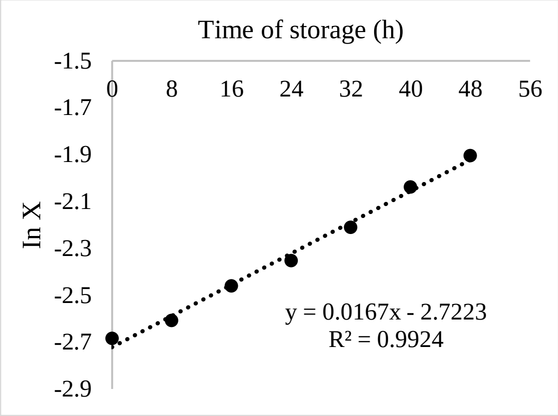
D-4			

Formulations: D (80%CL-corn starch, 2% extracted cellulose, 1% extracted hemicellulose); D-1 (80%CL-corn starch, , 3% extracted cellulose, 1% extracted hemicellulose); D-2 (80%CL-corn starch, 4% extracted cellulose, 1% extracted hemicellulose); D-3 (80%CL-corn starch, 2% extracted cellulose, 5% extracted hemicellulose); D-4 (80%CL-corn starch, 2% extracted cellulose, 10% extracted hemicellulose). All formulations contained 10% PVA and 5% MgS.

Table 5-9 Lines represent the first order fit (Eq. (2)) moisture content (dry basis) vs time of CL-corn starch biodegradable foams at three different relative humidity (%).

Trays	RH 53 %	RH 75%	RH 83%
D			
D-1			

D-2	Time of storage (h) In X 0 8 16 24 32 40 48 56 -2 -2.1 -2.2 -2.3 -2.4 -2.5 -2.6 -2.7 -2.8 -2.9 -3 y = 0.0033x - 2.9047 R² = 0.8825	Time of storage (h) In X 0 20 40 60 -1.6 -1.8 -2 -2.2 -2.4 -2.6 -2.8 -3 y = 0.0075x - 2.8687 R² = 0.8767	Time of storage (h) In X 0 20 40 60 -1.6 -1.8 -2 -2.2 -2.4 -2.6 -2.8 -3 y = 0.0099x - 2.8849 R² = 0.9152
D-3	Time of storage (h) In X 0 8 16 24 32 40 48 56 -1.6 -1.8 -2 -2.2 -2.4 -2.6 -2.8 y = 0.0022x - 2.3814 R² = 0.9114	Time of storage (h) In X 0 8 16 24 32 40 48 56 -1.6 -1.8 -2 -2.2 -2.4 -2.6 -2.8 y = 0.0047x - 2.3047 R² = 0.8311	Time of storage (h) In X 0 8 16 24 32 40 48 56 -1.6 -1.8 -2 -2.2 -2.4 -2.6 -2.8 -3 y = 0.0074x - 2.3944 R² = 0.9736

D-4			

Formulations: D (80%CL-corn starch, 2% extracted cellulose, 1% extracted hemicellulose); D-1 (80%CL-corn starch, , 3% extracted cellulose, 1% extracted hemicellulose); D-2 (80%CL-corn starch, 4% extracted cellulose, 1% extracted hemicellulose); D-3 (80%CL-corn starch, 2% extracted cellulose, 5% extracted hemicellulose); D-4 (80%CL-corn starch, 2% extracted cellulose, 10% extracted hemicellulose). All formulations contained 10% PVA and 5% MgS.

Table 5-10 Equation at relative humidity, R^2, k values, k_{eff} values, half-life of biofoams.

Biofoam	RH (%)	R^2	k	K_{eff}	$t_{½}$ (months)
D	53	0.8946	0.0023	0.0003	3
	75	0.9062	0.0069		
	83	0.9819	0.0128		
D-1	53	0.8884	0.0027	0.0001	9
	75	0.7499	0.0057		
	83	0.9497	0.0061		
D-2	53	0.8825	0.0033	0.0002	5
	75	0.8767	0.0075		
	83	0.9152	0.0099		
D-3	53	0.9114	0.0022	0.0002	5
	75	0.8311	0.0047		
	83	0.9736	0.0074		
D-4	53	0.9584	0.0028	0.0004	2
	75	0.8632	0.0068		
	83	0.9924	0.0167		

Formulations: D (80%CL-corn starch, 2% extracted cellulose, 1% extracted hemicellulose); D-1 (80%CL-corn starch, , 3% extracted cellulose, 1% extracted hemicellulose); D-2 (80%CL-corn starch, 4% extracted cellulose, 1% extracted hemicellulose); D-3 (80%CL-corn starch, 2% extracted cellulose, 5% extracted hemicellulose); D-4 (80%CL-corn starch, 2% extracted cellulose, 10% extracted hemicellulose). All formulations contained 10% PVA and 5% MgS.

5.4. **Conclusion**

Cellulose and hemicellulose fractions extracted from sorghum by-products were proved as suitable additives in biofoam formulations. However, the lignin fraction was not a compatible component due to its hygroscopic nature; also lignin inclusion imparted a darker colour on the developed biofoams. The water resistance of cross-linked corn starch foams decreased by the addition of cellulose and hemicellulose at levels greater than 2% and 1%, respectively. However, varying levels of cellulose and hemicellulose had different effect on the mechanical properties of the biofoams, as well as on their crystallinity. Despite the higher density and lower elasticity of biofoams compared to EPS, the present findings demonstrate that biofoams can be used as an alternative to EPS trays in applications where contact with moist food occurs for short periods (up to one week) in low relative humidity (~50%).

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Polat, S., Uslu, M. K., Aygün, A., & Certel, M. (2013b). The effects of the addition of corn husk fibre, kaolin and beeswax on cross-linked corn starch foam. *Journal of Food Engineering*, *116*(2), 267–276.

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Chapter 6: General Discussion

6.1. Introduction

Sweet sorghum plants are recognised as a versatile feedstock that can be used as food, fuel, animal feed, and fibre as well as raw chemicals and biomaterials (Jianliang Yu et al., 2012). Approximately 61.4 million tons were produced worldwide in 2013 (FAO, 2015). Sorghum by-products represent an example of lignocellulosic biomass residue, containing plant structure polymers such as cellulose, hemicellulose and lignin. With regards to their polysaccharide content (cellulose and hemicellulose), these can be extracted and subsequently developed into a range of added value products for food and agricultural applications. Sorghum by-products, such as bran and stalk, were selected to be used in this study. The fractionation of biomass was conducted by an optimised two-step alkaline treatment for the isolation of cellulosic (R), hemicellulosic (H) and lignin (L) fraction from sorghum stalk and bran. This process converted lignocellulosic biomass into polymeric components amenable for further processing and transformation into products for packaging and agricultural applications. As such, subsequent work investigated the potential applications of the alkali extracted hemicelluloses and cellulose (present in the residue after alkali extraction) within the agriculture and packaging sector, and more specifically as materials for the development of formulations for the controlled release of fertilisers, as well as biodegradable foams for food packaging applications. Within the first area, one approach, as discussed in Chapter 4, has generated hemicelluloses-based films that can be used as a formulation for the film packaging and film containing fertiliser in the soil and hydroponic planting purposes. To this end, different plasticisers (at various concentrations) were shown to influence the physical and mechanical properties of the films, including their strength, elongation at break, water solubility and water permeability. As relatively limited amount of work has been conducted in this area it was important to evaluate the effect of the hemicelluloses properties (e.g. different levels of A/X ratios) on the films' properties. Results showed that hemicellulosic fraction extracted from sorghum stalk had a low A/X (~0.15). On the other hand, the hemicellulosic fraction of sorghum bran contained mixed-linkage glucan, with the ratio of A/X equal to 0.93 and X/G to 0.07. The hemicellulosic fraction with high branching structure had high solubility in water and exhibited thickening properties. The modification of the hemicellulosic fraction of sorghum stalk with long chain anhydride improved the film performance and water vapour permeability. The second approach consisted of the investigation of films containing fertiliser based on a simple deposition method and the release of the fertiliser was assessed in model solutions and in the soil. This line of research is very promising with a view to reduce the accumulation of fertilisers in plants. Therefore, it is essential to develop new formulations of fertiliser, which would be eco-friendly and more effective. Among all films tested, 2-OSA-HS film had the slowest release of fertiliser as opposed to HB and AXC films at RH 80% and 20 ^{o}C with moisture content of the soil was 70%. However, all films have been diminished in one day in the soil. Regarding the release of NPK, it has been reported that N gives a more profound effect on the soil than P and K. The release of N was almost 100% after one day. Therefore, this film was characterised as a weak carrier for fertiliser. We suggest that xylan-based films were deemed as potential carriers for fertilisers particularly for acidifying soil with low nitrogen content and water (hydroponic system application).

Within the second area, the aim was to develop biodegradable foams (biofoams) using the insoluble fibre (cellulose) extracted from sorghum stalk, which are present in the residue fraction after alkali extraction, and hemicellulosic fraction from sorghum bran, which mostly contained of arabinoxyloglucan. The high concentration of cellulose in the residue fraction is needed in order to exert strong mechanical properties and water resistance to biofoams. The addition of cellulose and hemicellulose to the batter affected the thickness of trays, indicating that the filler addition changed the ability of the starch paste to foam. CL-corn starch mixed with 1 % of hemicellulose and 2% of cellulose obtained the lowest foam density (0.25 g/cm^{-3}). It can be concluded that the cellulose adheres well to the starch matrix, and thus acts as reinforcement component. Moreover, it has been observed that WAC values increased due to the higher water holding capacity of trays. Furthermore, we also found that the addition of hemicelluloses (i.e mixed-linkage glucan) reduced the density of foams since the swelling was restricted. We suggested that it is related to the function of xyloglucan as a thickener. On the other hand, the addition of hemicellulose increased the WAC due to a high water holding capacity property of xyloglucan (Mishra & Malhotra, 2009). In terms of lignin fraction, the latter was not compatible component due to its hygroscopic nature and the generation of a darker colour of biofoam.

6.2. Limitations

6.2.1. Fractionation

Various physical, chemical, and biological treatments have been applied to improve the utilisation of agricultural by-products including bran, cob, stem, stalks, straws, and husk. In this study, sequential two stages alkaline extraction with 1 M of NaOH at 50 ^{o}C for 3 h was used, in which yielded 100% of xylose and 81% of arabinose from initial amount of sorghum stalk. For sorghum bran, 0.75 M of NaOH at the same temperature and time was the best treatment which yielded 63% of hemicelluloses. Alkaline treatment constitutes a common method of fractionation. It is an effective agent to break the cell wall structure by dissolving hemicelluloses, lignin, and silica, by hydrolysing uronic and acetic acid esters, by swelling cellulose, and by decreasing the crystallinity of cellulose. Furthermore, it increases the biodegradability of the cell wall due to the cleavage of the bonds between lignin and hemicelluloses or lignin and phenolic acids.

NaOH is an effective chemical in terms of extractability as opposed to ammonium hydroxide and potassium hydroxide. However, the latter is more reactive than sodium hydroxide. The yield of fractions depends on the extraction process in which the acetyl groups linked to hemicelluloses are usually removed during the extraction of hemicelluloses using alkali. To increase the yield of hemicelluloses, successive or sequential treatments are needed due to the complexity of the hemicellulosic structure. Furthermore, pretreatment such as steam explosion, hot water, and organic solvents is also needed in order to increase the accessibility of alkali to the cell wall by decreasing the content of lignin, breaking the bonds among the components, and exposing cellulose and hemicelluloses. In addition, the treatments on the extraction process will also influence the hemicelluloses structure. Moreover, the understanding of the physical stage of the plant is also very important since the content and chemical composition of hemicelluloses in sorghum depend on the species, age, climate, and harvest time.

6.2.2. Purification

Purification of extracted fractions is needed to get a better understanding of the physical, chemical, and functional characteristics of the individual components and tailor potential applications. However, pure hemicellulosic fractions are a challenge due to the complexity of the plant cell wall. Differences in the structural features of hemicelluloses, due to spatial arrangement, the degree of branching, of substituents along the xylan backbone and the ferulic acid content, can affect the functional properties and biological activities of such fractions. In addition, each tissue or organ in the plant has a different structure of hemicelluloses and may bond to other polysaccharides of the plant cell wall.

6.3. Recommendations

Within the context of this study, a method for the successful fractionation of sorghum stalk and bran was developed, the characteristics of the obtained fractions were investigated and potential applications were evaluated. However, further modifications in fractionation and purification techniques could be pursued, in order to increase the extraction yield for each fraction and investigate the functionalities of key-compounds on film or biofoam formation. Additionally, the application of sorghum stalk hemicelluloses as plastic packaging was found to hold more potential compared to its use as carrier of fertiliser. In addition, due to the high water solubility, the 2-OSA-xylan based film can be used as food coatings such as in fruit or confectioneries. Using this modified 2-OSA xylan film in antimicrobial packaging holds also potential due to its low water vapour and oxygen permeability properties. On the other hand, hemicelluloses from sorghum bran have a potential as a thickener agent, oil in water emulsifier, hydrogels, and water holding agent. Therefore, targeted food applications of sorghum by-product fractions can be also considered in the future.

6.4. Conclusions

The findings of this study with regards to sorghum by-products valorisation are considered as novel in terms of compositional analysis, extraction, structural characterisation, and potential applications. Strategies of chemical modification were applied to widen the range of utilisation of sorghum stalk hemicelluloses. Such achievements could allow sorghum by-products as a valuable feedstock to be valorised within the context of a biorefinery concept, which can open an efficient way to convert sorghum by-products into high value-added products as biomaterials for food packaging and the agro-industry. However, there still challenges in the valorisation of plant biomass such as the identification of suitable approaches in order to obtain high yields of fractions, as well as the economic assessment of the suggested valorisation route in order to evaluate its cost-effectiveness. The valorisation of sorghum by-products could be further promoted by sustainable policies and economic subsidies by the Indonesian or any other government in countries with considerable amounts of sorghum plantation and exploitation.

Appendix

List of conferences/seminars, professional memberships publications and certificate during PhD programme:

A. Seminars/ Conferences:

1. Poster presentation on Renewable chemicals from waste-securing the molecular value from waste streams, Royal Society of Chemistry, Burlington House, London UK on November 20th, 2015 under title “Xylan Extraction from Sorghum by-products”

2. Oral presentation on Food Bioscience Research Seminar, Department Food and Nutritional Sciences, University of Reading on January 8th, 2016 under title “Physical, Chemical, and Structural Properties of Extracted Hemicellulose, Cellulose and Lignin from Sorghum Stalk”

3. Oral presentation on 2nd International Conference on Food Properties (ICFP2016) Bangkok, Thailand on May 31st – June 2nd , 2016 under title “Characterisation of Fractions Extracted from Sorghum By-products”

4. Oral presentation on Food Bioscience Research Seminar, Department Food and Nutritional Sciences, University of Reading on November 17th, 2016 under title “Characterisation of Fractions Extracted From Sorghum Stalk”.

5. Oral presentation on the 18th international conference on biomass, bioenergy, biofuels and bioproducts, Penang, Malaysia on December 1st-2nd , 2016 under title “Physical and structural properties of xylan-based biodegradable films from sorghum by-products”

6. Oral presentation at 25th European Biomass Conference and Exhibition 2017 on June 12th-15th, 2017 in Stockholm, Sweden under title “Physical and structural properties of xylan-based biodegradable films from sorghum by-products”.

7. Oral presentation at Total Food 2017 in Norwich, United Kingdom on October 31st – November 2nd, 2017 under title “Biodegradable foams based on extracted fractions from sorghum by-products”.

B. Professional Membership:

- Member of Food Waste Net, UK

C. Publications:

- P Luna, P., Chatzifragkou, A., Charalampopoulos, D. (2016). Physical, Chemical, and Structural Properties of Extracted Hemicellulose, Cellulose and Lignin from Sorghum Stalk. Proceedings of the 2016 International Conference on Food Properties (ICFP2016) (attached).

- P Luna, P., Chatzifragkou, A., Charalampopoulos, D. (2016). ‘Characterisation of Fractions Extracted from Sorghum Byproducts'. World Academy of Science, Engineering and Technology, International Science Index 120, International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering, 10(12), 776 – 781 (attached).

D. Certificate:

Reading Researcher Development Programme (RRDP) (attached)

SUGAR STANDARD CURVES