The incorporation of Curcuminoids in oat fibre extruded products

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Abstract

Curcuminoids are polyphenolic bioactive ingredients found in the roots of the plant Curcuma Longa. These compounds have health benefits and can be used to develop health promoting functional foods but the low water solubility of these compounds limits their bioavailability. One of the main approaches to overcome the low solubility and stability of curcuminoids in an aqueous environment is to make use of the interaction between curcuminoids and other food ingredients.

Oat dietary fibre is one such food ingredient that could potentially be used as a carrier for curcuminoids. Oat fibre is known to have health benefits for humans, including lowering cholesterol serum, the risk of coronary heart disease and blood pressure. Dietary fibre has a potential to interact with polyphenolic compounds, such as the curcuminoids, by a number of mechanisms but the possible interactions between curcuminoids and oat fibre ingredients have not been studied to date.

Extrusion cooking is one of the main technologies known to have a great potential for the manufacture of snack products. Bioactive components can be added to extruded snacks in order to improve their health benefits but these components can degrade during extrusion processing. The possible effect of bioactive addition on the physical properties of extruded products should be also considered.

This thesis aims to investigate the potential of oat fibre ingredients as a carrier for curcuminoids. It also aims to examine the feasibility of producing a curcuminoid-enriched oat fibre-corn based extruded product, with a focus on curcuminoid stability during extrusion

processing. It also aims to evaluate the effect of curcuminoid addition on the physical properties of extruded products.

The thesis is divided into three main sections:

1. The first section focuses on the use of oat fibre as a potential carrier material to increase the solubility and stability of curcuminoids. Studies are carried out to understand the interaction between curcuminoids and the oat fibre ingredients. The stability of the curcuminoids in the presence of oat fibre materials during storage was also determined.

2. In section two, the effect of extrusion technology on the physico-chemical properties of oat fibre containing 28 % β-glucan are examined. The physico-chemical characteristics of oat fibre including molecular weight, soluble solids content, water absorption index, dynamic vapour sorption, thermal and pasting properties were measured after extrusion using two feed or barrel moisture contents (50 % - 60 %) and two screw speeds (200 rpm – 300 rpm) and results were compared with the properties of non-extruded oat fibre.

3. In the third section, extrusion technology was used as a delivery method for the production of a curcuminoid enriched oat fibre-corn based extrudates. This section focuses on the stability of curcuminoids, as affected by extrusion conditions, including the two levels of feed moisture content and two levels of screw speed. In addition, the effect of curcuminoids on the physical characteristics of extrudates including bulk density, expansion, hardness and colour were investigated.

The spectroscopic experiments showed that both protein and β-glucan components of oat fibre are able to interact with curcuminoids. Solubility experiments indicate the curcuminoids in the supernatant fraction of a 1 % w/w oat fibre dispersion in 2 % v/v EtOH (88 μg/mL)

increased by a factor of 21 compared to only 2 % v/v EtOH (4.1 μg/mL). This concentration of curcuminoids in the supernatant is also much higher than that of the reported for the solubility of curcuminoids in aqueous media (11 ng/mL, pH 5).

In the presence of oat fibre materials, curcuminoids were converted from a crystalline to an amorphous state, as observed by Wide-angle X-ray powder diffraction. The amorphous state of the curcuminoids in the precipitate of (25.8 μg/mL) curcuminoids−oat fibre (1% w/w TS) dispersion with 2 % v/v EtOH resulted in higher stability for curcuminoids in the precipitate rather than supernatant of the oat fibre dispersion. These findings show the potential of oat fibre as a carrier for curcuminoids in functional foods.

Extrusion of oat fibre with high levels of β-glucan under conditions of high moisture did not significantly change the molecular weight of the soluble fraction, the total soluble solids, the water absorption index and thermal properties of oat fibre. A higher specific mechanical energy was found to result in an increased specific surface area and absorption of water vapour as a surface monolayer. The viscoelastic properties of oat fibre were also maintained after extrusion. This study indicates that extrusion processing is a promising technology to produce extruded products based on oat fibre where the functional properties and potential health benefits of oat fibre are preserved.

The physical properties of oat fibre based extrudates containing curcuminoids were significantly affected by feed moisture content, whereas the effect of screw speed and curcuminoid addition was not significant. Higher feed moisture resulted in darker extruded snacks with higher bulk density, hardness, 90 % retention of curcuminoids after extrusion and drying but lower expansion. Curcuminoids were also stable in dried extruded products during 80 days of storage at 25 °C. These studies provided information for the selection of process

variables for extrusion. The supporting compositional evidence will help with the introduction of curcuminoid-enriched extruded snacks as a new product category in the functional food market.

In conclusion, this study showed that both protein and β- glucan components of oat fibre are able to interact with curcuminoids and increase the solubility of curcuminoids in an aqueous solution of 2 % v/v EtOH. It is possible that curcuminoids also interact with proteins and dietary fibres in the precipitated fraction. These findings illustrate the potential for the curcumin carrying capacity of oat fibre to be capitalized upon in the fortification of food with curcuminoids. The application of extrusion processing to modify the functional properties of oat fibre, showed that current extrusion conditions can be used to produce extruded products from a commercially available oat fibre preparation with high β-glucan content where the properties of the extrudates were largely preserved and the health benefits are expected to be similar with non-extruded oat fibre. The ability to achieve high retention of curcuminoids (~ 90 %) at feed moisture content of 35% and screw speed of 200 rpm or 300 rpm during extrusion combined with the stability of the curcuminoids in the dried extruded snack shows the potential to improve the health benefits of oat fibre extruded products by the incorporation of curcuminoids.

Declaration

This is to certify that

(i) the thesis comprises only my original work towards the degree of Doctor of Philosophy,

(ii) due acknowledgement has been made in the text to all other material used,

(iii) the thesis is fewer than 100,000 words in length, exclusive of tables, maps, bibliographies and appendices.

Sara Sayanjali

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Preface

This thesis is submitted for the degree of Doctor of Philosophy at the University of Melbourne. The research described here was conducted under supervision of A. Prof Sally Gras in the department of Chemical Engineering, at The University of Melbourne and Dr. Roman Buckow, Dr. MaryAnn Augustin and Mrs. Luz Sanguansri from CSIRO Agriculture and Food. The experiments, data collection and analysis were performed by Mrs Sara Sayanjali.

All laboratory work was conducted at CSIRO Agriculture and Food, Werribee, except for some HPLC experiments that were carried out at Bio21 Molecular Science and Technology Institute, at The University of Melbourne.

Dr Danyang Ying and Mr. Keith F. Pitts from CSIRO Agriculture and Food assisted in extrusion trial. The cryo-SEM images were performed by Dr Lydia Ong (Bio21 Molecular Science and Technology Institute, The University of Melbourne). Sections of this thesis have been published, submitted or are in preparation for publication as described below:

Journal publications from this thesis

1. **Sayanjali, S.**, Sanguansri, L., Buckow R., Gras, S., and Augustin, M. A. (2014), Oat fibre as a carrier for curcuminoid. Journal of Agriculture and food Chemistry, 62, 12172−12177, (Chapter 3).

2. **Sayanjali, S.**, Sanguansri, L., Ying, D., Buckow, R., Augustin, M. A., and Gras, S. The effect of extrusion on the functional properties of oat fibre. Submitted to LWT, Food Science and Technology Journal, (chapter 4).

3. **Sayanjali, S.**, Ying, D., Buckow R., Augustin, M. A., Gras, S. And Sanguansri, L. Effect of curcumin addition and extrusion conditions on the properties of a curcumin-enriched oat fibre-corn based extruded product. Submitted to Journal of Functional Food, (Chapter 5).

Oral/Poster presentations at conferences

1. Sayanjali, S., Sanguansri, L., Buckow R., Gras, S., and Augustin, M. A. (2014), Oat fibre as a carrier for curcuminoid. 47th Australian Institute of Food Science and Technology. Melbourne, Australia, 22-25 June, 2014.

2. Sayanjali, S., Sanguansri, L.,Ying, D., Buckow R., Augustin, M. A. and Gras, S. (2016). The effect of extrusion process on functional properties of oat fibre. Second Food Structure and Functionality Forum Symposium, Singex, Singapore, 28th Feb – 2 March, 2016.

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Now it’s the moment; the last education carrier.

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Abbreviations

TS	Total Solid
GOPOD	Glucose Oxidase/Peroxidise
EtOH	Ethanol
IR	Infrared Spectroscopy
SME	Specific Mechanical Energy
MC	Moisture Content
Pd	Polydispersity
Mw	Average of Molecular Weight
Mn	Number of Average Molecular Weight
TSS	Total Soluble Solid
WAI	Water Absorption Index
DVS	Dynamic Vapour Sorption
RH	Relative Humidity
GAB	Guggenheim-Anderson-De Boer
S_{GAB}	Specific Surface area
Tg	Glass Transition Temperature
T_0	Onset Temperature
RVA	Rapid Visco Analyser
RPM	Revolutions Per Minute
BD	Bulk Density

Chapter 1

General introduction

Consumers are now more aware of the correlation between diet and health and are interested in nutritional based foods that can improve their health (Parr-Vasquez & Yada, 2012). Diet is also a known cause of several diseases including certain cancers, type 2 diabetes, cardiovascular disease, osteoporosis, hypertension and obesity (Nehir El & Simsek, 2012) and a program of optimised daily nutrition may prevent such diseases (Butt & Sultan, 2013). Healthy foods play a key role in this approach, together with suitable methods for food storage, the fortification of foods and the development of health promoting foods. The food industry can also assist by reducing the levels of trans fatty acids, saturated fats, salt and sugar in processed foods (Mohanty & Sahoo, 2010).

The terms " functional", "nutraceuticals" and "pharma" foods were first introduced in the 1980s (Raghuveer & Tandon, 2009). A functional food is defined as a food that is consumed as part of a normal diet but contains certain ingredients that can reduce the risk of chronic disease in addition the inherent nutritional functions of the foods (Parr-Vasquez & Yada, 2012). Such foods can be prepared by fortification of food products with vitamins and minerals, the addition of bioactive ingredients or via processing such as fermentation, which leads to an increase in bioactive components (Raghuveer & Tandon, 2009).

Curcuminoids are an example of a bioactive material that can be added to food products. Curcuminoids are polyphenolic compounds that are present in turmeric plants that are hydrophobic, low molecular weight and can be considered a functional food ingredient (Schaffer et al., 2011). Curcuminoids have traditionally been used for medicinal purposes for

at least 6000 years (Aggarwal et al., 2003). Clinical studies have also shown that the curcuminoids are potential effective agents against diabetes, Alzheimer's, cancer, multiple sclerosis, lung fibrosis, cardiovascular disease arthritis and inflammatory bowel disease (Beevers & Huang, 2011).

In spite of the above mentioned health benefits of curcuminoids, the low water solubility and low bioavailability of curcuminoids in the gastrointestinal tract, has limited the application of these functional ingredients in the pharmaceutical and food industries. Many investigations have examined the increase in bioavailability of curcuminoids using novel formulations. One of the most studied approaches is the use of water soluble compounds that can bind curcuminoids to increase their solubility. These soluble compounds can have a carrier role, allowing the curcuminoid complex to be more readily incorporated into food

Oat fibre is a potentially useful carrier for interacting with curcuminoids. It is rich in β-glucan, a soluble fibre known for its health benefits, including lowering cholesterol serum, the risk of coronary heart disease and blood pressure (Wood, 2007). In addition to fibre components, oat is also a notable source of proteins and lipids compared with other cereals (Sadiq Butt et al., 2008). This combination of oat fibre components can potentially promote interactions with the curcuminoids.

Extrusion cooking is one of the main technologies known to have a great potential for the manufacture of functional food products. Extruded products can be fortified with the addition of bioactive components such as the curcuminoids in order to address the need for a healthy ready to eat food products (Bisharat et al., 2013). Extrusion is a thermo-mechanical process where high heat, high pressure and shear forces are applied to an uncooked mass (Kim et al., 2006). During extrusion, the physico-chemical properties of raw ingredients change, this can

lead to improvements in the functional properties of foods, although the effects can also be detrimental. Extrusion processing is becoming more popular due to its low cost and versatility. This continuous process also offers high productivity and unique product shapes with the potential for low moisture products that are microbiologically safe (De Vos et al., 2010; Yılmaz et al., 2001). The quality of the final products are mainly dependent on extrusion variables such as raw materials composition, feed moisture, barrel temperature, screw speed, type of extruded and screw configuration which is further discussed in next chapter (Miller & Mulvaney, 2000).

In this research, the potential of oat fibre to interact with curcuminoids and improve the solubility and stability of curcuminoids is investigated. An extrusion process was applied to deliver a curcuminoid-oat fibre complex within a food product and the stability of curcuminoid in extruded snack products during 80 days of storage at 25 °C examined. In addition, the physico-chemical properties of extruded oat fibre without curcuminoids are also investigated. The characteristics of extruded products containing curcuminoids, including the expansion ratio, apparent viscosity, hardness and cross section examination are also evaluated. This study illustrates a potential route for the production of extruded foods that incorporate the benefits of oat dietary fibre and curcuminoids.

Research questions and objectives

From the literature review presented above it can be seen that there are several gaps in our understanding of curcuminoids and their interactions with potential carrier materials, such as oat fibre. The effect of extrusion processing on the bioactivity of curcumin and physico-chemical properties of curcuminoids and carrier materials are also not fully understood. The major research questions of this thesis are therefore:

1. Does oat fibre interact with curcumin?
2. Which components of oat fibre will interact with curcuminoids?
3. What is the solubility of curcuminoids in aqueous oat fibre dispersion?
4. What is the chemical stability of curcuminoids when bound to oat fibre ingredients?
5. Which physico-chemical properties of oat fibre are altered during extrusion?
6. What process variables significantly affect the physico-chemical properties of oat fibre?
7. Will incorporation of curcuminoids into extruded snacks affect their physico-chemical properties?
8. What process variables affect the physico-chemical properties of curcuminoids-enriched oat fibre extrudates?
9. What is the stability of curcuminoids during extrusion processing?
10. What is the storage stability of curcuminoids incorporated into extruded snacks?

To address these questions, several physico-chemical analyses were applied, from fluorescence spectroscopy and high performance liquid chromatography to the advanced techniques available for structural analysis including scanning electron microscopy and X-ray diffraction. In addition, extrusion trails were carried out to produce oat-fibre extruded

products, as well as curcuminoid-enriched oat fibre based extruded products. The physical properties of extrudates and curcumin stability during extrusion, drying and storage were also analysed.

This thesis aims to investigate the potential of oat fibre as a carrier for curcumin and to then introduce these components in an extrusion process to produce a new extruded functional food that combines the potential health benefits of curcuminoids and oat fibre. The thesis also aims to provide scientific insights into the mechanisms of curcuminoid interactions with oat fibre. This will potentially enable an improvement of extruded product quality. Another focus is on the physical properties of extrudates, which link to the physiological benefits of these ingredients. A final consideration is the stability of curcumin during and after processing.

Therefore the specific objectives of this thesis are as follows:

1. To understand the interaction between the carrier oat fibre and curcuminoids.
2. To study the effect of extrusion processing on the physico-chemical properties of the oat fibre carrier material.
3. To understand the effect of extrusion conditions on the physico-chemical properties of extruded products containing curcuminoids.
4. To investigate the effect of curcuminoids on the physical properties of extruded products.
5. To investigate the stability of curcuminoids during extrusion, drying and storage.

The study described in this thesis is presented in 6 chapters. A general introduction is given above in Chapter 1. A literature review of relevant studies on curcumin and oat fibre characteristics and also extrusion technology is also presented in the current chapter above. In Chapter 3, the potential interaction between curcumin and oat fibre is investigated. In addition the solubility, stability and structure of curcumin in presence of oat fibre dispersion are analysed. In Chapter 4, the physico-chemical properties of extruded oat fibre are investigated and compared with the properties of non-extruded oat fibre. In Chapter 5, the physico-chemical properties of extrudates containing curcumin are investigated. Also the stability of curcumin in extruded products during 80 days of storage at 25 °C is determined. The conclusions of this thesis and recommendations for the future studies are then presented in Chapter 6.

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Chapter 2

Literature review

A literature review regarding the topics discussed in current thesis is offered in this chapter. The specific objectives of the project are described in detail at the end of Chapter 2.

2.1. Polyphenols

Polyphenols are substances found in plants that are known to have more than one phenol unit per molecule (Landete, 2012). Their roles are to protect the plant from reactive oxygen species, photosynthetic stress and aggression by pathogens and herbivores (Beckman, 2000; Yang et al., 2001). Polyphenols are mainly responsible for the bitterness, astringency, colour, flavour and odour of foods (Pandey & rizvi, 2009).

Polyphenol compounds are products of the secondary metabolism of plants (Landete, 2012). The chemical structure of phenolic compounds typically contains phenylalanine amino acid, or its close precursor, shikimic acid (Shrivastava et al., 2013a). This structure can be varied from simple polyphenols, such as phenolic acids, to polymerized compounds, such as tannins (Kondratyuk & Pezzuto, 2004). Polyphenols also often occur in conjugated forms, including sugar residues (Harborne, 1994). Other compounds, such as organic acids, carboxylic, lipids and amines also differentiate the classes of polyphenols (Pandey & rizvi, 2009; Shrivastava et al., 2013a). The main classifications of polyphenols are shown in Figure 2.1.

Polyphenols are of increasing interest because of their possible beneficial effects on human health. The main health benefit of polyphenols is related to their radical scavenging activity (Munin & Levy, 2011). Polyphenols can act using different mechanisms including (a)

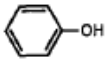
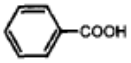
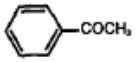
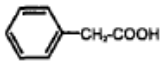
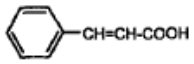
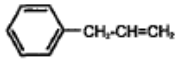
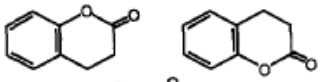
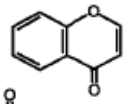
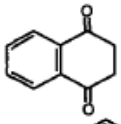
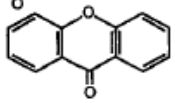
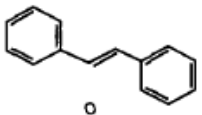
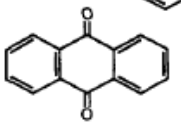
complexation with pro-oxidant proteins (Munin & Levy, 2011) (b) interacting with ions such as Fe^{3+}, Al^{3+} and Cu^{2+}, which are able to create free radicals (Handique & Baruah, 2002) or (c) by direct trapping the reactive oxygen species (ROS) (Latchman, 1997).

According to a broad range of clinical studies, it has been shown that the consumption of diets rich in polyphenols, results in protection against development anti-inflammation (Biswas & Rahman, 2008), cardiovascular disease (Suresh et al., 2006), cancer (Huang et al., 1991; Ramirez-Tortosa et al., 1999) and neurodegenerative diseases (Naidu & Thippeswamy, 2002). As a consequence, polyphenols can be regarded as a healthy food component with potential health promoting or disease-preventing properties that can potentially incorporated into food products.

In spite of all these health benefits, the bioavailability of polyphenols is limited due to the very low and inadequate gastric residence time and low solubility of these components (Fang & Bhandari, 2010; Munin & Levy, 2011). In addition, the high degree of sensitivity of polyphenols against environmental conditions (light, temperature, oxygen) during food processing, distribution or storage limits the bioavailability of polyphenols (Munin & Levy, 2011). Bioavailability is the portion of the nutrient that is consumed, absorbed and metabolized in the human gastrointestinal tract (Pandey & rizvi, 2009). The chemical structure of polyphenols determines this bioavailability by the rate and the extent of absorption. Consequently the specific biological characteristics of polyphenols varies from one polyphenol to another (Pandey & rizvi, 2009). For example, it has been shown that polyphenols in the form of esters, polymers and glycosides cannot be absorbed in native form (D'Archivio et al., 2007). Therefore, the application of phenolic compounds in food or

pharmaceutical industries requires considerable efforts in order to maintain the structural integrity of the polyphenol until the consumption.

Figure 2.1. Table of polyphenol classification (Bravo, 1998).

Class	Basic Skeleton	Basic Structure
Simple phenols	C_6	
Benzoquinones	C_6	
Phenolic acids	C_6-C_1	
Acetophenones	C_6-C_2	
Phenylacetic acids	C_6-C_2	
Hydroxycinnamic acids	C_6-C_3	
Phenylpropenes	C_6-C_3	
Coumarins, isocoumarins	C_6-C_3	
Chromones	C_6-C_3	
Naftoquinones	C_6-C_4	
Xanthones	C_6-C_1-C_6	
Stilbenes	C_6-C_2-C_6	
Anthraquinones	C_6-C_2-C_6	
Flavonoids	C_6-C_3-C_6	
Lignans, neolignans	$(C_6$-$C_3)_2$	
Lignins	$(C_6$-$C_3)_n$	

2.2. Curcuminoids

There has been extensive interest in using of polyphenols obtained from food sources to prevent human diseases. Tumeric, a rich source of phenolic compounds, specifically the curcuminoids, was widely used as a herbal medicine from second millenium bc (Brouk, 1975). The rhizome of turmeric plants contains turmerin (a water-soluble peptide), essential oils (such as turmerones, atlantones and zingiberene) and the major chemical components curcuminoids, which make the yellow colour characteristics (Potter et al., 2013). Curcuminoids include diferuloylmethane (curcumin I), demethoxycurcumin (curcumin II), bisdemethoxycurcumin (curcumin III) (Anand et al., 2007; Schaffer et al., 2011) and the recently discovered cyclocurcumin (Goel et al., 2008). Commercial curcumin typically consists of nearly 77 % diferuloylmethane, 17 % demethoxycurcumin and 6 % bisdemethoxycurcumin (Taylor & Leonard, 2011).

Curcumin is a bis-α, β-unsaturated diketone (commonly called diferuloylmethane) which consists of seven carbon chains with two benzene methoxy rings (Yang. et al., 2013) (Figure 2.2). Curcumin shows keto-enol structural isomerism depending on pH , ionic strength and polarity of solvent (Chignell et al., 1994). Its dominant form in acidic and neutral solutions is keto and in an alkaline environment the dominant form is the enol form as shown in Figure 2.3 (Orlando et al., 2012). The keto-enol forms of curcuminoid interacts with solvent through a strong intermolecular hydrogen bonding (Yang et al., 2011).

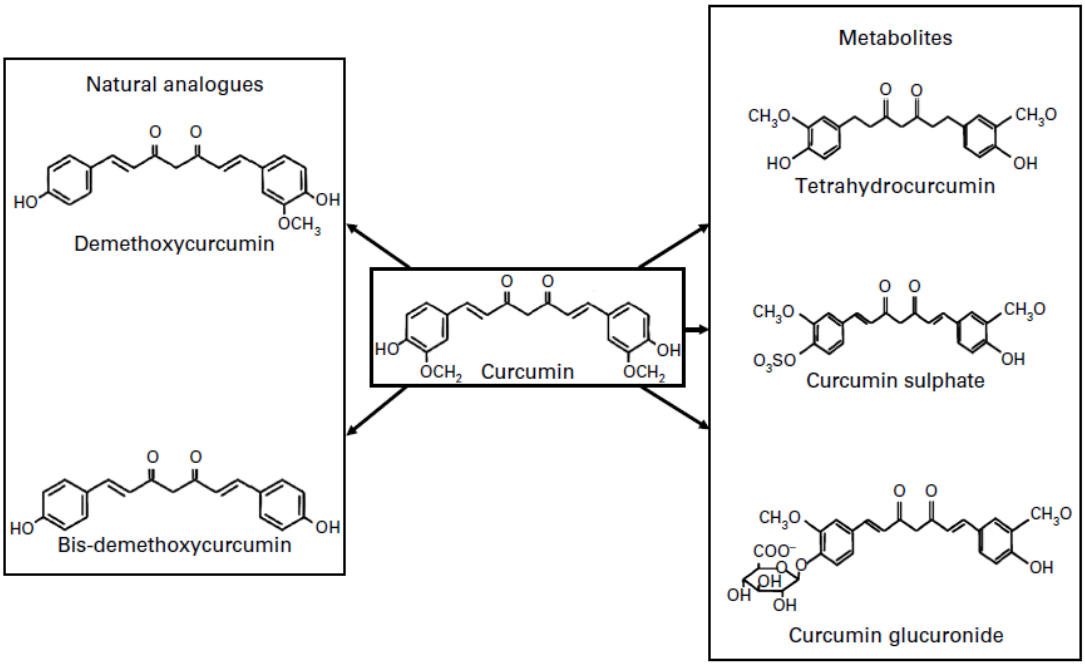


Figure 2.2. Chemical structure of curcumin and its natural isomer and main metabolites (Epstein et al., 2010).

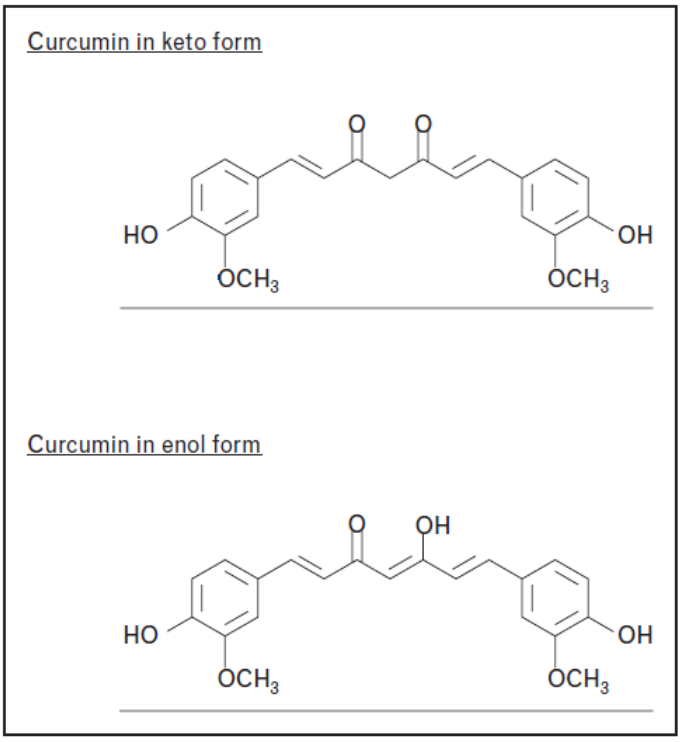


Figure 2.3. Different chemical structures of curcumin (Schaffer et al., 2011)

2.2.1. Fluorescence property of curcumin

Curcumin can absorb light in the visible region and emits fluorescence with low quantum yield (Patra & Barakat, 2011). The maximum optical absorption of curcumin ranges between ~ 408 — 430 nm depending on solvent characteristics, while the maximum emission wavelength of curcumin in either polar or non polar media, is in the range of 460 — 560 nm and the emission is more sensitive to the solvent properties (Mukerjee et al., 2010). In organic solvents, the diketone group of the curcumin molecule undergoes an enolisation, which provides the conjugation between π-electron clouds of the two rings sections (Patra & Barakat, 2011). This phenomena results in the formation of a conjugated chromophore (Patra & Barakat, 2011). The formation of this chromophore causes a reduction in the energy level (Jagannathan et al., 2012). Due to the low energy level of the π-π* excitation of the chromophore, the curcumin solution in organic solvents normally absorbs around 420 nm and shows a bright yellow-orange colour (Jagannathan et al., 2012) (Jagannathan, 2011).

The photochemical properties of curcumin, rely on the specific conditions of the microenvironment of the molecule, such as the presence of polar or non-polar solvents, for instance curcumin is soluble in polar organic compounds and insoluble in non-polar environments, such as water (Lee et al., 2008). It has been reported that water can suppress the fluorescence intensity of curcumin due to a reaction between H_2O molecules (which act as an electron donor) and the curcumin, resulting in the formation of a non fluorescent and more stable complex (Jasim & Ali, 1992). The maximum fluorescence intensity of curcumin shifts to a longer wavelength if the environment of curcumin changes from a non-polar to a polar solvent (Lee et al., 2008). For example, the maximum fluorescence of curcumin is about 439 nm in hexane, while it is about 518 nm in dimethylsulfoxide (DMSO) and 536 nm

in N-butyronitrile giving an indication of the relative polarity of these solvents (Patra & Barakat, 2011).

2.2.2. Health benefits of curcumin

The different biological and pharmacological effects of curcumin have been widely studied by researchers across the world. These studies have demonstrated the potential of curcumin as an anti-inflammatory, antioxidant, antimicrobial and anti-carcinogenic agent (Epstein et al., 2010; Schaffer et al., 2011; Song et al., 2012) (Figure 2.4). In recent years the role of oxidative stress in development of various diseases such as diabetes, chronic lung diseases, Alzheimer and cardiovascular diseases has been investigated. Oxidative stress occurs when there is an excess amount of reactive oxygen species (ROS) (Khurana et al., 2013). ROS are generated from the enzymatic reduction of oxygen (Hristova & Penev, 2014). The natural defense system of the body can usually regulate and monitor the presence of ROS (Brieger et al., 2012). Under the conditions such as stress, polluted environments and aging, the amount of ROS will increased gradually and the cellular defense mechanism of body is overwhelmed by the excessive level of ROS, resulting in cell deterioration (Selvaraju et al., 2012).

It is well established that polyphenols, such as curcumin, can prevent the oxidative stress and consequently protect body cells from detrimental effects (Khurana et al., 2013). It has been reported that consumption of curcumin is safe, tolerable and nontoxic for humans (Gupta, 2012). A diet including 500 mg curcumin a day can reduce the level of lipid peroxides, total serum cholesterol and increase serum HDL (Pari et al., 2008).

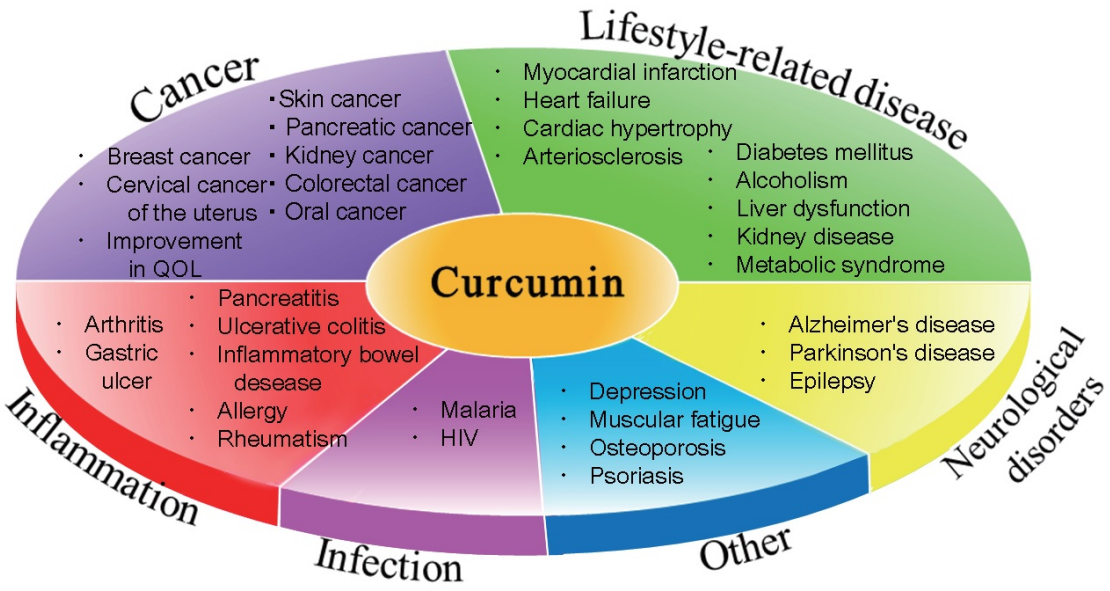


Figure 2.4. A range of therapeutic applications proposed for curcumin (Shimatsu et al., 2012)

In addition, some studies suggest that curcumin can prevent the oxidation of low density lipoprotein (LDL), which has a critical role in development of atherosclerosis (Naidu & Thippeswamy, 2002; Ramirez-Tortosa et al., 1999). Furthermore, curcumin has radical scavenging activity that can prevent proteins and lipids from oxidation (Kolodziejczyk et al., 2011). The mechanism of radical scavenging of curcumin is a subject of many studies. According to one study, 'the enolised diketone system of curcumin is involved in the scavenging of oxygen' (Tonnesen, 1995). On the other hand it has been reported that the phenolic group is essential for the free radical scavenging activity and also the presence of the methoxy group further increased this activity (Sreejayan & Rao, 1996).

In one study, the anticancer activity of curcumin was examined (Aggarwal et al., 2003). This characteristic of curcumin comes from its ability to prevent the reproduction of tumour cells

(Aggarwal et al., 2003), specifically by inhibiting transcription factors, proteins that can control the rate of transcription of genetic information from DNA to messenger RNA by binding to certain DNA sequences (Latchman, 1997).

Curcumin also possesses anti-inflammatory properties (Biswas & Rahman, 2008). Curcumin can inhibit two enzymes, called lipoxygenase (LOX) and cyclooxygenase 2 (COX-2), which are involved in the process of inflammation (Huang et al., 1991; Suresh et al., 2006). Curcumin has also a protective role against Alzheimer's disease, decreasing the inflammation of the brain and protecting the brain against β-amyloid formation, which promotes neurotoxicity (Potter et al., 2013). Curcumin decreases β-amyloid formation by inhibiting the formation and aggregation of amyloid precursor protein and β-amyloid respectively (Orlando et al., 2012).

2.3. Challenges for curcumin delivery

Despite the potential health benefits of curcumin, there are some limitations in the application of curcumin in the food and pharmaceutical industries. The first challenge is due to a very low bioavailability of curcumin. The recommended maximum daily intake of curcumin has been reported at 0–1 mg/kg body weight based on a Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) report (Potter et al., 2013). Clinical studies showed that curcumin can be administered safely at oral doses of up to 8 g per day, without toxic effects (Smith, 2001). Approximately 75 % of orally administrated curcumin, however, was excreted with faeces in rats (Hoehle et al., 2006).

The reasons attributed to the low bioavailability of curcumin are described here:

2.3.1. Low water solubility

Aqueous solubility is an important characteristic for any bioactive component. Water solubility can control the dissolution, absorption and in-vivo bioavailability of bioactive components (Meng & Ma, 2002). Curcumin is hydrophobic due to the lack of any polar group and the stretching of the conjugated back bone (Potter et al., 2013). This property makes curcumin insoluble in water (polarity: 1) ; however, it is completely soluble in ethanol (polarity : 0.654), dimethylsulfoxide (polarity : 0.444) and acetone (polarity : 0.355) (Jagannathan et al., 2012). The maximum water solubility of curcumin at pH = 5.0 was reported to be as 11 ng/mL (Aggarwal et al., 2003; Kaminaga et al., 2003; Yu & Huang, 2010) and 0.0004 mg/ml at the physiological pH of 7.4 (Tønnesen et al., 2002).

2.3.2. Rapid metabolism

Curcumin undergoes rapid metabolism immediately after absorption via glucuronidation or sulfation or reduction mechanisms (Colyer & Luthe, 1984). It has been reported that 60 – 70 % of orally administered curcumin is eliminated in faeces due to low solubility and rapid intestinal metabolism (Loponen et al., 2007) with only 11.78 % of curcumin metabolised during intestinal transport (Peterson, 1986).

2.3.3. Curcumin stability

2.3.3.1. pH stability

Curcumin is not soluble in water at acidic and neutral pH, while it is soluble in an alkaline environment (Kumavat, 2013). At alkaline pH, the acidic phenol group in curcumin donates its hydrogen, forming the phenolate ion that enables curcumin to dissolve in water (Kumavat, 2013). After a long period of time, however, curcumin degrades easily into compounds like

vanillin and ferulic acid (Wang et al., 1997) and turns to red color (Patel et al., 2010), leading to a decrease in the absorbance at 420 nm indicating a reduction in the concentration of pure curcumin (Dey & Sreenivasan, 2014). At acidic pH, curcumin primarily occurs in a protonated state and is degraded to trans-6-(4´-hydroxy-3´-methoxyphenyl)-2, ferulic acid, 4-dioxo-5-hexanal, feruloylmethane and vanillin (Tonneson & Karlsen, 1985).

Many have tried to improve the curcumin stability at different pH. In one study, pure and microencapsulated curcumin in maltodextrin were analysed spectroscopically at pH 1-9 (Sousdaleff et al., 2012). Encapsulated curcumin was stable in the pH range of 1- 8, with a slight increase in color at pH 9, while the absorbance of free curcumin reduced significantly at pH higher than 6. The formation of curcumin microcapsules with maltodextrins can protect curcumin from degradation across range of pH without precipitation and colour change (Sousdaleff et al., 2012). In another study, it was observed that encapsulation of curcumin in gelatine and starch reduces the absorbance of curcumin by 3.3 % and 2.6 % in the pH range of 1-6, while this value decreases up to 15 % for free curcumin (Wang et al., 2009).

In a further study, curcumin was embedded in zein polymeric particles (Patel et al., 2010). The results of this study showed that curcumin has higher stability in the pH range of 1-9 when incorporated in zein particles, compared with free curcumin, due to the effect of the zein particles which protected curcumin against degradation (Patel et al., 2010). These studies clearly demonstrate the effect of a carrier to protect curcumin against degradation in different pH range.

2.3.3.2. Temperature stability

Curcumin undergoes thermal degradation under different food processing conditions (Dahmke et al., 2014). For instance, it was reported that up to 53 % of curcumin content is lost due to the thermal degradation under pressure cooking (15 p.s.i) for 10 min (Suresh et al., 2009; Suresh et al., 2007). The thermal stability of curcumin, however, can be improved by interaction of the curcumin with a carrier that can protect curcumin against degradation. For example, the heat stability of encapsulated curcumin in gelatine before and after spray drying was determined (Wang et al., 2009). Temperatures below 70 ºC had no effect on stability of either free or encapsulated curcumin. Increasing the temperature higher than 70 ºC, however, decreased the absorbance value of free curcumin very rapidly, while this value decreased slowly for encapsulated curcumin, indicating the protective effect of encapsulation against thermal degradation. Encapsulation of curcumin, however, doesn't always protect curcumin against degradation. For example, curcumin degradation in free and encapsulated form was examined at -15 °C, 4 °C and 25 °C (Mangolim et al., 2014b). Retention of encapsulated curcumin with β-cyclodextrin was improved 9 % and 4 % after 90 days at -15 °C and 4 °C respectively and no improvement of curcumin stability was observed for both free and encapsulated curcumin at 25 °C, indicating the combined effects of encapsulation and environment temperature on curcumin stability.

The protective effect of a carrier on the thermal degradation of curcumin was further investigated in another study (Niu et al., 2012a). The reduction of relative intensity of encapsulated curcumin in liposomes complex during 180 min was significantly smaller than that of non-encapsulated curcumin (Niu et al., 2012a). Also in this study, it was observed that increasing the temperature from 25 °C to 80 °C resulted in fast degradation of liposomal

curcumin. Above the phase transition temperature of the liposome, the structure of the liposome changes from a gel phase to a liquid state, suggesting a weaker protection of curcumin (Niu et al., 2012a). The results of this study show that the physico-chemical properties of an ingredient as a carrier should be considered, since any change in such properties of carrier may affect the stability of curcumin.

The temperature also affects the interactions between curcumin and carrier molecules. For example, the association constant (Ka) of curcumin with β-casein increased when the temperature increased from 25 ºC to 37 ºC from 1.8 ± 0.4 $(mol/L)^{-1}$ to 8.3 ± 0.7 $(mol/L)^{-1}$ (Esmaili et al., 2011), showing that increasing the temperature is not always detrimental to curcumin stability. In another study, curcumin was encapsulated in yeast cells through freeze drying a yeast cell-curcumin suspension (Paramera et al., 2011). Encapsulation of curcumin in yeast cells was favoured at temperatures above 35 °C, rather than below 35 °C. A higher temperature between 35-45 °C, can cause more fluidity within the phospholipids of the yeast cell membrane, therefore curcumin can penetrate further into the plasma membrane and be more efficiently encapsulated.

2.3.3.3. Light Stability

Curcumin undergoes degradation when exposed to light in both solution and solid forms (Ansari et al., 2005). Curcumin is rapidly degraded into vanillin, vanillic acid, ferulic aldehyde and ferulic acid upon exposure to light (Meng & Ma, 2002).

The application of carriers to protect curcumin against light degradation has been widely studied (Abdul Aziz et al., 2007; Tønnesen et al., 2002). Microencapsulation of curcumin with gelatine can improve curcumin stability against light for 12 hr/day. The light

degradation of free curcumin powder was reported to be 0.95 ± 0.10 % per day, while curcumin microencapsulated in gelatin was degraded 0.13 ± 0.04 % per day (Abdul Aziz et al., 2007). In another study, the effect of ultra violet light (UV) on curcumin stability was investigated (Patel et al., 2010). More than 55 % of the curcumin in zein-curcumin particles was not changed when exposed to UV light, in comparison with less than 30 % of pure curcumin was retained when exposed to the equivalent UV light (Patel et al., 2010). These studies indicate the significant effect of a carrier in protecting curcumin against light degradation

In a separate study, the light stability of curcumin encapsulated with maltodextrin was investigated (Sousdaleff et al., 2012). Starchy materials, such as maltodextrin, were observed to protect curcumin against light degradation. These materials can form a layer around curcumin, due to their plastic properties. If the starch structure breaks down, curcumin is exposed to oxidation and light and then degrades quickly (Sousdaleff et al., 2012). Therefore the structural properties of the carrier need to be considered for selecting a carrier to protect curcumin.

In spite of above literature, the light stability of curcumin is not always improved by encapsulation. The results of one study showed that when curcumin was stored in the dark it degraded at a rate similar to the curcumin stored in daylight, likely due to the effect of oxygen (Aparecida Marcolino et al., 2011), indicating that light is not the only major factor in the degradation of curcumin. In addition, it was demonstrated that even complexation of curcumin with β-cyclodextrin didn't improve the stability of curcumin against light, since a degradation of ~40 % in colour intensity occurred in storage under light and dark, similar to the loss that occurs without complexation (Aparecida Marcolino et al., 2011).

2.4. Strategies to improve curcumin delivery

There have been a significant number of strategies trialled to overcome the poor bioavailability, limitations of stability and rapid metabolism of curcumin. These strategies are classified into four groups:

1. Metabolism interference via adjuvant

Adjuvants such as piperine generally improve the bioavailability of curcumin by blocking the metabolism of curcumin (Anand et al., 2007). For instance, it has been reported that the curcumin concentration in blood serum was either undetectable or very low after receiving a dose of 2 g of curcumin alone, while concomitant administration of piperine, produced a 2000 % increase in bioavailability (Shoba et al., 1998).

2. Liposomes, micelles and phospholipid complexes

Liposomes are excellent drug delivery systems, since they can carry both hydrophilic and hydrophobic molecules (Zuidam, 2010). Some studies have reported that liposomal encapsulation of curcumin can enhance the loading level of curcumin in lymphocyte cells (Kunwar et al., 2006), leading to higher gastrointestinal absorption and plasma antioxidant activity (Takahashi et al., 2009).

Micelles and phospholipid complexes are self-assembled mixtures of water, oil and surfactants and offer a promising method to improve the bioavailability of curcumin. For example, the results of one study showed a significant improvement in curcumin bioavailability due to the formation of a curcumin-phospholipid complex (Liu et al., 2006), by improving the gastrointestinal absorption to achieve higher curcumin concentration in plasma and lower kinetic elimination (Liu et al., 2006).

3. Nanoparticles

Incorporation in nanoparticles is one approach that can help to overcome poor aqueous solubility, dissolution and/or bioavailability of curcumin (Devalapally et al., 2007). Nanoparticles are stable colloidal particles that range in size from 10 nm to 1000 nm (Yen et al., 2010). The physicochemical properties of curcumin- polyvinylpyrrolidone (PVP) nanoparticles including the small particle size and amorphous state of curcumin in this nanoparticles complex, increase the release rate of curcumin from polyvinylpyrrolidone nanoparticles (Yen et al., 2010).

4. Derivatives and analogues

It has been shown that the chemical structure of curcumin plays an important role in its biological activity. For example, isomerization can influence the antioxidant activity of curcumin (Shen & Ji, 2007). Therefore, there is a possibility to improve the bioavailability of curcumin by structural modifications. However, this has not been studied in detail.

The potential of natural carriers, such as proteins (Kim et al., 2011; Patel et al., 2010; Sneharani et al., 2009; Wang et al., 2005), phospholipid complexes (Lin et al., 2009) and starch (Minpeng & Suhong, 2012; Yu & Huang, 2010) have also been investigated to improve the solubility and stability of curcumin. For instance, there have been many attempts to use milk proteins, such as caseins, as potential carriers for curcumin and other polyphenols (Esmaili et al., 2011; Rahimi Yazdi & Corredig, 2012; Sahu et al., 2008; Sneharani et al., 2010). The presence of hydrophobic and negatively charged regions and the lack of a folded structure makes casein a useful molecule for interacting with curcumin (Sneharani et al., 2009). In one study, the binding characteristics of curcumin with αs_1-casein were examined

(Sneharani et al., 2009). Curcumin was found to interact with αs_1-casein through hydrophobic bonding. In the presence of αs_1-casein, ~ 45 % curcumin was retained after 6 hours, while about 90 % of the curcumin without αs_1-casein is decomposed rapidly in buffer at the end of 30 min incubation (Sneharani et al., 2009).

The application of β-casein proteins to improve curcumin solubility and stability has also been investigated (Esmaili et al., 2011). β-casein forms micellar nanostructures due to its amphiphilic and self-assembling properties, which makes this protein a good carrier system for hydrophobic bioactive materials, such as curcumin (O'Connell et al., 2003). The hydrophobic interaction between of β-casein and curcumin increased the solubility of curcumin in aqueous solution at least 2500 fold (Esmaili et al., 2011).

Curcumin also interacts with β-lactoglobulin via a central hydrophobic part of the β-lactoglobulin molecule (Sneharani et al., 2010). This kind of interaction can improve curcumin stability up to 6.7 times in comparison with curcumin alone in aqueous solution. Also the solubility of curcumin in β-lactoglobulin nanoparticles was markedly enhanced, with aqueous solubility of 625 μM in compared to 30 nM without the carrier (Sneharani et al., 2010).

Apart from milk proteins, the role of other proteins such as corn protein (zein) as a carrier for curcumin have also examined (Patel et al., 2010). More than 55 % of curcumin in zein–curcumin composite colloidal particles was preserved after 6 hours, compared with less than 30 % for curcumin solution (Patel et al., 2010). In another study, the application of soy protein as a carrier for curcumin was investigated (Tapal & Tiku, 2012). Curcumin solubility in water was enhanced from 11 ng/ml to 8.9 μg/ml when curcumin incorporated into soy protein complex (Patel et al., 2010).

The potential of carbohydrates ingredients to increase the solubility and stability of curcumin has been also investigated. Hydrophobic interactions between curcumin and hydrophobically modified starch have been shown to increase curcumin solubility about 1670 fold in comparison with aqueous solubility of curcumin (Yu & Huang, 2010). In another study, the incorporation of curcumin in some steviol glycosides, such as stevioside and rubusoside, was investigated (Zhang et al., 2011a). Curcumin solubility was enhanced from 61 µg/ml to 2.318 mg/ml in the presence of 1 % to 10 % rubusoside (w/v) respectively. Rubusoside is an amphyphilic compound, which has both a lipophylic steviol unit and a hydrophilic glucose unit that facilitate curcumin solubilisation in aqueous solution (Zhang et al., 2011a). In another study, nanoparticles of curcumin and chitosan were also prepared by spray drying (O'Toole et al., 2012). The encapsulation efficiency of curcumin in chitosan/tween 20 particles was reported to be nearly 100 %. Furthermore, *in vitro* experiments showed that curcumin could be totally released from chitosan particles within 2 hours (O'Toole et al., 2012).

Despite broad research on the interactions between polyphenolic compounds, such as curcumin, with different types of carrier materials, there are very few studies on the use of dietary fibre as a potential carrier. A large proportion of polyphenols are associated with dietary fibre in plants and fruits. In general, fibre-polyphenol complexes may be present as soluble polymers, insoluble macromolecular complexes or as hydrated networks (Ma, 1987). Interactions of polyphenols with fibre components of the food matrix potentially impacts on polyphenol metabolism and should be considered and exploited in future studies.

In a recent review about the potential of dietary fibre as a carrier for polyphenolic compounds (Saura-Calixto, 2011), it has been reported that polyphenols can be linked with dietary fibre

by a number of mechanisms including hydrogen bonding (between the hydroxyl group of polyphenols and oxygen atoms of the glycosidic linkages of fibre), hydrophobic interactions and/or covalent bonds such as ester bonds between phenolic acids and polysaccharide (Mirmoghtadaie et al., 2009). In one study, the interaction between polyphenolic proanthocyanidins and apple cell wall materials in aqueous solutions was attributed to non-covalent bonding between these two components (Bourvellec et al., 2004). In a separate study, the potential of β-glucan as a carrier for tea polyphenols was investigated (Wu et al., 2011). The interaction between β-glucan and hydrophobic polyphenolic compounds of tea was facilitated by hydrogen bonding and hydrophobic interactions (Wu et al., 2011). These studies promisingly demonstrate the potential and efficient interaction between polyphenolic compounds and fibre materials.

The interaction between polyphenols and fibre materials are influenced by the ionic strength of the environment. For example, the effect of ionic strength and ethanol concentration on the strength of interactions between β-glucan and tea polyphenols has been studied (Umetskaya et al., 1984). Ethanol in high concentration forms a hydrogen bond with tea polyphenols at the OH group of the polyphenol ring that results in the reduction of binding between β-glucan and tea polyphenols (Umetskaya et al., 1984; Veverka et al., 2014). The results of this study show that the concentration of ethanol or ionic strength of solvents should be considered while studying the interaction of fibre materials with polyphenols.

In spite of above studies, there have been few investigations examining interaction between curcumin and dietary fibre. In one study, the incorporation of curcumin into soluble dietary fibre from fenugreek was investigated (Im et al., 2012). The bioavailability of curcumin

increased 20 fold to 250 mg/kg dosage in Wistar rats, indicating the potential of fibre materials to interact with curcumin.

In another study, the stability of bioactive materials including curcumin was studied when incorporated in a β-glucan complex (Veverka et al., 2014). Interaction with β-glucan increases the oxidative, thermal and light stability of curcumin by incorporation of curcumin into the helical structure of β-glucan and the formation a stable complex (Veverka et al., 2014). This study shows promising results suggesting the potential of oat dietary fibre, specifically β-glucan, as a suitable carrier for curcumin.

2.5. Oat dietary fibre

Dietary fibre (DF) consists of the residue of edible plant cells, polysaccharides, lignin, and substances resistant to digestion by the digestive enzymes of gastrointestinal tract. The constituents of dietary fibre include cellulose, hemicelluloses, lignin, gums, mucilage, oligosaccharides, pectin, and other associated minor substances (Dai & Chau, 2017; Lunn & Buttriss, 2007). Dietary fibre are divided into soluble or insoluble DFs. Insoluble dietary fibre IDF consists mainly of cell wall components (e.g., cellulose, lignin, hemicelluloses), while SDF consists of noncellulosic polysaccharides (e.g., pectin, gums, mucilage) (Dai & Chau, 2017).

Oat fibre is known as a healthy food and consumption of 3 g of soluble fibre per day from whole oats is thought to reduce the risk of heart disease (FDA, 1997). Several reviews have focused on the health benefits of oat fibre (Othman et al., 2011; Sadiq Butt et al., 2008). These benefits are mainly related to β-glucan, the main soluble dietary fibre within oat grain (Schultz, 2004).

2.5.1. β-glucan

Oat β-glucan is a soluble fibre that occurs mainly in the cell wall of the endosperm of oats (Sadiq Butt et al., 2008). It consists of units of D-glucose linked by either β-1,3 linkages (30 %) along the backbone or β-1, 4 linkages (70 %) between the backbone and the side chains (Figure 2.5) (Mantovani et al., 2008).

Any change in molecular structure of β-glucan can result in a change in functional properties of β-glucan including solubility and viscosity. For instance, the β-(1→4) linkages within β-glucan can lead to interchain aggregation (and hence lower solubility) due to strong hydrogen bonds that occur along the cellulose-like segments, while the β-(1→3) links break up the regularity of the β-(1→4) linkage sequence, making the molecule more soluble and flexible (Liu & White, 2011).

β-glucan is typically viscous and forms gels similar to other food hydrocolloids (Lazaridou & Biliaderis, 2007b). β-glucan is therefore a useful food ingredient that can increase the gelling capacity and viscosity of aqueous solutions, modifying the texture and appearance of food formulations (Pan et al., 2015). β-glucan has been also incorporated into products such as breakfast cereals, pasta, noodles, bread, muffins, dairy and meat products to develop a range of healthy functional foods (Cavallero et al., 2002; Duss & Nyberg, 2004).

The ability of oat β-glucan to form highly viscous solutions in water can also lead slower intestinal transit and delays gastric emptying (McClements, 2012; Wood, 2004). Consumption of 3 g oat β-glucan per day (0.75 g per serving) can also reduce blood cholesterol and normalize blood sugars (Lazaridou & Biliaderis, 2007b; Mahdavi et al., 2014).

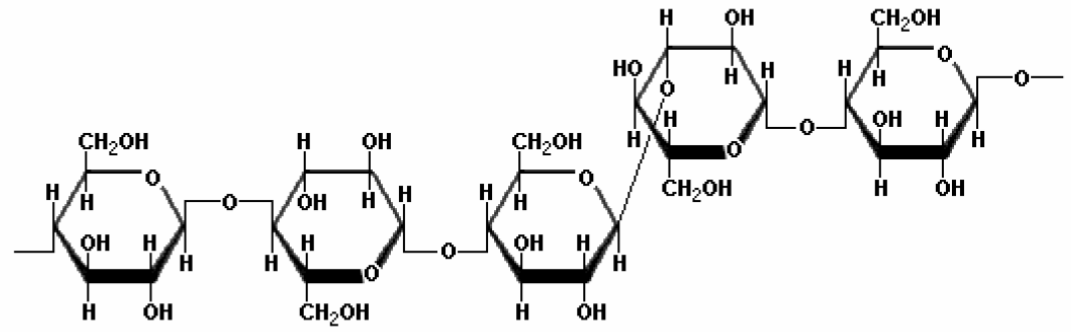


Figure 2.5. Chemical structure of β-glucan (Othman et al., 2011)

2.6. Extrusion

The health benefits of oat dietary fiber, are known to be related to its (1→3)/(1→4) chemical bond ratio, viscosity and molecular weight (Schultz, 2004), properties which are influenced by the processing applied before consumption. One the other hand processing methods can also improve the functional properties of oat dietary fibre (Zhang et al., 2011b), illustrating the balance that must be stuck between processing conditions to ensure optimal properties in the processed product.

Extrusion processing is a technology that has the potential to affect oat fibre properties. This process applies high temperature, high pressure and mechanical forces in a short time to an uncooked mass (Navale, 2015). The main operative body in the extruder is a screw or a pair of screws fitted within a barrel. During thermal processing a pressure up to 20 MPa and temperature of 200 ° C are applied to a mixture of feed materials and water in order to be mixed, compressed, melted and plasticized (Guy, 2001).

Extrusion cooking is an energy efficient method that has been widely applied to produce ready-to-eat cereals and snack products (Brennan et al., 2013a). It can also be applied for applications such as applying temperature or shear that can induce morphological changes,

blending, separation, reducing the water and volatile content, flavour entrapment or encapsulation (Yuliani et al., 2006). In addition, extrusion can be increased in scale to an industrial scale. The process has the advantage of being high throughput, cost effective and environmentally friendly process relative to other food processing technologies, such as spray drying and freeze drying (Yılmaz et al., 2001). A schematic figure of an extruder is shown in Figure 2.6.

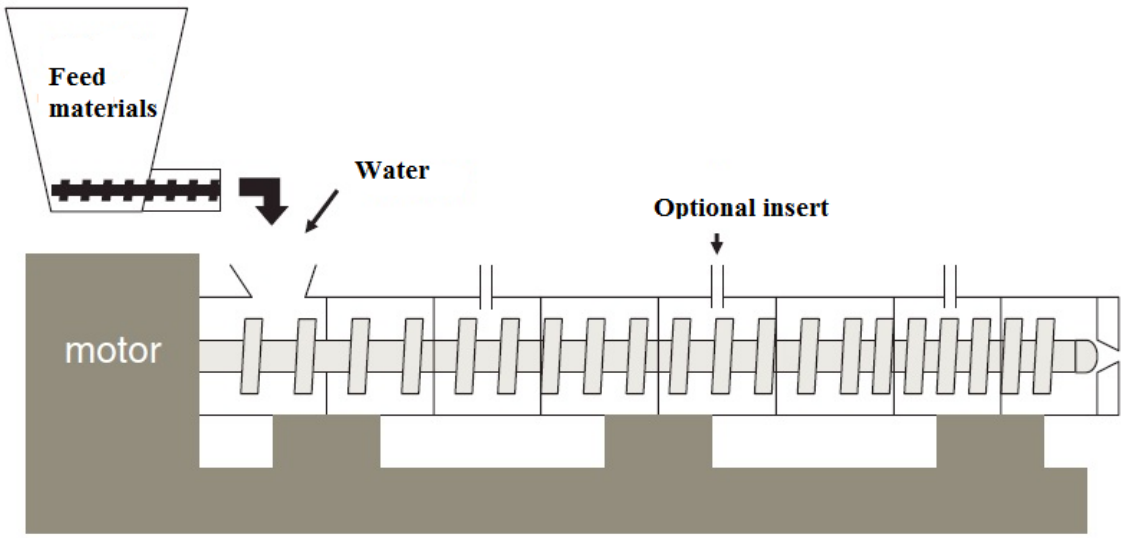


Figure 2.6. Schematic figure of cooking extruder.

2.6.1. Effect of extrusion processing on raw ingredients (oat fibre)

During extrusion processing the microstructure, chemistry and macroscopic properties of the raw material are affected (Wolf, 2010a). For instance, chemical changes such as starch gelatinization and protein denaturation may occur, although the former depends on the moisture content (Basediya et al., 2013; Kim et al., 2006). In addition, extrusion cooking may adversely affect the nutritional quality of foods, for example by destruction of vitamins, or

favourably, for example by inducing the inactivation of anti-nutritional factors (Singh et al., 2007). These changes mainly rely on process factors such as the extrusion temperature, screw speed, barrel temperature and screw configuration including length of screw, helix angel, screw pitch, channel depth) (Ali et al., 1996; Cindio et al., 2002; Miller & Mulvaney, 2000). Variables like feed moisture content and feed formulation also play an important role in determining the extrudate characteristics (Miller & Mulvaney, 2000). The connections between different variables that have an influence on the extrusion cooking process are illustrated in Figure 2.7 (Brennan et al., 2013a).

Extrusion can produce considerable changes in the structure and properties of oat fibre, with subsequent changes in physiological responses (Wan et al., 2009a). In one study, the effect of different processing including steam heated, superfine ground and extrusion (600 rpm, 180 °C, 40 % moisture content of feeding materials) on the molecular weight of oat bran was investigated (Zhang et al., 2009). The results of this study indicated that the proportion of fractions with a molecular weight more that 5×10^5 in oat soluble dietary fibre (OSDF) was 97.4 % in extruded oat bran in comparison with 31.3 % , 30.3 % and 37.3 % OSDF for untreated, steam heated and superfined ground oat bran respectively. This result has been attributed to the lack of large degradation of oat fibre during extrusion (Zhang et al., 2009).

In another study, opposing results have been reported regarding the degradation of oat fibre during extrusion (Tosh et al., 2010). Increasing the specific mechanical energy from 84 Wh/kg to 135 Wh/kg resulted in reduction in the molecular weight of β-glucan from 1,930,000 to 251,000 g/mol using die temperatures in the range of 181 °C to 237 °C and feed moisture of 7 % w/w (Tosh et al., 2010), indicating that the degradation of β-glucan is dependant to the extrusion variables such as temperature and specific mecjanical energy.

The molecular structurre of oat fibre can also change during extrusion. For example, it was shown that the β (1→4) linkage level of oat soluble dietary fibre (OSDF) from extruded oat bran is the lowest in compared with β (1→4) linkage level of oat soluble dietary fibre from other processings including steam heated, superfine ground (Zhang et al., 2009). Also the OSDF obtained from extruded oat bran had the lowest solubility values in compared with solubility values of OSDF from other processing methods (Zhang et al., 2009). This lower solubility was related to the reduced amount of β-(1→4) linkages in the extruded oat bran samples. It was further explained that β-(1→4) units of β-glucan tend to aggregate with each other through strong hydrogen bonds, which make the β-glucan molecule less soluble, while the β-(1→3) linkage makes the molecule more soluble and flexible (Buliga, 1986). These findings indicate the potential of extrusion processing to change the molecular structure of oat dietary fibre and finally the functional properties of that.

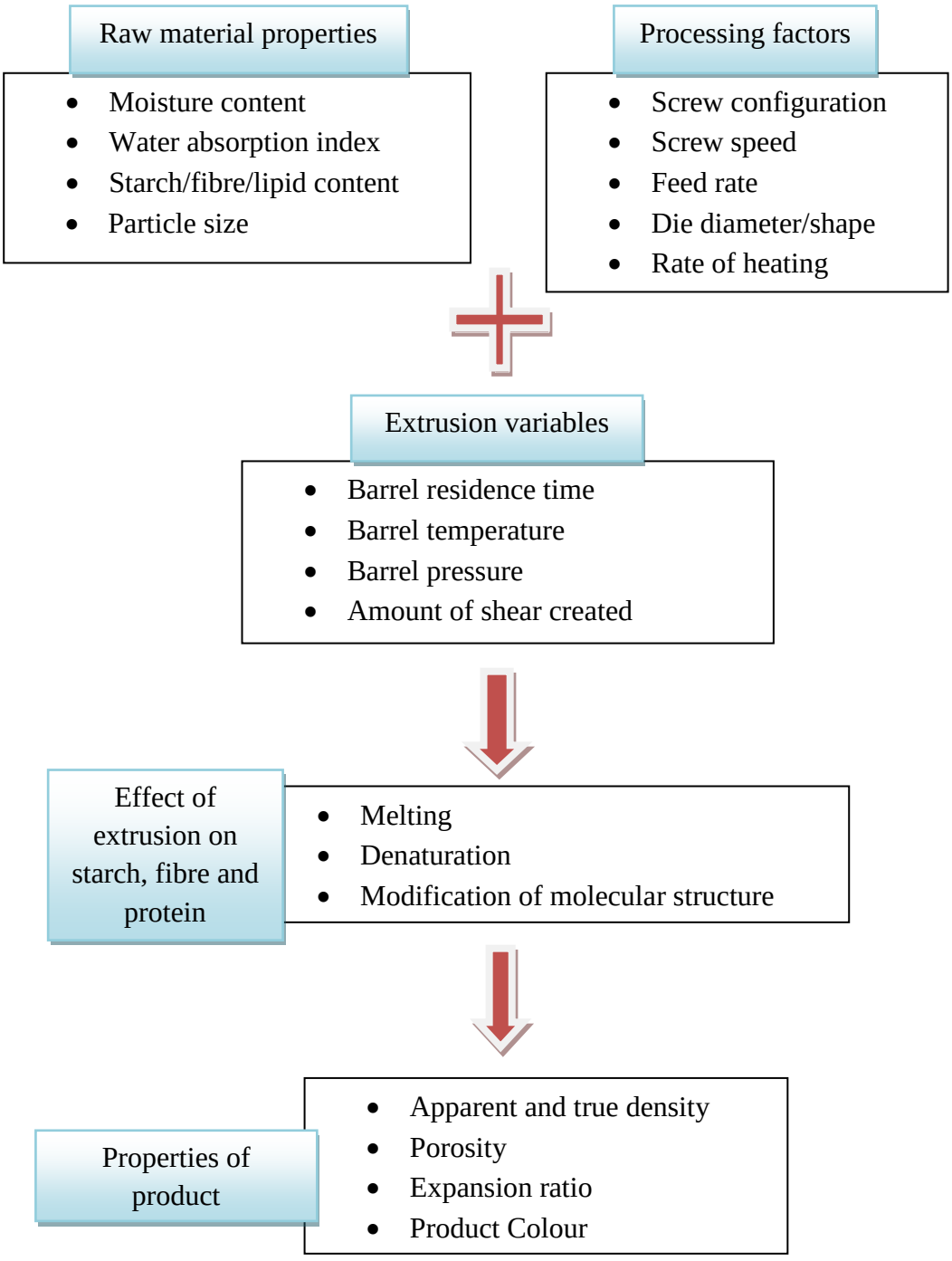


Figure 2.7. Potential interactions of raw materials and extrusion process variables on an extrudate product.

The effect of feed moisture inside the extruder on the yield of soluble dietary fibre (SDF) of oat bran has been investigated (Zhang et al., 2011b). Increasing the moisture content of the feed from 10 % to 20 % and 30 % at a constant barrel temperature of 140 °C, reduced the SDF content of extrudate products from 14.2 to 12.6 and 11.5 g/100g, respectively (Zhang et al., 2011b), suggesting that optimizing the barrel moisture content is necessary to obtain a high yield of soluble dietary fibre.

The particle size distribution of oat dietary fibres can also be affected by extrusion processing. For example, it was reported that the soluble dietary fibre from extruded oat bran can have a larger particle size distribution in comparison with non-extruded oat bran (Zhang et al., 2011b). This was attributed to the fact that extrusion processing can result in the extraction of high molecular weight soluble dietary fibre from oat bran (Zhang et al., 2011b).

Other properties of fibres that might be influenced by extrusion are water absorption index (WAI) and water solubility index (WSI). These properties are indicators of molecular fragmentation during processing (Hagenimana et al., 2006; Xu et al., 2014). WAI and WSI are dependent on extrusion variables, such as temperature and barrel feed moisture (Gutkoski & Eldash, 1999). For example, it was shown that increasing the barrel temperature from 77 °C to 140 °C resulted in a decrease of the WSI of oat flour from 8.95 to 5.45 % of dry matter but the WAI of oat flour increased from 2.75 g gel/g to 6.35 g gel/g of dry matter across the similar range of temperatures (Gutkoski & Eldash, 1999). WSI depends on the amount of soluble molecules and starch gelatinization (Gomez & Aguilera, 1983); however, the lower WSI of extruded oat flour was attributed to the presence of lipids, proteins, and other dietary fibre that may influence on oat starch cooking levels, and therefore the extent of soluble fraction release.In some examples, however, increasing the extruder temperature from 160 °C

to 237 °C and specific mechanical energy (SME) from 84 Wh/kg to 148 Wh/kg enhanced the water solubility of β-glucan in oat bran from 66.8% to 100%, due to the higher depolymerisation of β-glucan (Tosh et al., 2010).

Another factor affecting the water absorption capacity of extruded materials is the barrel moisture content. At a lower barrel moisture content, the dough inside the extruder is more viscose resulting in a higher specific mechanical energy input to the dough (Ding et al., 2006). This can result in a higher degree of biopolymer disintegration, allowing for better penetration of water into macromolecules into the structure (Marzec & Lewicki, 2006; Mesquita et al., 2013).

2.6.2. Effect of extrusion processing on phenolic compound (curcumin)

The high temperature and mechanical forces during extrusion processing can stimulate the degradation of phenolic compounds, due to the decarboxylation of phenolic acids (Brennan, 2011). For example, it was found that 30 % of β-carotene was degraded during extrusion in a maize starch matrix at temperatures of 135-170 °C and screw speeds of 300-800 rpm (Lee et al., 1978). Extrusion variables such as screw speed, temperature and feed moisture content can also influence the degradation of bioactive components. For example, the total anthocyanin content of grape pomace decreased during extrusion at the lowest screw speed (100 rpm) and the highest temperature (190 °C) from 1134 mg/kg to 530 mg/kg (Khanal et al., 2009b). At a higher screw speed of 200 rpm, the residence time of grape pomace inside the barrel and the exposure to conditions of high temperature was reduced, resulting in higher retention of anthocyanins (Khanal et al., 2009b). These results clearly indicated that residence time is another important factor that can affect on bioactive materials degradation.

Subsequently optimising the extrusion variables in order to obtain the best condition for protecting the bioactive components should be highly regarded.

The effect of barrel temperature (160 °C and 180 °C) and screw speed (150 and 200 rpm) on anthocyanin content of blueberry pomace after extrusion was also investigated (Khanal et al., 2009a). The temperature and screw speed had no significant effect on the total anthocyanin content after extrusion due to the high barrel moisture content (45 %). A higher moisture content inside the extruder can prevent extensive losses of anthocyanin during extrusion due to the decrease in mechanical energy (Khanal et al., 2009a). The protective effect of moisture, has been also reported to enable the retention of cyanidin glycosides, which improved from 25 % to 65 % by increasing the barrel moisture content from 15 g/100 g to 22 g/100 g during extrusion at a constant barrel temperature (100 °C) and screw speed (300 /min) (Hirth et al., 2015). These results clearly show the protective effect of moisture on the degradation of polyphenolic compounds, since higher moisture content can reduce the specific mechanical energy applied during extrusion.

In some cases, the content of detectable polyphenolic compounds increased after extrusion. For instance, the ferulic acid content increased from 25 to 104 mg/100 g dry matter of rye grains after extrusion at a feed moisture content of 20 % (w/w) and barrel temperature of 180 °C (Gumul et al., 2010). This was attributed to the release of phenolic compounds from the cell matrix during extrusion (Brennan, 2011).

Different strategies can be applied to protect bioactive components against extrusion conditions (Ying et al., 2015a). For instance, the method of delivery into the extruder is an important factor that can influence the stability of bioactive components. It was shown that up to 90 % of β-carotene can be preserved during extrusion with 25 % (w/w) feed moisture

content and barrel temperature of 140, 160 or 170 °C when it is added in an oil in water emulsion stabilized by a heated protein-carbohydrate matrix, while 70-85 % of β-carotene was retained when stabilized by Tween 80 (Ying et al., 2015a). These findings indicate that the delivery method of bioactive components into extrusion should be considered as it can affect on stability of bioactive components.

2.6.3. Effect of extrusion variables on physical properties of extruded products

Physical characteristics of extrudates such as expansion, bulk density and texture are important parameters to evaluate the consumer acceptability of extruded products (Patil et al., 2007a). Some of the main physical properties of extruded products are described below:

2.6.3.1. Bulk Density and Expansion ratio

Bulk density and expansion ratio are interdependent physical characteristics. The increase in expansion corresponds to a decrease in bulk density and vice-versa. The barrel moisture content and extrusion temperature are the two most important factors affecting extrudate expansion and density (Alam et al., 2016). Further, the dough viscosity and elastic force (i.e., the die swell) affect the expansion and density of the extrudates (Moraru & Kokini, 2003). The bubbles inside the viscoelastic melt grow when the melt exits the extruder die due to moisture flash-off (Patil et al., 2007a). Increasing moisture content of the feed materials reduces the elasticity of the dough due to the plasticization effect of water on the dough (Baljit et al., 2015). A reduction of in dough elasticity subsequently causes the air bubbles to collapse inside the dough, resulting in a lower expansion and higher density in the extruded product (Gulati et al., 2016).

Reducing the moisture content inside the extruder barrel increases the melt viscosity, which often leads to an increase in the expansion of extrudates and a reduction in the bulk density of extrudates. There is an optimum moisture content, however, for achieving the maximum expansion of extrudates (Baik et al., 2004). At a moisture content below a certain level, the shear rate inside the barrel increases, resulting in molecular degradation that can cause a reduction of the expansion ratio (Baik et al., 2004).

The effect of barrel moisture content on the bulk density and expansion has already been reported for expanded snacks made from waxy and regular barley flour, which were extruded at a moisture content ranging from 17 – 21 % and screw speeds of 50, 75, 100, 125 and 150 rpm (Baik et al., 2004). The expansion ratio of extrudates increased from 1.81 to 2.68, but the bulk density decreased from 0.46 g/mL to 0.18 g/mL as the moisture content decreased from 21 % to 17 % (Baik et al., 2004). Similar trends have also been reported for extrudates produced from corn starch, where the bulk density and expansion ratio increased from 0.2 to 0.7 g/cm^3 and 2 to 4 respectively after extrusion at 150 or 250 rpm using a die temperature of 100 °C or 260 °C and feed moisture contents of 12 or 25 kg/100 kg (wet basis), respectively (Thymi et al., 2005). These studies clearly demonstrate the significant effect of barrel moisture content on desirable expansion and density of extruded products.

In another study, the expansion ratio of expanded snacks made from rice flour, wheat bran and corn grits decreased up to 25 % due to the presence of tomato paste (containing ~ 2.5 % w/w lycopene on a dry basis) (Dehghan-Shoar et al., 2010). The authors attributed the reduced expansion to the lubricating effect of tomato paste on the dough, leading to a reduction in specific mechanical energy and die pressure. The tomato paste also contained fibres, resulting in higher water absorption, leading to a higher bulk density compared with

snacks without tomato paste (Dehghan-Shoar et al., 2010). The results of this study demonstrate the effect of bioactive material addition on the physical properties of extruded products.

2.6.3.2. Texture

The sensory attributes of extruded products are directly related to the physical properties of these foods, where texture plays a major role. In such foods, where expansion is desired, texture is also of major importance, with crispness or hardness being of the most important attributes (Valles Pamies et al., 2000).

Hardness and crispness of ready-to-eat extrudates are often directly affected by the expansion ratio (Sharma et al., 2015). Larger expansion can reduce the thickness of the air cell walls, so less force is required to break the extrudates (Zarzycki et al., 2015). Similar to expansion and density of extruded products, the hardness and crispness of extrudates are also affected by extrusion conditions. For instance, the effect of extruder barrel moisture content on the hardness of extrudates has been widely studied (Liu et al., 2000; Seth et al., 2015; Zarzycki et al., 2015). In one study, the hardness of the extrudates made from oat-corn flour increased as the barrel moisture content increased from 18 % (w/w) to 21 % (w/w), whereas an increase in the screw speed from 200 rpm to 400 rpm resulted in a decrease in hardness (Liu et al., 2000). In another study, increasing the barrel moisture content from 14 % (w/w) to 16 % (w/w) resulted in an increase in the hardness of rice-corn extrudates from 12 to 18 Newton (N). These results indicate the effect of extrusion conditions including the barrel moisture content and screw speed on hardness of extrudates.

The hardness of extrudate products is also affected by the size of the air cells and thickness of the cell walls, which are often affected by the extruder barrel moisture content (Jin et al., 1995). At a higher moisture content of the feed, the viscosity of starchy dough and mechanical energy in the extruder decreased, resulting in decreased bubble growth (Kristiawan et al., 2016). Under these conditions extrudates became more dense and hard (Ding et al., 2005; Kristiawan et al., 2016). The feed moisture content of 16 % (w/w) at a barrel temperature of 150 °C resulted in thicker cell walls and harder rye expanded snacks, in comparison with snacks produced at 12 % (w/w) moisture content and barrel temperature of 190 °C (Saeleaw et al., 2012). Furthermore, increasing the feed moisture content from 13 % (w/w) to 19 % (w/w), in corn-lentil extrudates, resulted in thicker cell walls and a reduction in the number of air cells (Lazou & Krokida, 2010). These results indicate that the bubble growth and expansion ratio should be considered and optimized to achieve a suitable hardness/crispiness of extrudates.

2.6.3.3. Color

Colour in extruded products is influenced by extrusion conditions such as temperature, residence time, pressure and moisture content (Guy, 2001). Combination of extrusion conditions, facilitate the most common colour changing reactions during extrusion which are non-enzymatic browning (Maillard and caramelization) and pigment degradation (Steel et al., 2012). The colour parameters of an extruded product is expressed as L*, a* and b* values. L* value gives a measure of the lightness of the product from 100 for perfect white to zero for black. The redness/greenness and yellowness/blueness are presented by a* and b* values, respectively (Stojceska et al., 2009).

The effect of feed moisture content (18, 19.5 and 21%) and screw speed (200, 300, and 400 rpm) on colour of oat-corn extrudates has been already studied (Liu et al., 2000). The increase in moisture content from 18 to 21% led to the decrease of lightness (L*) and redness (a*) and increase of yellowness (b*) in the extrudates du to the. Screw speed, however, showed no significant effect on L* and b* values of corn-oat extrudates, but significantly affect on redness on samples (Liu et al., 2000).

In another study, increasing the feed moisture content from 12% to 17% led to significant reduction in lightness of extrudates from wheat flour extruded at screw speed of 200 rpm. The a* and b* did not change significantly by increasing the moisture content from 12% to17% (Stojceska et al., 2009). These results indicate the effect of extrusion conditions including the feed moisture content and screw speed on colour parameters of extruded products. It is worth mentioning that increases in feed moisture content results in reducing the residential time, which lead to less non-enzymatic browning of extruded product (Gutkoski & Eldash, 1999).

The mentioned results from literature indicated the significance of evaluation of extrudates' colour as an important factor affected by extrusion processing.

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Chapter 3

Oat fibre as a carrier for curcuminoids

3.1. Introduction

Foods, rich in polyphenols, are an important source of dietary antioxidants considered important for prevention of cancer and the alleviation of diabetes, cardiovascular diseases, neurodegenerative diseases, and osteoporosis (Arts & Hollman, 2005; Landete, 2012; Pandey & rizvi, 2009; Yang et al., 2001). The bioavailability of polyphenols is affected by many factors including interactions with the food matrix and its ingredients, the gut transit time, and colonic microflora (Visioli et al., 2011). Encapsulation of polyphenolic compounds has been suggested as a possible strategy to address issues related to their poor bioavailability (Fang & Bhandari, 2010; Munin & Levy, 2011).

Curcuminoid (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadience- 3,5-dione), the major component in turmeric roots (*Curcuma longa* L.) has been traditionally used for medicinal purposes (Aggarwal et al., 2003). There is increasing interest in curcuminoids because of its purported antioxidative, antiatherosclerotic, and anti-inflammatory properties and its potential role in prevention of diabetes, Alzheimer's, multiple sclerosis, cardiovascular disease, lung fibrosis, arthritis, and inflammatory bowel disease (Beevers & Huang, 2011). However, the low solubility of curcuminoids in aqueous solutions and its poor bioavailability limits its application (Anand et al., 2007). Approaches to overcome the low solubility of curcuminoids in an aqueous environment include the complexation of curcuminoids with food proteins (Rahimi Yazdi & Corredig, 2012; Sahu et al., 2008; Tapal & Tiku, 2012), or encapsulation of curcuminoids in starch (Yu & Huang, 2010).

Recently, it was reported that the bioavailability of curcuminoids was improved by formulating curcuminoids with fenugreek-derived soluble dietary fibre (Im et al., 2012). Oats contain protein, fibre, and fat in amounts that are generally higher than that of other cereals. Fractionation of the oat grain enables the preparation of a range of ingredients such as oat fibre concentrates and oat protein isolates. Oat fibre is a rich source of soluble fibre such as β-glucan. This makes it attractive as a functional food ingredient and as a carrier for bioactives; however, its application as a carrier material for curcuminoids is unknown. In this work, we examined the potential of oat fibre as a carrier for curcuminoids for functional food applications. In characterizing its potential as a carrier for curcuminoids, we also examined the partitioning of curcuminoids between the soluble and insoluble fractions of curcuminoids−oat fibre dispersions.

3.2. Materials and Methods

3.2.1. Materials

A powdered turmeric extract (Biocurcum, BCM- 95CG, total curcuminoids complex, purity: 95.7 %) was a gift from Arjuna Natural Extracts Ltd (Kerala, India). Previous analysis indicated that this material consists of 70 ± 0.5 % curcumin, 15.0 ± 0.2 % demethoxycurcumin, and 1.8 % bisdemethoxycurcumin (Fu et al., 2014). Concentrated oat fibre obtained from oat bran that was provided by CreaNutrition (Zug, Switzerland). This product contained 21.89 ± 0.02 (% w/w) protein, 27.54 ± 1.12 (% w/w) β-glucan, 5.2 ± 0.02 (% w/w) lipid, 6.5 ± 0.2 (% w/w) moisture, and 33.5 ± 0.5 (% w/w) other components (not measured components that include carbohydrates and other dietary fibre). Oat β- glucan (> 95 %) was purchased from Megazyme (Bray, Ireland). In this thesis the product name “oat well

28% or oat fibre" was used as general terminology to refer to these two materials respectively.

Quantification of β-glucans, lipids and total solids were carried out based on the AOAC Official Method 995.16 (AOAC, 2005), Australian Standard method AS 2300.1.3 (Standard, 2008) and AOAC Official Method 990.20 (AOAC, 1993), respectively, which will be explained in details in this chapter.

3.2.2. Protein

Protein content was analysed using a LECO FP-2000 Nitrogen analyser (LECO Australia Pty Ltd., Castle Hill, NSW, Australia). The measured nitrogen content was converted to total protein content by applying a conversion factor of 5.83. Measurements were performed in triplicate.

3.2.3. Fat

The content of fat was measured based on the Australian Standard method AS 2300.1.3 (Standard, 2008). A certain amount of oat fibre (0.5 g) was weighed into a Mojonnier fat extraction tube and then 2-3 mL warm water was added to dissolve the oat fibre fat. Then 10 mL HCl (1 N) was added and mixed properly. The tube was placed in a 70 °C water bath for a few minutes with gentle mixing. After cooling down to room temperature, 10 mL of ethanol was added to the mixture, followed by adding 25 mL of diethyl ether and 25 mL of petroleum spirit, and shaking for two min. The mixture was kept at room temperature for 40 min for digestion. Then the ether fat solution was drawn off to a pre-weighed, 250 mL flat bottomed flask. Two more extractions using 30 mL 1:1 diethyl ether and petroleum spirit mixing solution were carried out and stirred for 1 min. The third extraction was kept for another 40

min at room temperature for digestion and then mixed with the previous extracts. The extractions were evaporated in a water bath at 60 °C for 5 min by using the rotary evaporator (Scitek, Victoria, Melbourne, Australia). The residual was dried in an oven ((Thermotec 2000, Contherm, Lower Hutt, Wellington, New Zealand) at 102 °C for one hour.

The percentage of total fat was calculated using the following equation (3.1):

$$\text{Total fat (\%)} = (\text{weight of fat} - \text{blank*}) \times 100 / \text{weight of powder} \quad (3.1)$$

* Blank may be omitted if the total fat (%) is high.

3.2.4. Total solids

The total solids were determined according to the AOAC standard method (AOAC, 1993). The sample (M_1) was placed in a pre-weighed plate (M_2) and dried in a forced convection oven (Thermotec 2000, Contherm, Lower Hutt, Wellington, New Zealand) at 104 °C overnight. After drying, the plate containing the sample was cooled in desiccators before weighing (M_3). This procedure was repeated until the weight of the samples remained unchanged. The total solids content was expressed as the percentage relative to the initial weight of the sample using following equation (3.2):

$$Total\ solids\ \left(\%\frac{w}{w}\right) = \frac{M3-M2}{M1} \times 100 \quad (3.2)$$

3.2.5. β-glucan

Quantification of β-glucan, was carried out based on the AOAC Official Method 995.16,17 (AOAC, 2005). A β-Glucan Assay Kit (Mixed Linkage) was purchased from Megazyme (Wicklow, Ireland). A portion of oat fibre (20 mg) was directly transferred to the centrifuge tubes with caps. One mL of EtOH (50 % v/v) was added to the tube and vortexed vigorously. 5 mL of sodium phosphate buffer (pH 7.4, 20 mM) was added to the tube and immediately mixed. The content of the tube was heated for 5 min in a boiling water bath and then vortexed vigorously. Subsequently, sample tubes were equilibrated in a 50 °C water bath for 2 min. 200 µL of Lichenase solution (10 U) was added to the tube and mixed immediately before incubation at 50 °C for 60 min. Samples were vortexed every 15 min to ensure that no material is stuck to the tube wall. After incubating, samples were centrifuged at 1000 g for 10 min. 300 µL of supernatant was transferred to the bottom of three test tubes with caps (100 µL to each tube). 100 µL of sodium acetate buffer (pH 4.0, 50 mM) was added to the first tube (sample blank). 100 µL of β-glucosidase solution (0.2 U) was added to the two other tubes. All three tubes were gently vortexed in order to mix enzyme and sample. All three tubes were incubated for 15 min at 50 °C in a water bath. After incubation, three mL of GOPOD (glucose oxidase/peroxidise) reagent was added to the bottom of each test tube and vortexed gently. All capped tubes were incubated for 20 min at 50 °C. Finally all samples were equilibrated at room temperature before absorbance measurement at 510 nm.

The β-D-glucan content of oat fibre was calculated (percent, on as is basis) as follows:

$$\beta\text{-D-glucan } \% = \Delta A \times F \times 94 \times 1/1000 \times 100/W \times 162/180$$

$$= \Delta A \times F / W \times 8.46$$

Where ΔA is the absorbance of the reaction solution (i,e., after lichenase treatment minus blank absorbance for the same test solution); F is the factor to convert absorbance values to μg glucose (100 μg glucose/absorbance values for 100 μg glucose); 94 is the volume correction factor (0.1 of solution from 9.4 mL was analysed); 1/1000 is the conversion from μg to mg; 100/W is the conversion to 100 mg test tube; W is the test portion weight (mg); and 162/180 is the factor to convert from free glucose, as determined, to anhydroglucose, as occurs in β-D-glucan.

The β-D-glucan content of oat fibre (percent, on dry weight basis) is calculated as follows (3.3):

$$\beta\text{-D-glucan \%} = \beta\text{-D-glucan (as is basis)} \times 100 / (100 - \text{moisture content (\%)}) \quad (3.3)$$

3.3. Interaction and solubility experiments

3.3.1. Solubility in aqueous ethanol

A dispersion of curcuminoids (25.8 μg/mL) in 2 % v/v EtOH was prepared by diluting a stock solution of curcuminoids (1280 μg/mL) in 100 % EtOH with water.

3.3.2. Solubility in oat fibre dispersions

Curcuminoids (0−368 μg/ mL) − oat fibre (1 % total solid (TS), w/w) dispersions were prepared by dispersing the required volume of curcuminoids (dissolved in 100 % EtOH) into the oat fibre dispersion (1 % TS, w/w) in water. The EtOH concentration in the final dispersions was 2 % v/v. The dispersions were sonicated in an ultrasonic bath (Unisonic Australia Pty, Brookvale, NSW, Australia) at 20 kHz and 30 °C for 10 min followed by

homogenization at 13 500 rpm for 10 min with an Ultra Turax T25 homogenizer (Crown Scientific Pty, Murrarie, QLD, Australia).

3.3.3. Enhancing curcuminoids's solubility through increasing oat fibre concentration and homogenization

A curcuminoids (88 µg/mL) − oat fibre (1 % total solid (TS), w/w) dispersion was prepared by dispersing the required volume of curcuminoids (0.6 mL) from stock solution of curcuminoids (220 µg/mL) dissolved in 100 % EtOH into the 1-6 % w/w oat fibre dispersion. The dispersions were sonicated in an ultrasonic bath (Unisonic Australia Pty, Brookvale, NSW, Australia) at 20 kHz followed by homogenization at 300, 800, 9500, 13500 and 20500 rpm for 10 min with an Ultra Turax T25 homogenizer (Crown Scientific Pty, Murrarie, QLD, Australia). Dispersions were ultracentrifuged (20400 g, 20 min, 22 °C) to obtain a soluble fraction (supernatant). Curcuminoids were extracted from the supernatant by mixing 5 g of the supernatant with 100 % EtOH to obtain an extract in 80 % (v/v) EtOH. The dispersion was stirred for 20 min and then centrifuged at 12100 g at 22 °C for 20 min. A second extraction was carried out and the supernatants from the first and second extractions were combined and curcuminoids concentration was obtained using fluorescence analysing.

3.3.4. Partitioning and binding experiments

Freshly prepared dispersions (pH 6.8 ± 0.1) of curcuminoids (25.8 µg/mL) with oat fibre (1 % TS, w/w) were used for the partitioning experiments. The curcuminoids were solubilised in 100 % EtOH prior to addition to the oat fibre dispersion. The EtOH concentration in the final dispersions was 2 % v/v. To separate the soluble from insoluble fractions of the dispersions, samples were ultracentrifuged (20,400 g, 20 min, 22 °C) to

obtain a soluble fraction (supernatant) and an insoluble fraction (precipitate) (Figure 3.1). Each fraction was weighed. The supernatant and precipitate compositions were analysed separately for total solids, protein, and β-glucan content.

Curcuminoids were also extracted from the supernatant by mixing 5 g of the supernatant with 100 % EtOH to obtain an extract in 80 % (v/v) EtOH. The dispersion was stirred for 20 min and then centrifuged at 12 100 g at 22 °C for 20 min. A second extraction was carried out, and the supernatants from the first and second extractions were combined and used for fluorescence measurements. For the extraction of curcuminoids in the precipitate, 0.2 g of the precipitated fraction was mixed with 1.6 mL of 80 % (v/v) EtOH.

The extraction was repeated twice with 1.6 mL of 80 % (v/v) EtOH. After each extraction, the dispersion was stirred for 45 min and centrifuged at 12 100 g and 22 °C for 20 min, and the supernatant was collected from each extraction and combined for analysis. The concentration of curcuminoids from the extracts was analysed by fluorescence spectroscopy. The curcuminoids concentration was quantified using a calibration curve of curcuminoids in 80 % (v/v) EtOH at concentrations of 0.4−5.5 μg/mL. Spiking experiments with a known amount of curcuminoids were also carried out to confirm the recovery of curcuminoids from each fraction. The results showed a recovery rate of ~93 %.

Figure 3.1. Supernatant and precipitate of 1 % (w/w) curcuminoids-oat fibre dispersion

3.3.5. Preparation of curcuminoids−β-Glucan dispersion in 2 % EtOH

β-glucan (84 mg) was dispersed in 29.08 mL of Milli-Q water, and an aliquot of 0.6 mL of the curcuminoids stock solution (1280 μg/ mL) in pure EtOH was added to this dispersion to obtain the final mixtures of curcuminoids (25.8 μg/mL)−β-glucan (0.28 % TS, w/w) in 2 % (v/v) EtOH. A 0.28 % (w/w) β-glucan concentration was equivalent to the amount of β-glucan present in a 1 % (w/w) oat fibre dispersions used to examine the interaction of oat fibre with curcuminoids in this work. The dispersions were sonicated, homogenized, and centrifuged as previously described. The pH value of the mixtures was 7.0. The amount of curcuminoids in the supernatant was analysed by fluorescence measurements after extraction.

3.3.6. Fluorescence measurements

The fluorescence spectra of curcuminoids, oat fibre dispersion (1 % TS w/w), curcuminoids-oat fibre dispersions (1 % TS, w/w), and curcuminoids−β-glucan dispersions (0.28 % TS, w/w), all in aqueous EtOH (2 % v/v), were measured using a spectrofluorometer (Varioskan Flash microplate reader, Thermo Fisher Scientific Inc., Waltham, MA, USA). Samples (350 μL) were loaded into a 96-well microplate (OptiPlate-96, PerkinElmer, Santa Clara, CA, USA). The fluorescence emission spectra were recorded from 450−700 nm at a fixed excitation wavelength (λ_{ex}) of 420 nm.

To probe the quenching of the intrinsic fluorescence of proteins in oat fibre dispersions, emission spectra were recorded from 315−600 nm at an excitation wavelength (λ_{ex}) of 280 nm.

3.3.7. X-ray Diffraction

The powdered curcuminoids (1 g) and the precipitate (1.36 g) obtained after centrifugation of the curcuminoids (25.8 μg/mL)−oat fibre (1 % TS, w/w) dispersions in aqueous EtOH (2 % v/v) were analysed by an X-ray (D8 Advance) diffractometer (Bruker AXS Inc., Madison, WI, USA) with Cu Kα radiation. The machine was equipped with backgroundless sample holders. The X-ray diffraction (XRD) scanning was performed at a rate of 0.02 degrees step size and 5 s per step with the scanned angle set from $10° \leq 2\theta \geq 70°$. Measurements were performed at a voltage of 40 kV and 30 mA.

3.3.8. Fourier transformed infrared spectroscopy (FTIR) experiment

Fourier transformed infrared (FTIR) spectra of samples were recorded on a Varian 7000 FTIR spectrometer with a Specac MKII Golden Gate single reflectance diamond ATR attachment equipped with KRS-5 optics and a heated top plate maintained at 30 °C. Whole dispersion of curcuminoids (18.4 mg/mL) − oat fibre (1 % w/w) was dried at air oven dryer (Thermotec 2000, Contherm, Lower Hutt, Wellington, New Zealand) for 24 hours (100 °C). Curcuminoids powder, oat fibre powder and a physical mixture of oat fibre and curcuminoids were also prepared. Samples were placed onto the diamond ATR crystal, and spectra were collected in the absorbance mode from 4000 to 400 cm^{-1} at a resolution of 2 cm^{-1}.

3.3.9. Differential Scanning Calorimetry (DSC) experiment

DSC measurements of curcuminoids powder (0.1 and 10 mg), oat fibre powder (10 mg) and dried curcuminoids (18.4 mg/mL)-oat fibre (1 % w/w) dispersion were conducted on a differential scanning calorimeter (STAR System DSC I, Mettler Toledo, Australia). Samples were heated from 10 °C to 200 °C at 3 °C/min. Hermetically sealed Medium Pressure (stainless steel) pans were used with a Viton O-ring (ME-00026929, Mettler Toledo Ltd., Melbourne, Australia). An empty pan was used as a reference. Data collection and analysis was performed using the STARe software version 9.30. The list of samples which were analysed by DSC is shown in Table 3.1.

Table 3.1. Samples used in DSC experiments

Sample	Amount of curcuminoids in DSC pan	Amount of oat fibre material in DSC pan	Moisture content of sample in DSC pan	Amount of sample in DSC pan
Dried oat fibre powder	0	9.58 mg	4.15 %	10 mg
Curcuminoids powder	10 mg	0	0	10 mg
Curcuminoids powder	0.1	0	0	0.1 mg
Physical mixture	0.18	9.81	4.5 %	10 mg
Dried dispersion of 1 % oat fibre with 18.4 mg/mL curcuminoids	0.18 mg	9.81 mg	4.45 %	10 mg

3.4. Storage Stability of Curcuminoids

The storage stability of curcuminoids in the curcuminoids (25.8 μg/mL)−oat fibre (1 % TS, w/w) dispersions in 2 % (v/v) EtOH and the supernatant fraction obtained upon centrifugation at 20,400 g (Beckman J2-MC, Ramsey, MN, USA) for 20 min at 22 °C was determined. The curcuminoids content in the precipitated fraction was obtained by difference. Sodium azide was added to all samples (0.02 % w/w) to prevent microbial spoilage. Samples (5 g) were placed in glass vials and were stored at 25 °C for 11 days. Samples were taken at intervals for the analysis of curcuminoids. Curcuminoids were extracted using EtOH and the

curcuminoids concentration was estimated using fluorescence spectroscopy. Furthermore, the storage stability of curcuminoids in the curcuminoids (25.8 μg/mL) − oat fibre (1 % TS, w/w) dispersions in 2 % (v/v) EtOH was determined at 4 °C during 24 hours in order to investigate the rate of curuminoids degradation before extrusion processing.

3.5. Results and Discussion

3.5.1. Fluorescence measurements of curcuminoids in 2 %, 10 %, 50 % and 100 % v/v ethanol

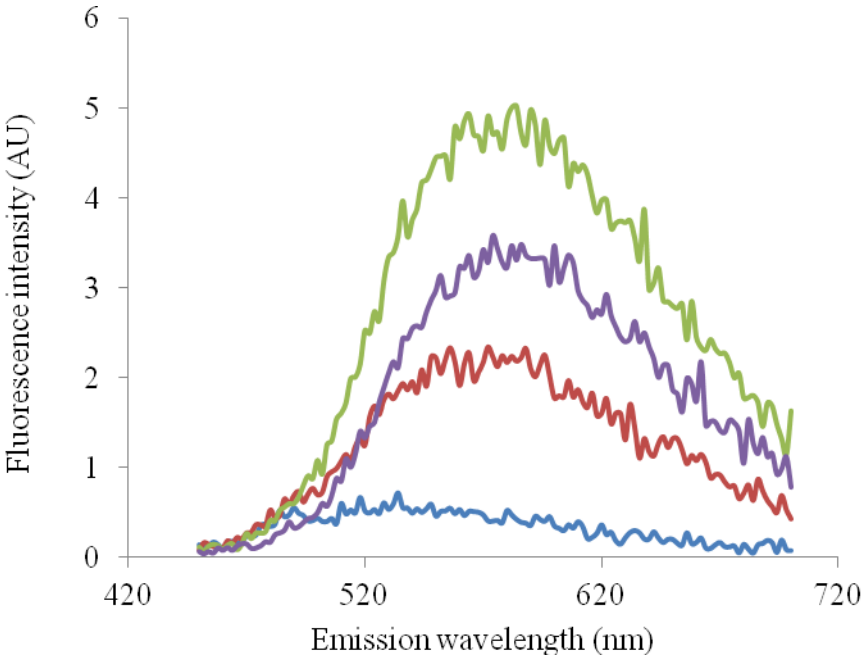
The florescence intensity of curcuminoids (0−36.8 μg/mL) in 2 % (v/v) EtOH is shown in Figure 3.2.A. The maximum emission wavelength of curcuminoids in 2% EtOH was at 566 nm. The fluorescence intensity of samples containing 3.7, 18.4, and 36.8 μg/mL was 2.7, 4.0, and 3.6, respectively. One factor that may contribute to the nonlinear increase in fluorescence intensity with increased curcuminoids concentrations from 3.7−18.4 μg/mL is the super-saturation of curcuminoids at high concentrations. In addition, with increasing curcuminoids concentration in 2 % v/v EtOH, there is likely to be self quenching of curcuminoids. Self-quenching is particularly prevalent at higher concentrations. Self quenching of curcuminoids was also observed in sodium phosphate buffer at pH 7.0 (Niu et al., 2012b). In this study it was observed that by increasing the curcuminoids concentration from 2 to 15 μM, the fluorescence intensity of curcuminoids increased. However, increasing the curcuminoids concentration from 15−20 μM resulted in a reduction of fluorescence intensity for liposomal curcuminoids due to self quenching of curcuminoids (Niu et al., 2012b).

The fluorescence intensity of curcuminoids in 10 % (v/v) EtOH was slightly increased in comparison with that in 2 % (v/v) EtOH. Further increasing in the EtOH concentration to 50

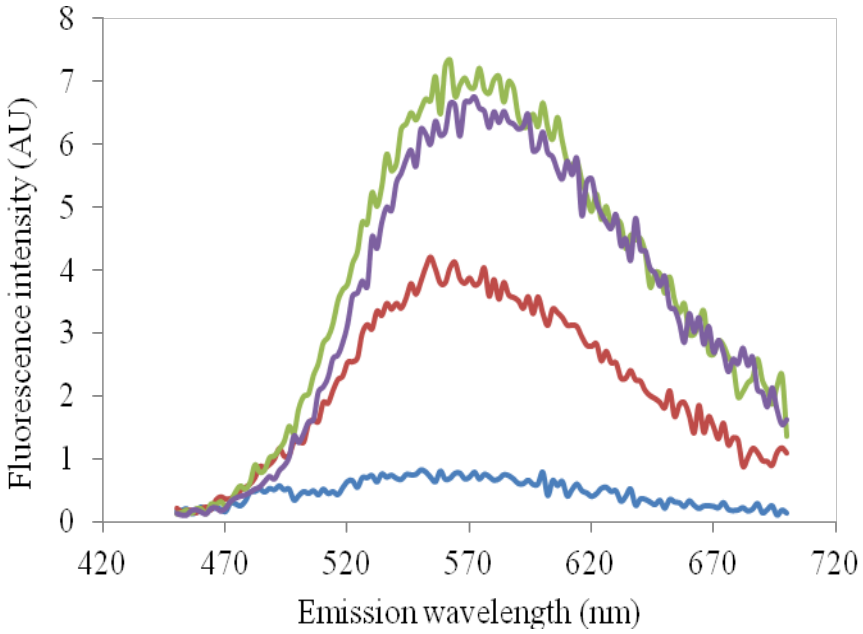
and 100 % (v/v), resulted in a significant increase in fluorescence intensity of curcuminoids and the self quenching effect was not observed (Figure 3.2 C and D).

At lower EtOH concentration (2 and 10 % (v/v)), the maximum emission wavelength of curcuminoids was at ~566 nm. Increasing the EtOH concentration resulted in a reduction of the maximum emission wavelength to ~530 nm. Higher EtOH concentrations changed the polarity of the environment leading to a lower emission wavelength of curcuminoids. It was reported that the maximum fluorescence of curcuminoids shifted to lower wavelength if the environment of curcuminoids changes from polar to non-polar solvents (Priyadarsini, 2009).

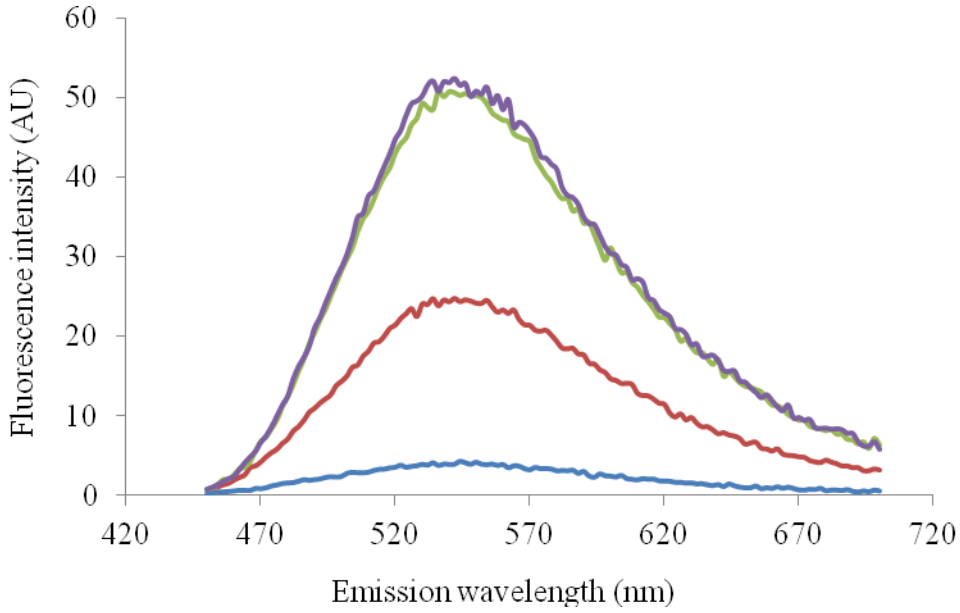
(A)


Fluorescence intensity (AU)
0
1
2
3
4
5
6
420
520
620
720
Emission wavelength (nm)

(B)


Fluorescence intensity (AU)
0
1
2
3
4
5
6
7
8
420
470
520
570
620
670
720
Emission wavelength (nm)

(C)



(D)

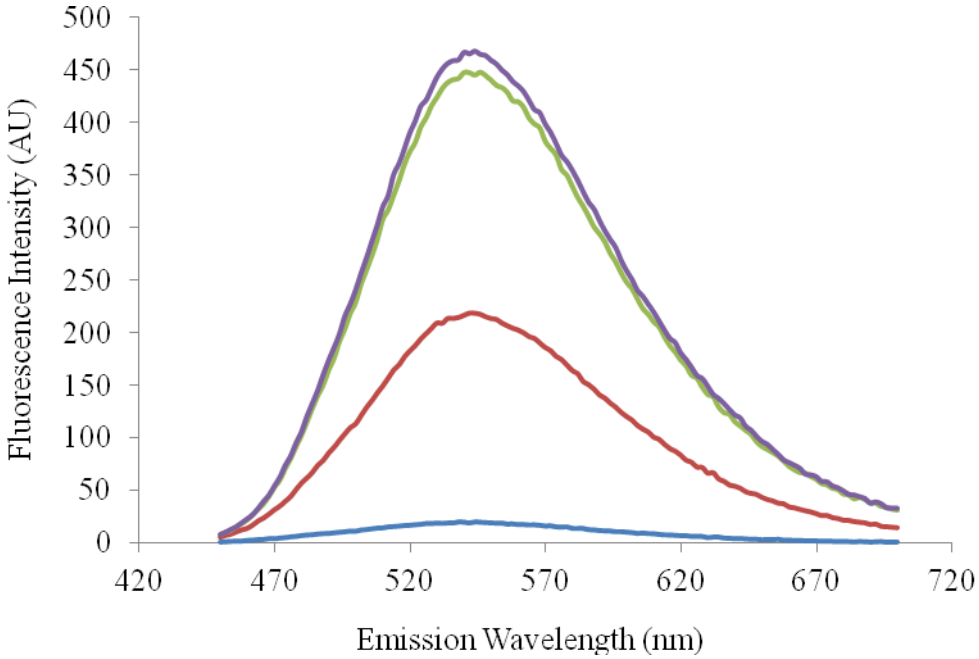


Figure 3.2. Fluorescence spectrum of 0.4 µg/mL (blue), 3.7 µg/mL (red), 18.4 µg/mL (green) and 36.8 µg/mL (purple) curcuminoids in (A) 2 %, (B) 10 % (C) 50 % and (D) 100 % (v/v) EtOH.

3.5.2. Fluorescence of curcuminoids in the presence of pure β- glucan in 2 % v/v EtOH

The fluorescence spectra of curcuminoids in the presence of pure β-glucan (0.28 % TS, w/w) are shown in Figure 3.3. There was a blue shift in the position of the maxima when curcuminoids were dispersed in the presence of β-glucan in 2 % (v/v) EtOH (542 nm) compared to curcuminoids in 2 % (v/v) EtOH alone (566 nm). At an equivalent concentration of added curcuminoids (36.8 μg/mL), the fluorescent intensity of the curcuminoids−β-glucan dispersions in 2 % (v/v) EtOH was greater than that in 2 % (v/v) EtOH alone (Figure 3.2.A). These observations may be interpreted as an increased solubility of curcuminoids due to binding to the β-glucan, possibly due to hydrogen bonding and complexation of the phenolic groups of curcuminoids to β-glucan (Wu et al., 2011).

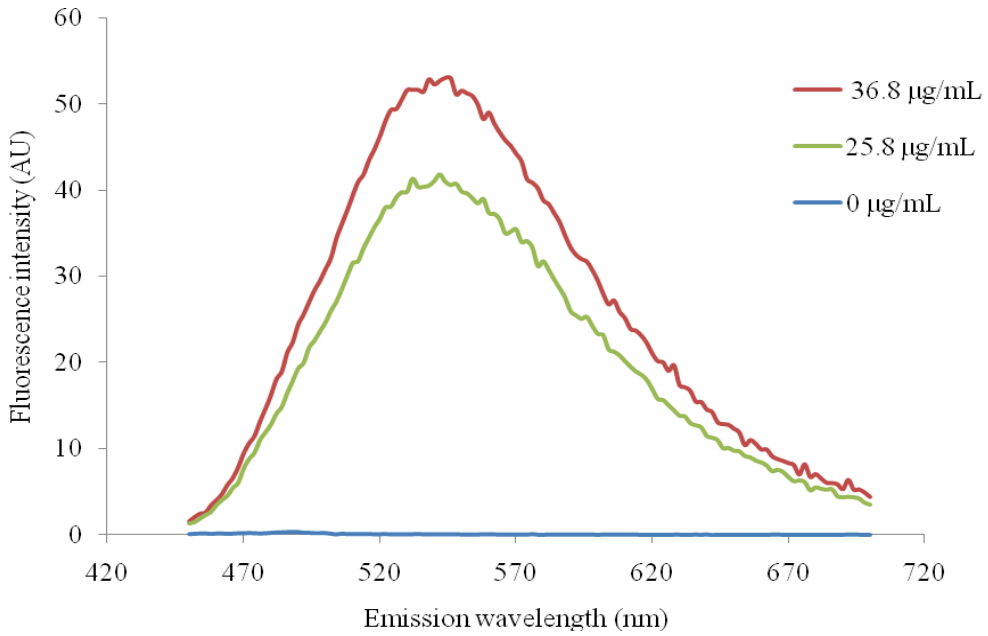


Figure 3.3. Fluorescence spectra of curcuminoids in a dispersion of β-glucan (0.28 % TS, w/w) in 2% (v/v) EtOH.

3.5.3. Fluorescence of curcuminoids in the presence of oat fibre in 2 % v/v EtOH

In curcuminoids−oat fibre (1 % TS, w/ w) dispersions containing 2 % v/v EtOH, a blue shift in the wavelength of maximum emission was observed from about 566 nm for curcuminoids in 2 % v/v EtOH (Figure 3.2.A) to about 490 nm in oat fibre dispersions with 2 % (v/v) EtOH (Figure 3.4). This blue shift in the emission wavelength is consistent with a transfer of the curcuminoids into a less polar microenvironment. At corresponding levels of added curcuminoids (36.8 μg/mL), the fluorescence intensity of the curcuminoids in the presence of oat fibre in 2 % v/v EtOH was approximately 47, whereas it was only approximately 3.6 in 2 % v/v EtOH.

Blue shifts in the emission spectra of curcuminoids have been observed in mixtures containing casein micelles (Sahu et al., 2008), hydrophobically modified starch (Yu & Huang, 2010), β-casein (Esmaili et al., 2011), β-lactoglobulin (Sneharani et al., 2010) and for liposomal curcuminoids preparations (Niu et al., 2012b) and are consistent with the transfer of curcuminoids into a less polar environment. It was not possible to ascribe the relative binding affinities of the various curcuminoids to components of the oat fibre from the fluorescence spectra obtained in this study.

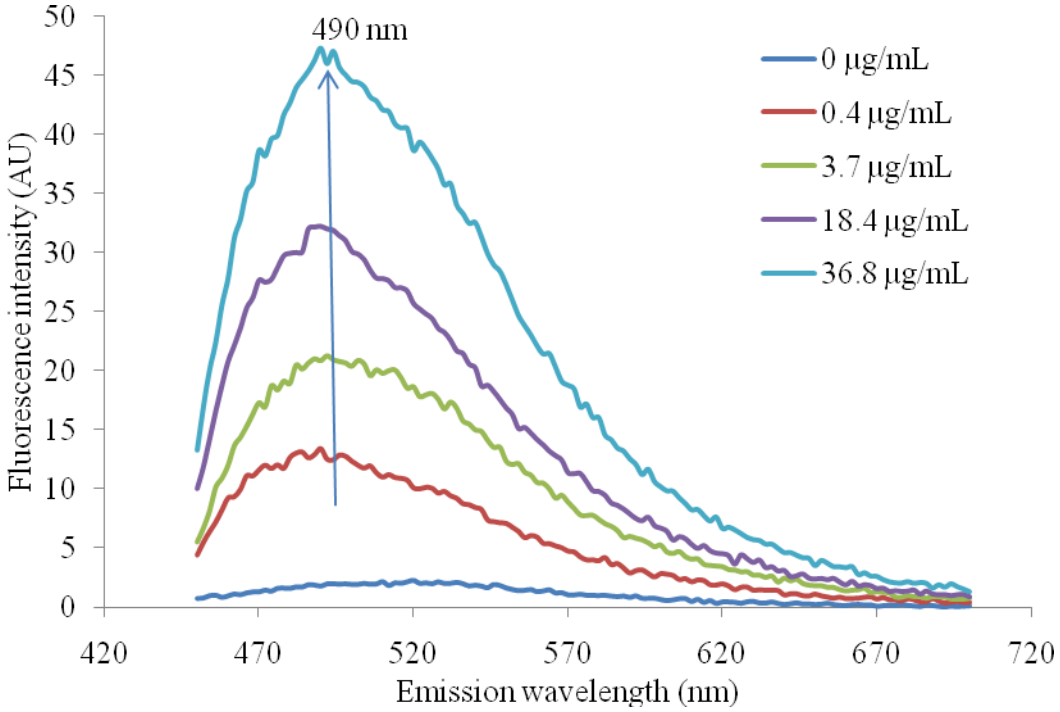


Figure 3.4. Fluorescence spectra of curcuminoids in a dispersion of oat fibre (1 % TS, w/w) in 2% (v/v) EtOH.

3.5.4. Fluorescence of proteins in curcuminoids−oat fibre mixtures.

The quenching of the intrinsic fluorescence of proteins within the curcuminoidss−oat fibre mixture and the blue shift in the wavelength of maximum emission (Figure 3.5) are evidence of binding of the curcuminoidss to protein. An increase in the curcuminoids concentration from 0−44.2 µg/mL resulted in a progressive increase in quenching, but there was no further increase when the concentration was raised to 55.3 µg/mL (Figure 3.5). Quenching has been observed for binding of curcuminoids to various types of proteins including milk proteins (Rahimi Yazdi & Corredig, 2012), soy protein (Tapal & Tiku, 2012) and plasma proteins (Leung, 2009).

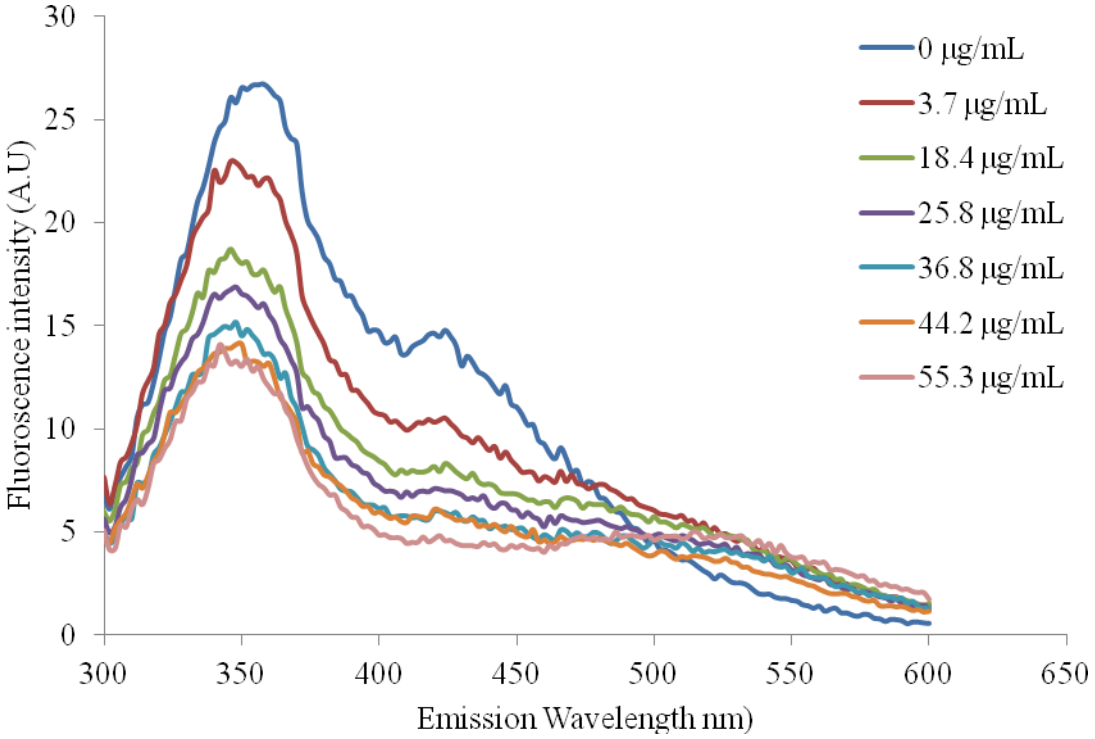


Figure 3.5. Protein fluorescence spectra of curcuminoids−oat fibre (1 % TS, w/w) dispersions in 2 % (v/v) EtOH at λ_{ex} = 280 nm.

3.5.5. Partitioning of oat fibre components and curcuminoids between soluble and insoluble fractions upon centrifugation

3.5.5.1. Partitioning of oat fibre components

The partitioning of oat fibre components and curcuminoids in the supernatant and precipitated fractions of curcuminoids−oat fibre dispersions was examined to estimate the distribution of curcuminoids in the soluble and insoluble fraction. For these experiments, we used 25.8 µg/mL curcuminoids in a 1 % TS (w/w) oat fibre dispersion in 2 % (v/v) EtOH.

Upon centrifugation of the oat fibre dispersion (1 % TS, (w/w)) in 2 % (v/v) EtOH, a supernatant (soluble) fraction (95.3 % (w/w), 0.28 % TS) and a precipitated (4.7 % (w/w), 0.73 % TS) fraction were obtained (Table 3.2). Of the total protein in the oat fibre dispersion, 38 % partitioned into the supernatant, while 62 % partitioned into the precipitate. Most of the β-glucan (61 % of the total β-glucan) partitioned into the supernatant, with 39 % found in the precipitated fraction (Table 3.2). Both soluble and insoluble proteins and β-glucans present in unprocessed oats are expected to be present in the oat fibre ingredient used in our experiments. The solubility of the proteins and β-glucan components and their distribution between the soluble and insoluble phases will depend on the isolation methods used to extract and process the oat fibre ingredient. Oat proteins comprise globulins, albumins, prolamins, and glutenins, and of these, the albumins are the most water-soluble proteins (Ma & Harwalkar, 1984). The insoluble β-glucans have lower molecular mass than the soluble β-glucan but remain in the insoluble fibre fraction because of entanglement with arabinoxylan (Johansson et al., 2004).

Table 3.2. Composition of Fractions Obtained upon Ultracentrifugation (20 400 g, 20 min, 22 °C) of Curcuminoids (25.8 μg/mL) − Oat Fibre 1 % (w/w) Dispersion in 2 % (v/v) EtOH

Components	Supernatant	Precipitate	total (supernatant + precipitate)
wt fraction (g)	95.25 ± 0.13	4.74 ± 0.15	99.99
total solid (g/100 g dispersion)	0.28 ± 0.03	0.73 ± 0.03	1.01
protein (g/100g)	0.11 ± 0.02	0.18 ± 0.01	0.29
β-glucan (g/100g)	0.17 ± 0.01	0.11 ± 0.02	0.28
curcuminoids (%)	38.63 ± 2.71	41.10 ± 2.15	79.7[b]

[b] An additional 13.4 ± 0.7 % of curcuminoids were recovered from the washings from the beaker and the probe used for homogenization.

3.5.5.2. Partitioning of Curcuminoids

The curcuminoids were approximately equally partitioned between the supernatant and precipitated fractions obtained upon centrifugation of the curcuminoids (25.8 μg/mL) − oat fibre (1 % TS, w/w) dispersion (pH 6.8 ± 0.1) in aqueous 2 % v/v EtOH. The supernatant contained 38.6 ± 2.7 % and the precipitated fraction contained 41.1 ± 2.15 % of the total added curcuminoids. Thus, the total amount recovered from both the supernatant and precipitated fractions accounted for approximately 79.7 % of the total curcuminoids originally added (Table 3.2). The overall amount of recovered curcuminoids from the homogenizer probe and glass beaker was 13.4 ± 0.7 % of the added total curcuminoids, which amounts to a total curcuminoids recovery of 93.1 %.

3.5.6. Solubility of curcuminoids

3.5.6.1. Solubility in 2 % (v/v) EtOH

Measurement of the supernatant fractions obtained upon centrifugation of 25.8 μg/mL curcuminoids dispersed in 2 % (v/v) EtOH without oat fibre showed that the highest concentration of curcuminoids in the supernatant was 4.1 μg/mL. This result showed that the solubility of curcuminoids in aqueous EtOH (2 % v/v) exceeds that of the reported solubility of curcuminoids in aqueous media (11 ng/mL, pH 5) (Tønnesen et al., 2002).

3.5.6.2. Solubility in the presence of oat fibre (1 % TS, w/w) in 2 % (v/v) EtOH

The solubility of the curcuminoids in the oat fibre dispersion was taken as the concentration of curcuminoids in the supernatant. An increase in the concentration of curcuminoids in the oat fibre dispersion from 0 to ~220 μg/mL resulted in an increased amount of curcuminoids in the supernatant after centrifugation (Figure 3.6). However, an increase in the amount of curcuminoids in the dispersion beyond 220 μg/mL did not result in a further increase of curcuminoids in the supernatant, which indicates a saturation of the sites in the soluble components (i.e., soluble protein and β- glucan components) of oat fibre. The highest amount of curcuminoids extracted from the supernatant corresponded to a concentration of ~88 μg/mL (Figure 3.6). The solubility of curcuminoids in the soluble supernatant component of the oat fibre dispersion (in 2 % v/v EtOH) increased by a factor of 21 over that in 2 % v/v EtOH (4.1 μg/mL; this work) without oat fibre. This concentration of curcuminoids in the supernatant is also much higher than that of the reported solubility of curcuminoids in aqueous media (11 ng/mL, pH 5) (Tønnesen et al., 2002). This result clearly demonstrated the ability of the soluble oat fibre components to carry curcuminoids in an aqueous system.

The combined effects of hydrophobic interactions and hydrogen bonding that govern these reported interactions are likely responsible for the interactions with oat fibre and increased solubility observed. The increase in curcuminoids solubility in the presence of biopolymers and synthetic polymers has been reported. For example, the addition of curcuminoids to soy protein (5 % (w/v) in water) increased the curcuminoids solubility by 812-fold because of the formation of a complex, driven by hydrophobic interactions (Tapal & Tiku, 2012).

Micelles of hydrophobically modified starch have also been used to encapsulate curcuminoids which resulted in a 1670-fold increase in curcuminoids solubility (Yu & Huang, 2010). It was suggested that this increase in curcuminoids solubility was due to the combined effects of the transfer of curcuminoids into a more hydrophobic environment and hydrogen bonding between curcuminoids and the starch. Synthesized chemical polymers, including hydroxypropylated derivatives of β (HP-β-CD) and γ (HP-γ-CD) cyclodextrin (CD), also increased the solubility of curcuminoids to about 1700- and 4700-fold, respectively, due to their ability to form inclusion complexes with curcuminoids (Baglole et al., 2005).

The solubilisation of curcuminoids with various materials appears to be related to the binding of curcuminoids to hydrophobic regions of molecules. Compared to hydrophobically modified starch and soy protein, oat fibre is less hydrophobic in nature. In another examples, CDs have a hydrophobic cavity and form inclusion complexes with curcuminoids (Tønnesen et al., 2002). The reduced availability and affinity of hydrophobic sites in oat fibre for curcuminoids compared to hydrophobically modified starch, soy protein, and CDs possibly explains the lower ability of curcuminoids to bind to oat fibre and, hence, it's reduced ability to solubilise curcuminoids.

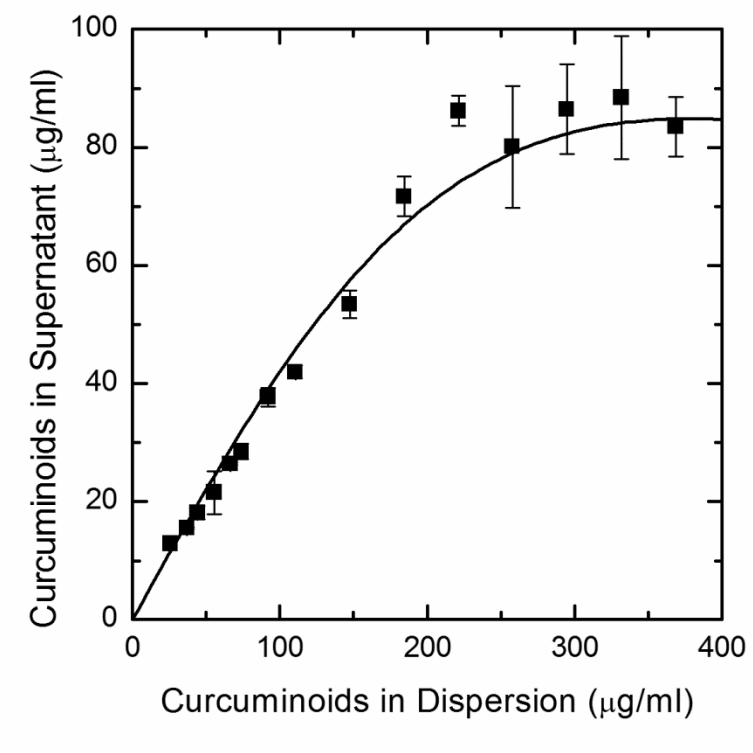


Figure 3.6. Concentration of curcuminoids in supernatant of 1 % (w/w) oat fibre dispersion in 2 % (v/v) EtOH as a function of total added curcuminoids in initial dispersion.

3.5.6.3. Effect of oat fibre concentration and homogenizer speed shear rate on curcuminoids' solubility

The maximum solubility of curcuminoids in the presence of 1 % (w/w) oat fibre was obtained at a curcuminoids concentration of approximately 88 μg/mL (Figure 3.6). However, the effect of increasing the oat fibre concentration (1-6 % w/w), homogenizer shear rate (300, 800, 9500, 13500 and 20500 rpm) on the curcuminoids' solubility was not considered.

Increasing the oat fibre concentration from 0.5 % to 1 % (w/w) resulted in an increase of the curcuminoids concentration in the supernatant of a curcuminoids (220 μg/mL) — oat fibre dispersion in 2 % (v/v) EtOH from approximately 53 to approximately 75 μg/mL(Figure 3.7). However, further increasing the oat fibre concentration resulted in a major reduction in

curcuminoids concentration in the supernatant. Increasing the oat fibre concentration enhances the viscosity of the dispersion notably, which possibly results in self assembly of β-glucan molecules (Morgan et al., 1999) and entrapment of curcuminoids in the spherical aggregates of β-glucan (Wu et al., 2011), leading to precipitate curcuminoids.

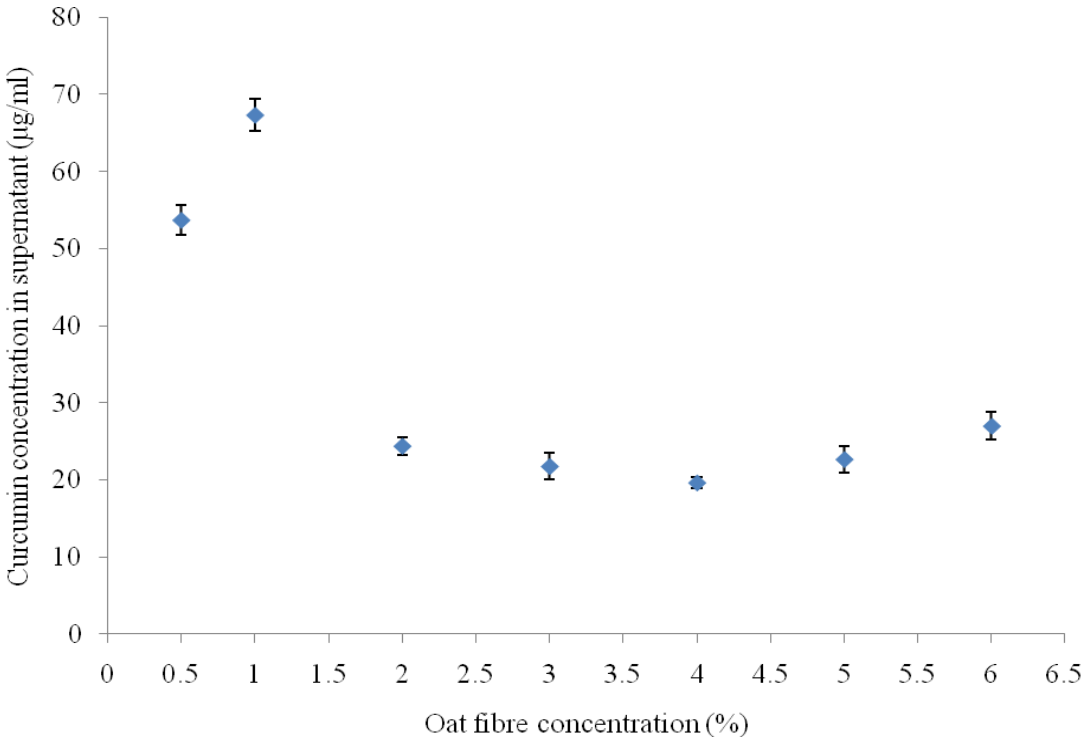


Figure 3.7. Concentration of curcuminoids in the supernatant of 0.5% - 6 % (w/w) oat fibre dispersion in 2% (v/v) EtOH.

Increasing the homogenizer speed from 300 rpm to 20000 rpm, increased curcuminoids solubility in the supernatant of curcuminoids (220 µg/mL) — (1 % w/w) oat fibre dispersion in 2 % (v/v) EtOH from 33 to 80 µg/mL (Figure 3.8). This is possibly related to the reduction of curcuminoids and possibly fibre particle size at higher homogenization speeds. It has been reported that using high shear dispensers increased the water dispersity of curcuminoids due

to the breakdown of curcuminoids particles (Donsì, 2010). In addition; the application of high shear dispensers resulted in a transition of curcuminoids from the crystalline to the amorphous form which favours curcuminoids solubility (Donsì, 2010).

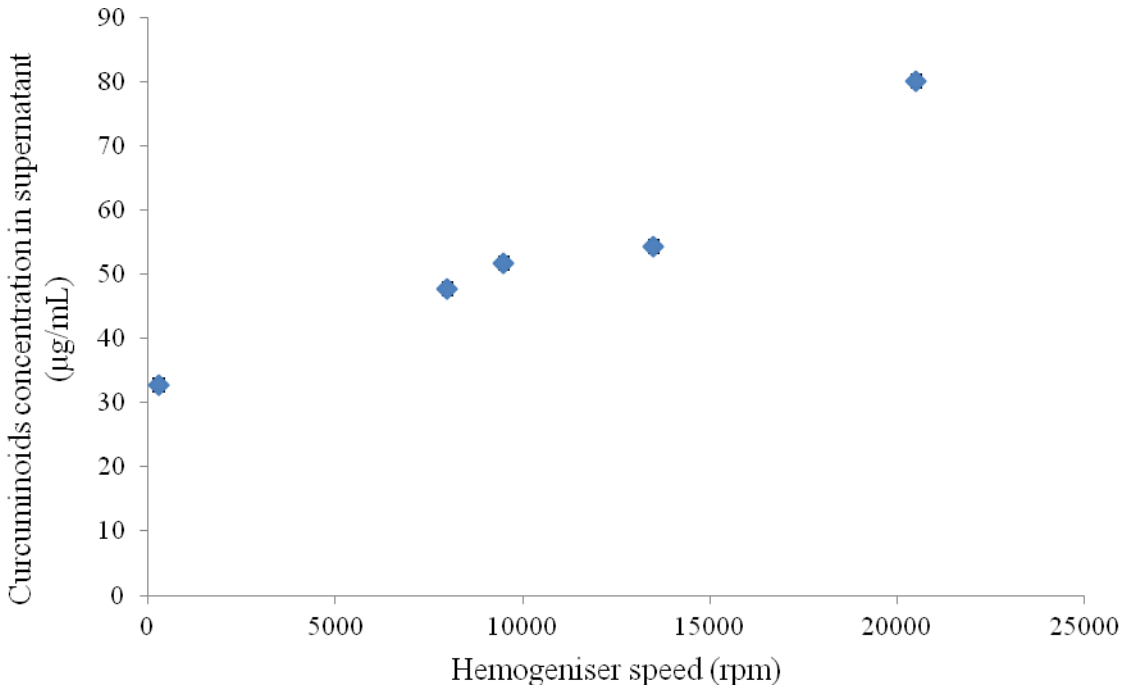


Figure 3.8. Concentration of curcuminoids in the supernatant of 1 % (w/w) oat fibre dispersion in 2 % (v/v) EtOH as a function of homogenizer speed.

3.5.7. Characterization of complex between curcuminoids and oat fibre ingredients

3.5.7.1. Determining the crystallinity of curcuminoids by X-ray diffraction

Wide-angle XRD studies on the precipitated fraction obtained upon centrifugation of the curcuminoids (25.8 μg/mL) − oat fibre (1 % w/w TS) dispersion in aqueous EtOH (2 % v/v) were carried out to investigate if crystalline curcuminoids was entrapped in the precipitated fraction. A powder dispersion of curcuminoids has characteristic peaks between 7 and 30 degrees, which suggest that the powder contains crystalline components (Table 3.3). The major reflection at ~17 degrees and relative intensities of reflections are largely consistent with previous reports of curcuminoids in a crystalline form (Table 3.3).

The differences observed are possibly due to the different preparations of curcuminoids used in the various studies. The XRD spectrum of the precipitate obtained upon centrifugation of the curcuminoids−oat fibre dispersion has an absence of sharp peaks (Figure 3.9), which suggests that curcuminoids in the precipitate were less crystalline in character than the original curcuminoids powder. The broad reflection observed in the precipitated fraction of the curcuminoids−oat fibre dispersions at ~19 degrees is a typical pattern of a more amorphous substance. The results suggest that the curcuminoids is in the form of an amorphous solid dispersion. Others have shown that amorphous solid dispersions are formed in a range of cellulose derivative matrices (Li et al., 2013).

The curcuminoids in an amorphous form are desirable from a delivery viewpoint as it improves bioavailability (Li et al., 2013). The position of the reflection (2θ value) observed for curcuminoids in the presence of oat fibre was at 19.4 deg. The corresponding values for

curcuminoids encapsulated with various materials were 11−12 and 10−20 deg with sodium caseinate (Pan et al., 2013), 19 and 23 deg with soy protein (Zi et al., 2012), 8 and 19 deg with zein (Patel et al., 2010) and 16 and 22 deg with CD (Mohan et al., 2012), which show similar trends for the XRD spectrum of encapsulated curcuminoids in different encapsulant materials.

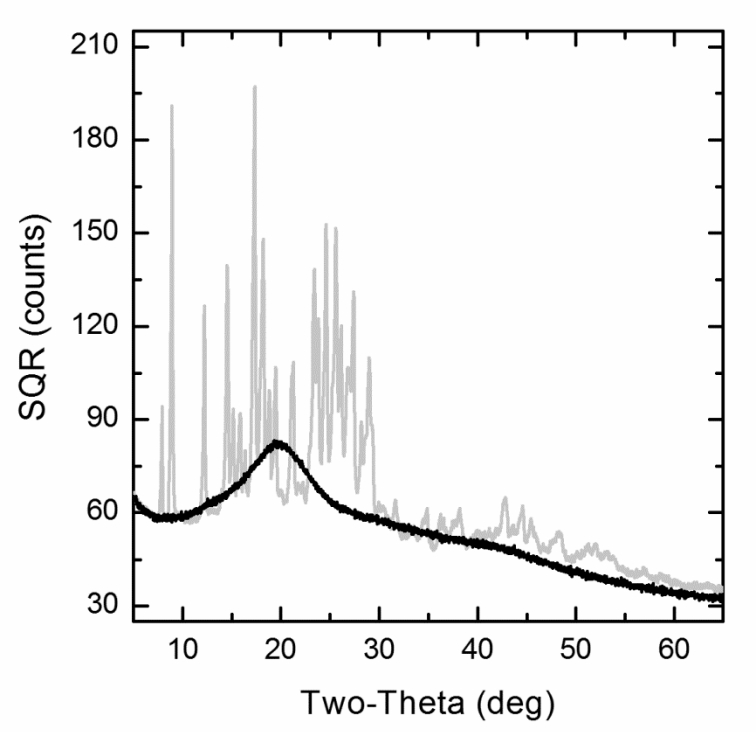


Figure 3.9. XRD spectra of curcuminoids present in the precipitate obtained upon centrifugation of a curcuminoids (25.8 μg/mL) − oat fibre (1% TS, w/w) dispersion (black) and spectra of the original curcuminoids powder (gray).

Table 3.3. Comparison of major reflections observed in the XRD spectra of curcuminoids crystals and encapsulated curcuminoids preparations from various studies.

This study		(Pan et al., 2013)		(Zi et al., 2012)		(Patel et al., 2010)		(Mohan et al., 2012)	
2θ (deg)	Intensity	2θ (deg)	Intensity	2θ (deg)	Intensity	2θ (deg)	Intensity	2θ (deg)	Intensity
7.9	94	~7	~490	-	-	-	-	-	-
8.9	191	~8-9	~1500	8.	~12500	8.9	~500	-	-
12.2	127	~12	~1350	12.10	~2500	12.26	~350	12	-
14.5	139	~15	~2000	14.39	~3000	14.54	~490	14.3	-
15.1	93	-	-	-	-	-	-	-	-
15.8	92	-	-	-	-	-	-	-	-
16.3	80	~16	~1300	-	-	-	-	-	-
17.3	197	~17	~4200	17.20	~13000	17.24	~1100	17.1	-
18.2	148	~18	~3000	-	-	-	-	18.2	-
19.5	107	~19	~1600	-	-	-	-	18.6	-
21.2	108	~21	~2250	-	-	-	-	21	-
23.4	138	~23	~3600	23.30	~2800	23.33	~600	23	-
24.6	153	~25	~3400	24.50	~2200	24.60	~800	24.4	-
25.6	152	~26	~4100	25.52	~2000	25.52	~790	25.5	-
26.1	120	-	-	-	-	-	-	-	-
26.8	107	-	-	-	-	-	-	-	-
27.4	131	~27	~3300	-	-	-	-	27.2	-
29.0	110	~28	~2800	28.87	~1500	-	-	-	-

3.5.7.2. FTIR analysis

FTIR is a suitable technique for investigating the specific intermolecular interaction (Mohan et al., 2012). The changes of interaction behaviour between different molecules can be characterized through the identification of the IR spectral features in intensity, bandwidth and position with which it allows to quantitatively study the interaction (Yadav et al., 2009b).

The FTIR peak assignments of the curcuminoids and oat fibre are shown in Figure 3.10. FTIR studies on a dried curcuminoids (18.4 mg/mL) − oat fibre (1 % w/w TS) in aqueous EtOH (2 % v/v) dispersion were carried out to investigate the interaction of curcuminoids with oat fibre ingredients. In the IR spectrum of curcuminoids, a broad peak at 3294 cm^{-1} and the sharp one at 3508 cm^{-1} were observed, indicating the presence of phenolic group and OH stretching (Shrivastava et al., 2013a). Another specific IR peak for curcuminoids is located at 1600 cm^{-1}, attributed to the symmetric aromatic ring stretching vibrations (C Cring) (Mohan et al., 2012). The IR spectrum of the physical mixture of oat fibre-curcuminoids contained peaks corresponding to both oat fibre and curcuminoids. In the spectrum of dried curcuminoids (18.4 mg/mL) - oat fibre (1 % w/w) dispersion (Figure 3.11), curcuminoids IR peaks at 1600 and 1625 cm^{-1} were masked by the protein spectra of oat fibre at 1641 cm^{-1}; however, the absorbance of oat fibre protein at 1641 cm^{-1} was reduced from 0.1390 to 0.04330 in the IR spectrum of the dispersion. In addition, the spectrum of oat fibre at around 1153 cm^{-1}, which has been assigned to amide II, shifted to 1151 cm^{-1} and overlayed the curcuminoids band at 1151 cm^{-1}. These observations show the possible interaction between the functional group of oat protein with curcuminoids.

The C=O IR band of curcuminoids at 1504 cm^{-1} , which was present in the physical mixture, disappeared in the IR spectrum of the oat fibre-curcuminoids dispersion, assuming that the

C=O group of curcuminoids is involved in the interaction (Mohan et al., 2012). The band at 1427 cm^{-1}, which is related to C-C-C vibration of the aromatic ring in curcuminoids, was absent in the IR spectrum of oat fibre-curcuminoids dispersion, indicating a possible complexation formation.

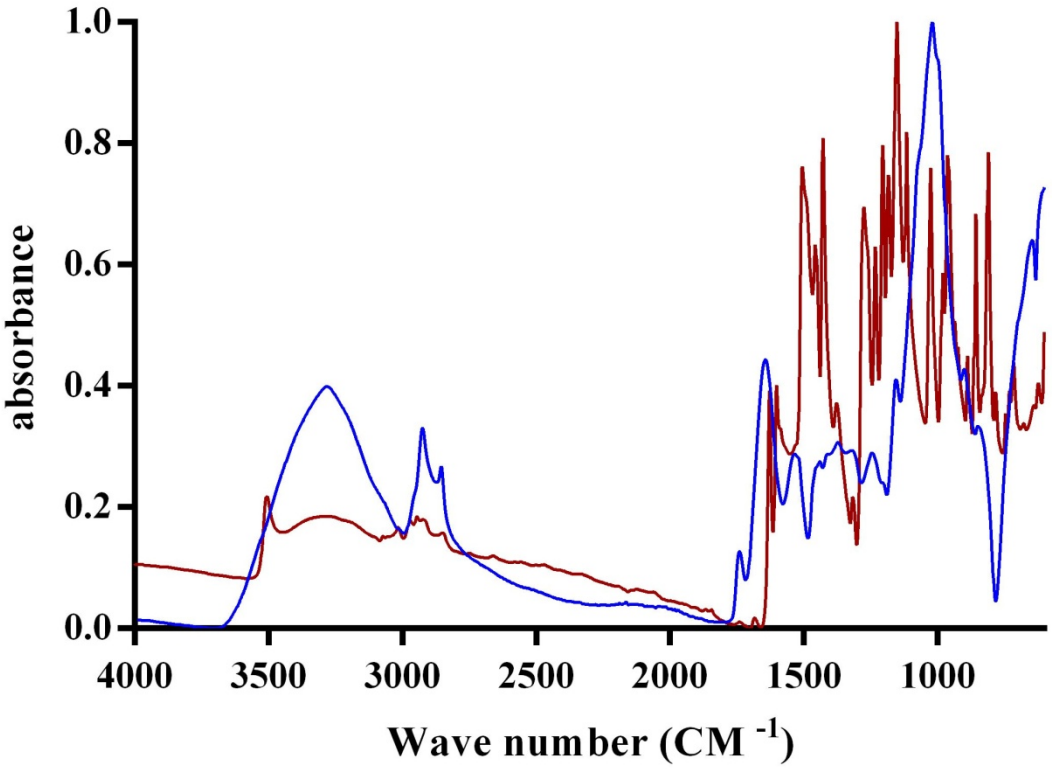


Figure 3.10. FTIR spectrum of curcuminoids (red) and oat fibre powder (blue).

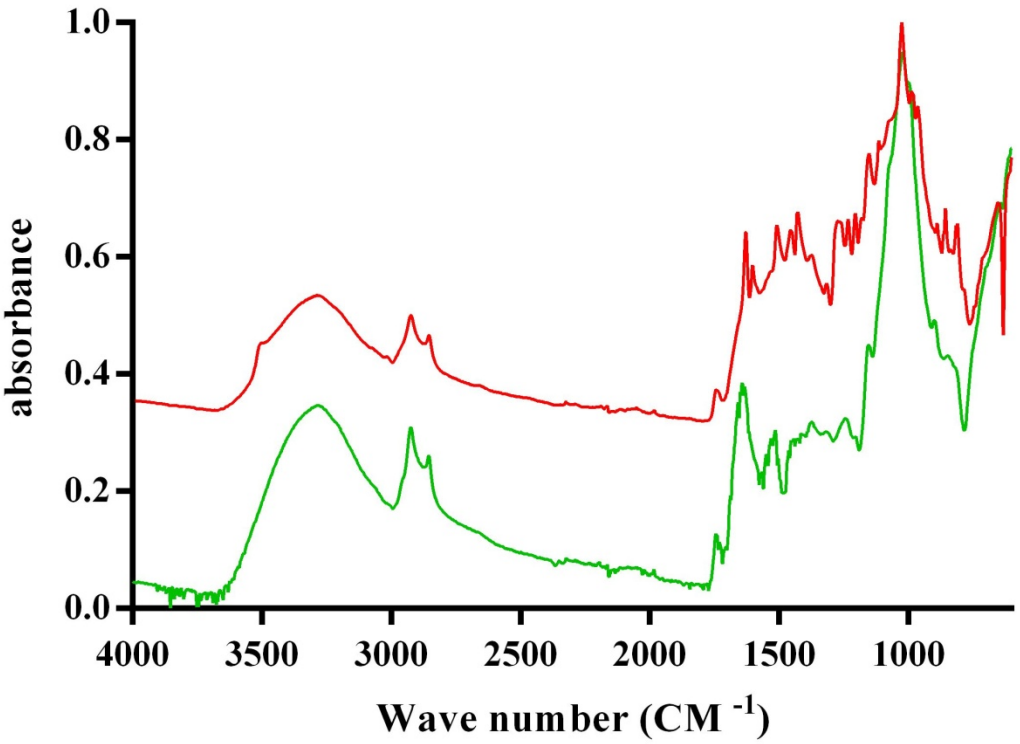


Figure 3.11. FTIR spectrum of physical mixture of curcuminoids-oat fibre (red) and dried curcuminoids (18.4 mg/mL) - oat fibre (1% w/w) dispersion (green).

3.5.7.3. Differential Scanning Calorimetry analysis

Formation of a complex of curcuminoids with carrier materials can be also monitored by differential scanning calorimetry (DSC). An interaction between two compounds is confirmed by elimination of endothermic peaks, appearance of new peaks, change in peak shape, peak temperature/melting point, and relative peak area (Gupta & Dixit, 2011; Karathanos et al., 2007).

The DSC thermogram of curcuminoids powder (10 mg) showed an endothermic peak at 170 °C indicating the melting temperature of curcuminoids (Figure 3.12) (Kakran et al., 2012; Mohan et al., 2012; Shrivastava et al., 2013b; Sousdaleff et al., 2012).

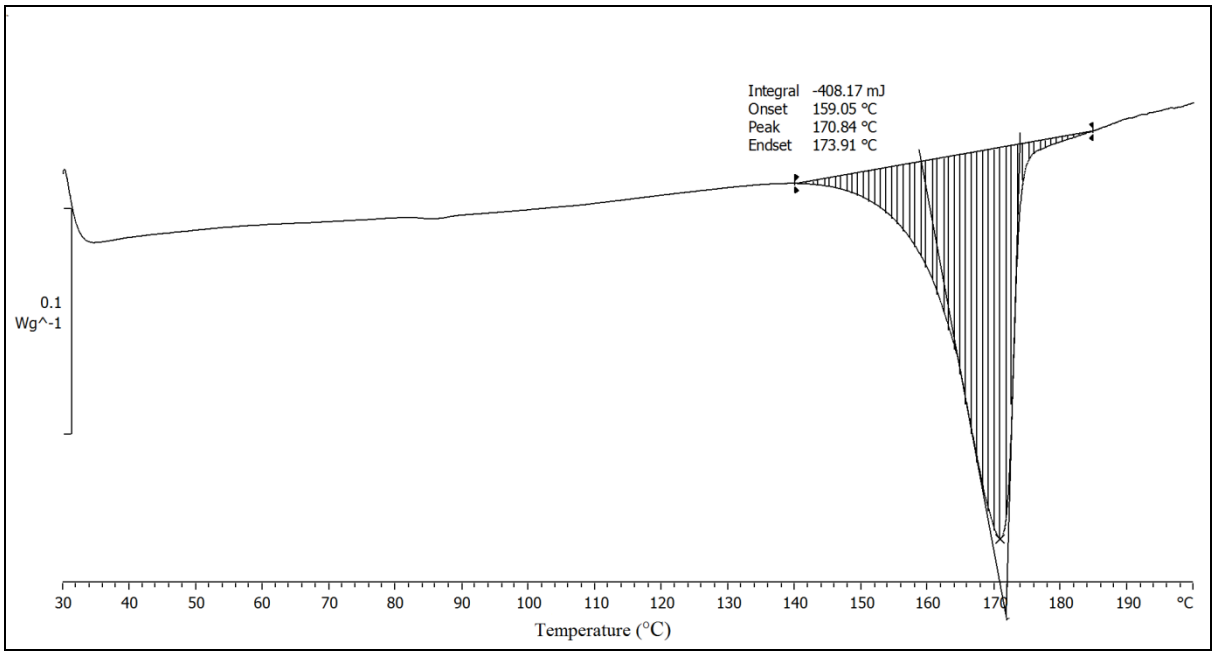


Figure 3.12. DSC thermogram of curcuminoids powder.

In case of oat fibre powder, two exothermic peaks were observed at 189 °C and 229 °C (Figure 3.13). It is suggested that the presence of these two peaks is related to decomposition phenomena. This observation was confirmed by cooling and re-heating of the sample as the exothermic peak disappeared in the second run of heating. It has been reported that the exothermic peak before 205 °C is due to the loss of bound water in the fibre materials and the second peaks can be attributed to the decomposition of the fibre backbone and dehydration of saccharide rings (Min et al., 2009; Wan et al., 2009b).

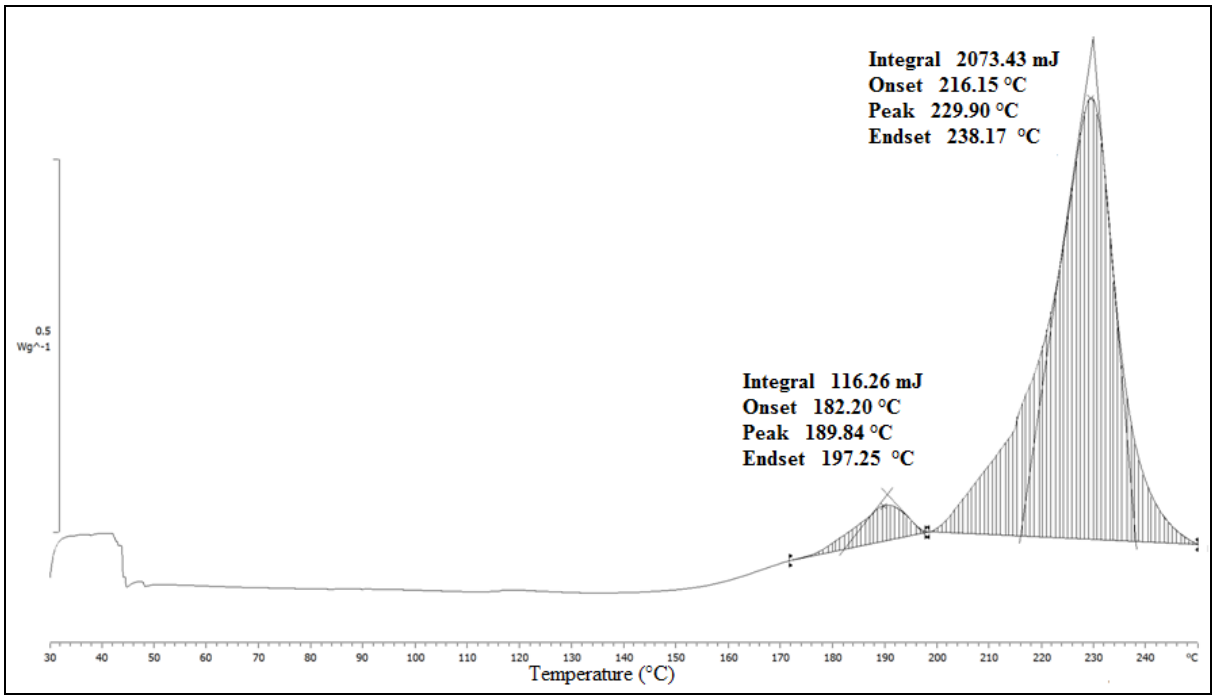


Figure 3.13. DSC thermogram of oat fibre powder.

The physical mixture of curcuminoids with oat fibre at a similar ratio of curcuminoids to the oat fibre in curcuminoids-oat fibre dispersion, showed both the endothermic peak of curcuminoids and exothermic peaks of oat fibre components (Figure 3.14). The melting point of curcuminoids slightly shifted to a lower temperature (168 °C). This result is in good agreement with another study that showed the endothermic peak of curcuminoids changed to lower temperature in the physical mix with hydroxypropyl-β-cyclodextrin (Yadav et al., 2009a).

In the DSC thermogram of the dried curcuminoids (18.4mg/mL) ─ 1 % (w/w) oat fibre dispersion (Figure 3.15), the endothermic peak of curcuminoids disappeared, which is probably indicating the formation of an oat fibre – curcuminoids complex. The absence of an endothermic peak of curcuminoids was also reported when curcuminoids were entrapped

with hydroxypropyl-β-CyclodextrinD (Yadav et al., 2009a), sodium caseinate (Pan et al., 2013), and maltodextrin (Sousdaleff et al., 2012).

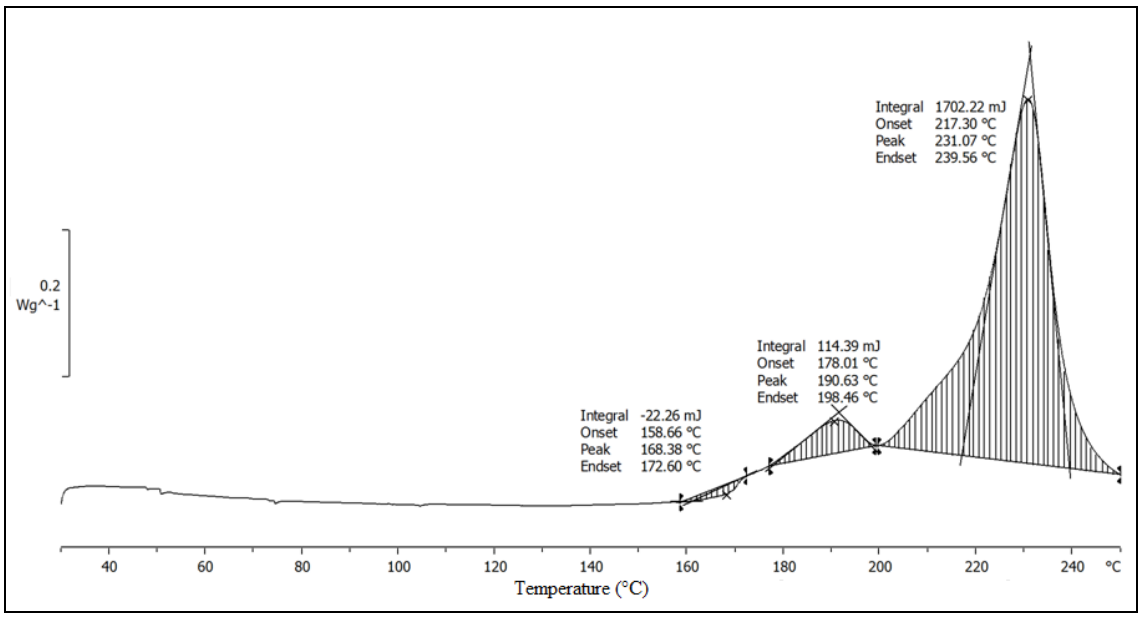


Figure 3.14. DSC thermogram of a physical mixture of oat fibre and curcuminoids powder.

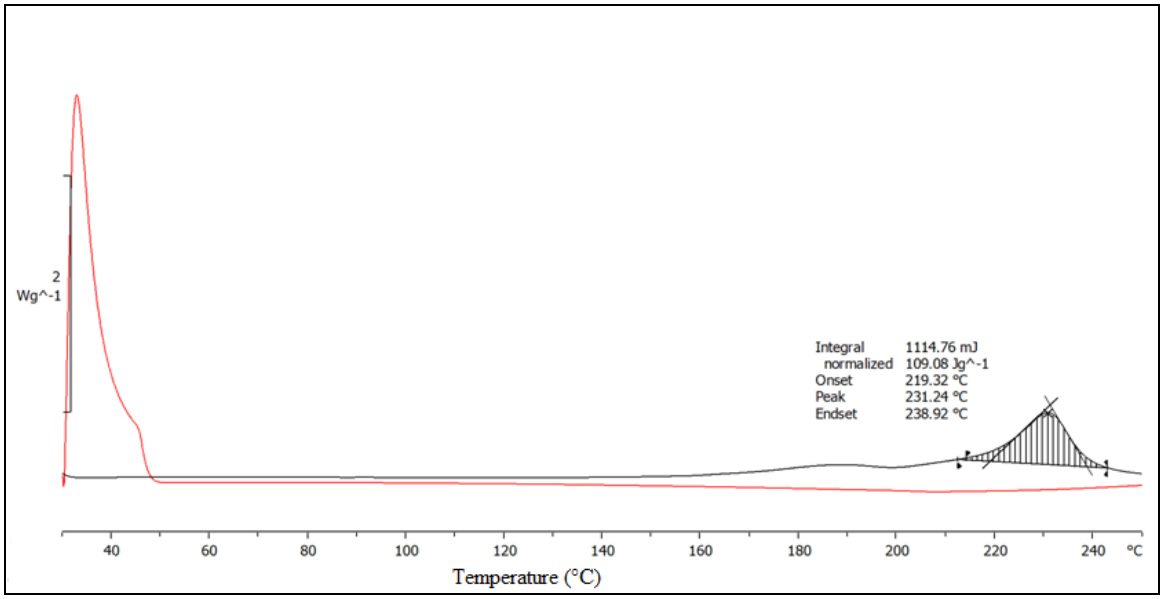


Figure 3.15. DSC thermogram of dried curcuminoids (18.4 mg/mL) ─1 % (w/w) oat fibre dispersion.

3.5.8. Storage Stability

Figure 3.16 shows the stability of curcuminoids in the presence of the whole curcuminoids−oat fibre dispersion in 2 % (v/v) EtOH and the supernatant and precipitated fractions of this dispersion during 11 days of storage at 25 °C. The concentration of curcuminoids decreased from 23.42 to 8.27 μg/mL in the whole oat fibre dispersion after 11 days. The degradation of the curcuminoids in the supernatant was faster than that in the precipitate (Figure 3.16). It is suggested that the difference between the stability of curcuminoids in the precipitate and supernatant is due to the insoluble curcuminoids in the amorphous state in precipitate. Others have shown that curcuminoids is stabilized against degradation when it is in the amorphous state (Li et al., 2013).

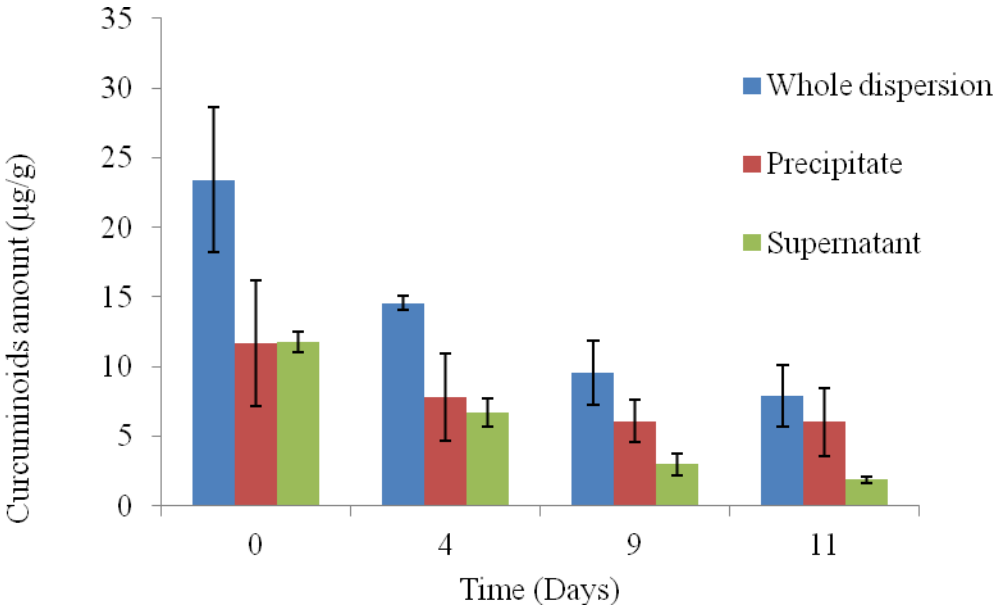


Figure 3.16. Amount of curcuminoids remaining during storage at 25 °C in the dispersion of curcuminoids-oat fibre (1 % TS, w/w) and in the supernatant and precipitated fractions obtained upon centrifugation. Values are expressed as the content per g of the original dispersion.

In addition, the stability of curcuminoids in the curcuminoids (25.8 µg/mL) − oat fibre (1% TS, w/w) dispersions in 2 % (v/v) EtOH was not affected by storing at 25 °C or 4 °C during 24 hours (Figure 3.17). Therefore, it was possible to prepare the curcuminoids-oat fibre dispersion a day before the extrusion trial without curcuminoids degradation.

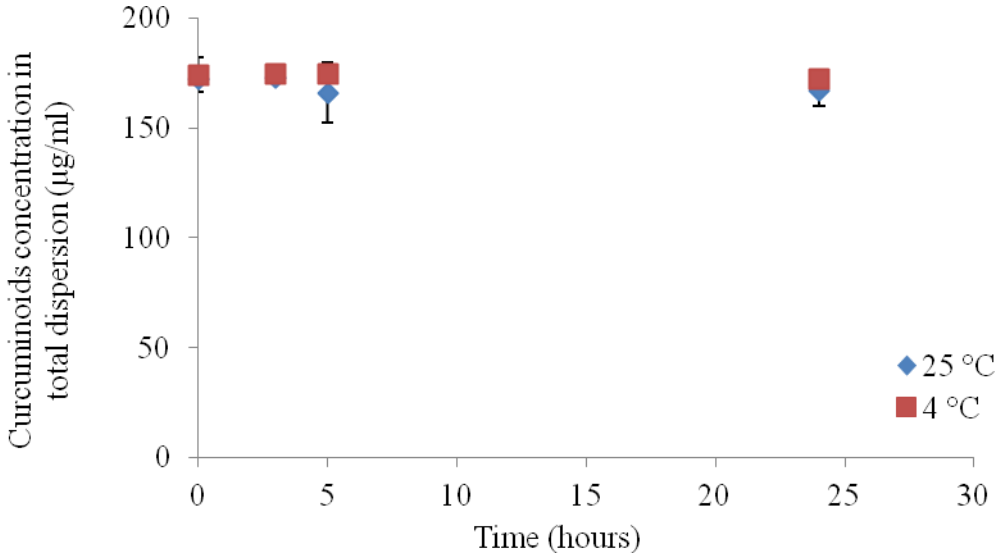


Figure 3.17. Storage stability of curcuminoids in the curcuminoids (25.8 µg/mL) − oat fibre (1% TS, w/w) dispersions in 2 % (v/v) EtOH stored at 25 °C and 4°C during 24 hours.

3.6. Conclusion

Curcuminoids have always been considered as a healthy polyphenolic compound; however, its poor solubility in an aqueous environment (11 ng/mL) is a major challenge for curcuminoids application in food and pharmaceutical applications. The results of fluorescence studies showed that the fluorescence intensity of different concentrations of curcuminoids increased in presence of oat fibre components (1 % w/w), indicating a potential interaction between curcuminoids and oat fibre ingredients.

Partitioning of curcuminoids and oat fibre components in the supernatant and pellet fractions obtained upon ultracentrifugation of 1 % (w/w) oat fibre dispersion showed that curcuminoids are approximately equally partitioned between supernatant and precipitate. In addition, protein and β-glucan fractions of oat fibre were mainly present in the supernatant, indicating their role to interact with curcuminoids and increase the solubility of curcuminoids. The intrinsic protein fluorescence of oat fibre – curcuminoids dispersion was quenched in the presence of curcuminoids, showing that the protein component of oat fibre interacts with curcuminoids. The results of fluorescence spectroscopy of pure β-glucan – curcuminoids dispersions also indicated that there is an interaction between curcuminoids and β-glucan.

The maximum concentration of curcuminoids in the supernatant of 1 % (w/w) oat fibre was obtained at 88 µg/mL, comprising both curcuminoids bound to soluble components of the oat fibre and free curcuminoids, in the aqueous phase. This amount of curcuminoids solubility exceeded that of curcuminoids solubility in water (11 ng/mL) as well as curcuminoids solubility in 2 % (v/v) EtOH (4.1 µg/mL). XRD experiments demonstrated that the

curcuminoids structure converted from a crystalline state to an amorphous state when incorporated into oat fibre components.

FTIR and DSC experiments also provided evidence of curcuminoids-oat fibre complex formation. The results of stability tests indicated that curcuminoids degradation in the supernatant of 1 % (w/w) oat fibre is faster in comparison with curcuminoids degradation in the precipitate. Therefore, it is suggested to add curcuminoids in a solid state in a food product rather that in solution or dispersion.

These findings illustrate the potential for the curcuminoids carrying capacity of oat fibre to be capitalized upon in the fortification of food with curcuminoids.

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Chapter 4

The effect of extrusion on the functional properties of oat fibre

4.1. Introduction

Oat dietary fibre, specifically β-glucan, has been shown to provide a number of health benefits, such as lowering cholesterol (Chen et al., 2006), reducing the incidence of coronary heart disease and reducing blood pressure (Berg et al., 2003; He et al., 2004). The high solubility and viscosity of oat fibre ingredients act by increasing the viscosity of food in the gastrointestinal tract, reducing the transit of food through the intestines and delaying gastric emptying and the intestinal absorption of nutrients (Lazaridou & Biliaderis, 2007a). Characteristics such as the molecular weight and water binding capacity determine the viscosity of oat fibre (Skendi et al., 2003a) and are key criteria in the selection of these functional food ingredients. The method of food processing should also be selected to preserve these characteristics.

Extrusion processing is a popular low cost technique that can efficiently produce a broad range of versatile food shapes from oat fibre or similar ingredients but this process can chemically and physically alter the feed (Brennan et al., 2013a). The high temperatures, high pressures and mechanical forces applied during extrusion can break covalent bonds and disrupt physical structures, leading to a change in functional properties (Kim et al., 2006; Singh et al., 2007). Extrusion variables such as the screw speed, screw configuration, barrel temperature and feed moisture can all impact on food ingredients (Ali et al., 1996; Cindio et al., 2002; Miller & Mulvaney, 2000). Consequently extrusion conditions, particularly

variables such as feed moisture and temperature, should be optimised for the food or ingredient of interest.

While extrusion processing is known to impact on the functional characteristics of oat flour (Gutkoski & Eldash, 1999) and oat bran (Zhang et al., 2009), the effect of oat fibre composition, such as dietary fibre, proteins and lipids, on the changes experienced during processing is not fully understood. The effect of individual process variables, such as barrel moisture content and screw speed on the functional properties of oat fibre preparations has also not been fully established. Conditions of low moisture (15-18 % (w/w)) and high temperature (150-180 °C) are known to reduce the viscosity of β-glucan in oat fibre by 27 % (Sharma & Gujral, 2013). High temperatures and high screw speeds (180 °C and 600 rpm) also decreased the solubility and swelling properties of oat soluble dietary fibre from 69 % to 47% and 1.08 to 1.03, respectively (Zhang et al., 2009). Such chemical and structural changes can also potentially influence the functional characteristics and nutritional properties of oat ingredients (Lazaridoua, 2004).

Oat fibre preparations with enhanced levels of β-glucan are of particular interest, as they can increase dietary soluble fibre and make it easier to deliver the recommended 3 g β-glucan per day, which has been shown to reduce blood cholesterol and normalize blood sugar levels (Lazaridou & Biliaderis, 2007; Mahdavi, Jafari, Ghorbani, & Assadpoor, 2014). Previous studies have typically examined oat fibre with low levels of β-glucan (3.44-4.76 % (w/w) in oat flour (Choi et al., 2012), 5 % (w/w) in commercial oat fibre (Rosell et al., 2009) and 14 % (w/w) in oat bran (Tosh et al., 2010). While the potential of high levels of oat soluble dietary fibre (OSDF) has been demonstrated with an extracted preparation containing 82.5 % (w/w)

OSDF (Zhang et al., 2009), this preparation lacked other oat fibre components, such as oat protein that are known to contribute functionality to oat ingredients (Kim & White, 2012).

This study aims to investigate the effects of extrusion processing on whole oat fibre preparations containing 28 % (w/w) β-glucan. The limited time and instrument availability restricted the experimental extrusion plan conducted here. The preliminary experiments, however, indicated that the constant temperature profile chosen provides a good condition under which the affect the physico-chemical properties of the formulation could be tested. The effect of two barrel moisture contents (50 % - 60 %) and two screw speeds (200 – 300 rpm) were examined. The molecular weight, total soluble solids and water sorption index were assessed, together with dynamic vapour sorption analysis. This later method has been applied to a wide range of dried foods but not to oat fibre; it provides a more sensitive method to assess interactions with water that are governed by structure and can potentially provide novel insights into the extrusion process. Thermal and pasting properties were also measured after extrusion to assess the impact of this processing technology.

4.2. Materials and Methods

4.2.1. Materials

Oat fibre (Oatwell 28 % β-glucan) was purchased from Brenntag Pty Ltd (Victoria, Australia). The oat fibre composition is shown in Table 4.1. The protein content was analysed using a LECO FP-2000 Nitrogen Analyser (LECO Australia Pty Ltd., New South Wales, Australia). The quantification of β-glucan, lipid and total solids was carried out based on the AOAC Official Method 995.16 (AOAC, 2005), Australian Standard Method (Standards Australia, 2008) and AOAC official method 990.20 (AOAC, 1993) respectively. All gel permeation chromatography solvents were purchased from Merck (Victoria, Australia).

Table 4.1. Oat fibre composition

Components	Amount (% w/w)
protein	21.8 ± 0.0
β-glucan	27.5 ± 1.1
lipid	5.2 ± 0.0
moisture	6.5 ± 0.2
carbohydrates and dietary fibre	33.5 ± 0.5

4.2.2. Extrusion processing

The oat fibre was extruded by a co-rotating twin-screw extruder (Figure 4.1) (MPF 19:25, APV Barker Ltd., Peterborough, United Kingdom). The barrel diameter was 19 mm and the length to diameter ratio (L/D) was 25. The dry feed (100 % oat fibre) was fed with a twin-screw volumetric feeder (K-MV-KT20; K-Tron LLC, Niederlenz, Switzerland). Melt pressure was monitored with a pressure transducer (Terwin 2076, Terwin Instrument Ltd., Nottinghamshire, United Kingdom) fitted into the die block. The temperature in the barrel increased from 80 °C in the first zone of the barrel to 90 °C and 100 °C in successive zones before reaching 110 °C in the final zone at the die. These barrel temperature profiles were selected based on our preliminary results. These range of barrel temperature profile was lower in compared with other studies that applied 180°C or 140 °C (Ding et al., 2006; Zhang et al., 2009). Deionised water was injected into the extruder to achieve two levels of total moisture content in the barrel. These were 50 % or 60 % moisture on a dry basis for the low and high moisture treatments respectively. These levels of moisture content were higher than

what usually used in extruder because of the strong water absorption capacity of oat fibre (Skendi et al., 2003b). The selection of higher feed moisture content and lower barrel temperature provides moderate SME applied on raw materials in comparison with other studies such as (600 rpm, 180°C) or (180-320 rpm, 100-140 °C) (Ding et al., 2006; Zhang et al., 2009). By changing the barrel moisture content (50% and 60%) and two screw speeds, a range of specific mechanical energy was obtained. This gave us a reasonable scope to obtain a picture on how the extrusion process will affect the physico-chemical properties of our formulations. Regarding the limitation of time and extruder accessibility, two factors (feed moisture and screw speed) were selected to study the effect of extrusion conditions on physico-chemical properties of raw materials.

The screw speed is a further process variable that was also adjusted to 200 rpm (low speed) or 300 rpm (high speed), as extruders commonly run within this range (Ding et al., 2006; Zhang et al., 2011b).

Extruded oat fibre samples were made in duplicate for each treatment. The extrusion process variables and measured parameters are presented in Table 4.2.

The rate of dry feed and water addition were adjusted so that the rate at which the material entered the extruder (total dry feed plus water) was 4 kg/h. After extrusion, samples were dried at 50 °C for 4 hours in a hot air oven (Contherm, Thermotec 2000, Wellington, New Zealand). Samples were sealed in high vapour barrier plastic bags and kept at 4 °C prior to analysis.

Figure 4.1. Twin screw extruder

Table 4.2. Extrusion process variables and measured parameters used in this study

Description	Sample name	Moisture content of feed materials (%)	Screw speed (rpm)	Specific mechanical energy (Wh/Kg)	Torque (N.m)
Non-extruded	Non-extruded oat fibre	-	-	-	-
High moisture - Low screw speed	Extruded oat fibre - 60 % MC – 200 rpm	60 %	200	70	35
High moisture - High screw speed	Extruded oat fibre – 60 % MC - 300 rpm	60 %	300	75	25
Low moisture - Low screw speed	Extruded oat fibre – 50 % MC - 200 rpm	50 %	200	100	50
Low moisture - High screw speed	Extruded oat fibre – 50 % MC - 300 rpm	50 %	300	117	39

4.2.3. Specific mechanical energy (SME)

For each treatment, the specific mechanical energy (SME) input was calculated using the following equation (4.1) (Ryu & Ng, 2001):

$$\text{SME} = \frac{\text{rpm (test)}}{\text{rpm (rated)}} \times \frac{\text{\% Motor load}}{100} \times \frac{\text{Motor power (rated)}}{\text{Feed rate}} \quad (4.1)$$

The unit for SME is Wh/kg. The set screw speed during extrusion is the "rpm (test)" and the "rpm (rated)" is the rated screw speed of the drive motor for the extruder (500 rpm). The rated motor power is 2.0 kW, and the feed rate is total mass input of dry-feed and water injection rate (kg/h).

4.2.4. Moisture content

The moisture content (MC) of the dry feed and the extrudates (either before or after drying) were measured with a moisture analyser (HB43-S, Mettler Toledo, Victoria, Australia). The extrudates were ground using a grinder (CG2B, Breville, New South Wales, Australia) before analysing.

4.2.5. Molecular Weight

The molecular weight of the soluble fractions of oat fibre was determined before and after extrusion. The aqueous phase of the dispersion was collected after centrifugation at 12000 g for 10 min. The supernatants were then used for molecular weight measurements (McKenzie et al., 2014). A Shimadzu liquid chromatography system fitted with a Shimadzu RID-10 refractometer (λ = 633 nm), using three waters ultrahydrogel columns in

series ((i) 250 Å porosity, (ii) 6 μm diameter bead size; and (iii) 10 μm diameter bead size)) operating at room temperature. The eluent was Milli-Q water containing 20 % v/v Acetonitrile and 0.1 % w/v Trifluoroacetic acid (TFA) at a flow rate of 0.5 mL min^{-1}. Astra software (Wyatt Technology Corp., Version 5.3.4.14) was used to process the data to determine the molecular weights of samples. Polydispersity (Pd), a measure of the distribution of individual molecular masses, was calculated as a ratio between the average of molecular weight (Mw) and the number average molecular weight (Mn). These numbers were calculated based on the peaks obtained from chromatography.

4.2.6. Soluble solid content

The total soluble solids (TSS) present in the aqueous phase of the 1 % w/w oat fibre dispersion were measured according to our previous method (Sayanjali et al., 2014b). Briefly a 1 % (w/w) oat fiber dispersion was sonicated in an ultrasonic bath (Unisonic Australia Pty, Brookvale, New South Wales, Australia) at 20 kHz and 30 °C for 10 min, followed by homogenization at 13500 rpm for 10 min with an Ultra Turax T25 homogenizer (Crown Scientific Pty, Murrarie, Queensland, Australia). To separate the soluble from insoluble fractions, the dispersions were then ultra-centrifuged at 20400 g for 20 min at a temperature of 22 °C to obtain a soluble fraction (supernatant). Each fraction was then weighed. The soluble solids content of the supernatant was then analyzed according to the AOAC Official Method 990.20 (AOAC, 1993).

4.2.7. Water absorption index

The water absorption index (WAI) was measured based on previous method with some modification (Anderson, 1982). Briefly, 2.5 g of extruded and non-extruded oat fibre was

dispersed in 30 ml of distilled water and the samples heated in a water bath at 60 °C for 1 hour. The aqueous phase of the dispersion was separated by centrifugation at 3000 g for 15 min.

The WAI was then calculated based on following equation:

$$WAI = \frac{\text{weight of hydrated gel (g)}}{\text{weight of oat fibre ,g)}} \quad (4.2)$$

4.2.8. Dynamic vapour sorption

The water vapor sorption properties of the extruded and non-extruded oat fibre were determined at 25 °C using a controlled-atmosphere microbalance (Dynamic Vapor Sorption Series 2000, Surface Measurement System Ltd., London, United Kingdom) housed in a controlled temperature incubator (controlled with an accuracy of ± 0.1 °C). The required relative humidity (RH) was generated by mixing continuous dry air and water saturated vapor gas flows where the flow of each stream was controlled. Humidity and temperature probes situated just below the sample and a reference holder gave independent verification of system performance. The microbalance was equipped with an electro balance for mass determination and a humidity sensor for relative humidity measurement.

The air flow in the DVS was set at a constant controlled atmosphere for the experiment. A fine ground ~50 mg sample of non-extruded and extruded oat fibre was loaded onto a quartz flat bottom sample pan. The sample was pre-equilibrated at 0 % RH with a continuous flow of dry air for 2000 min. The sample mass in this step was determined to be the dry mass (M_0). The flow of water saturated vapor was next increased to achieve the desired relative humidity. The sample was exposed sequentially to 5 %, 10 %, 20 %, 30 %,

40 %, 50 %, 60 %, 70 %, 80 % and 90 % RH for 600 min at each humidity setting and the change in sample mass as a function of time was recorded by the microbalance. The moisture isotherm was then calculated using the DVS Analysis Macro V6.1 software within the system. The Guggenheim-Anderson-De Boer (GAB) model, which is recognized as the most versatile sorption model available for the sorption of food (Rao et al., 1994), was then used to fit the moisture isotherm data:

$$\frac{M}{M_0} = \frac{CKa_w}{(1-Ka_w)(1-Ka_w+CKa_w)} \qquad (4.3)$$

Where M is the moisture content (g of water / 100 g of dry mass), M_0 is the monolayer water content (g of water / 100 g of dry mass), K is the adsorption constant, a_w is the water activity and C is the Guggenheim constant.

The specific surface area (S_{GAB}), defined as the accessible area of solid surface per unit mass of material (Itodo et al., 2010), calculated based on monomolecular adsorption of water vapor from the following Equation (Labuza, 1975):

$$S_{GAB} = \frac{M_0 \sigma_0 N_0}{100.M} \qquad (4.4)$$

Where M_0 is the monolayer water content (g H_2O/100g dry mass) (Mathlouthi, 2001), σ_0 is area occupied by a molecule of water ($10.8 \times 10^{-20} m^2$/ molecule), N_0 is Avogadro's number (6.023×10^{23}) and M is the molecular weight of water (18 g/mol).

4.2.9. Thermal properties

The thermal properties of samples were measured using a differential scanning calorimeter (STAR System DSC I, Mettler Toledo, Victoria, Australia) based on a published method (Homer et al., 2014). Analyses were performed on 40 mg of non-extruded oat fibre powder or dried extruded oat fibre, where all samples were dried at 104 °C for 12 h before analysis so the moisture content was ~ 4.5 % ± 0.2. Samples were then heated from -20 to 220 °C at a rate of 10 °C per min within a hermetically sealed medium pressure stainless steel pan containing a Viton O-ring. An empty pan of the same type was used as a reference. Data collection and analysis were performed using the STARe software version 9.30 (Mettler Toledo, Victoria, Australia).

4.2.10. Pasting properties

The pasting characteristic of extruded and non-extruded oat fibre samples was measured with a rapid viscosity analyser (RVA) based on the previous method (Ying et al., 2015b). Briefly, samples were ground using a grinder (CG2B, Breville, New South Wales, Australia) and screened through a 250 μm sieve. A 25 ml aliquot of distilled water and 3 g of extruded or non-extruded oat fibre were placed inside a RVA container.

The test included: (1) a stirring speed of 960 rpm for the first initial 10 seconds and 160 rpm for the remainder of 12.5 min test, (2) a temperature profile where the samples were equilibrated at 50 °C for 1.0 min, the temperature was then increased to 95 °C over a period of 3.8 min, held at 95 °C for 2.7 min and then the temperature decreased to 50 °C over a period of 3.8 min, and a final holding temperature of 50 °C was then applied for 1.5 min.

4.2.11. Statistical analysis

The experimental data were analysed using an analysis of variance (One-way ANOVA) using VassarStats. A probability of $p < 0.05$ was considered to be statistically significant. Results are presented as the average of duplicate analysis of each formulation produced in duplicated extrusion trials (n=4).

4.3. Results and Discussion

4.3.1. Molecular weight measurements

The properties of the oat fibre samples, including average molecular weight and polydispersity, were relatively unaffected by extrusion, as shown in Table 4.3. Neither the moisture content of the feed materials nor the two screw speeds significantly changed the average molecular weight from the $7.5 \times 10^5 \pm 2.2$ g/mol measured in the non-extruded material. Any small changes resulting from the increase in specific mechanical energy (SME) applied with the different treatments were masked by the high variability in molecular weight measurements. The low polydispersity also indicates a relatively uniform size distribution within the soluble oat fibre fraction both before and after extrusion.

Extrusion cooking can break the structure of oat fibre β-glucans, resulting in smaller water soluble fibre fragments (Gutkoski & Eldash, 1999; Tosh et al., 2010), but the conditions applied here including the low SME, high moisture and lower temperature likely reduced the impact of extrusion. Parameters such as screw speed (included within the expression for SME, see Eqn 4.1), feed moisture content and barrel temperaure can all influence fragmentation. The SME applied here was significantly lower (117 Wh/kg c.f. 135 Wh/kg), the moisture content significantly higher (50-60 % c.f. 7 %) and temperature

lower (80-110 °C c.f. 181 °C to 237 °C) than in previous studies, where the average molecular weight of β-glucan extracted from oat bran decreased from 1,930,000 g/mol to 251,000 g/mol (Tosh et al., 2010). The high β-glucan content of the oat fibre formulation used here (28 % (w/w) and high water absorption capacity of β-glucan lead to much higher moisture content in the extrusion conditions applied here.

Table 4.3. The average molecular weight and polydispersity of the water soluble fraction obtained from a 1% w/w aqueous dispersion of non-extruded or extruded oat fibre.

Sample	Average molecular weight ($\times 10^5$) (g/mol)	Polydispersity Index (Pd)
Non-extruded oat fibre	7.5 ± 2.2[a]	0.7 ± 0.2[a]
Extruded oat fibre – 60 % MC - 200 rpm	6.5 ± 2.3[a]	0.5 ± 0.3[a]
Extruded oat fibre – 60 % MC - 300 rpm	5.1 ± 1.3[a]	0.7 ± 0.0[a]
Extruded oat fibre – 50 % MC - 200 rpm	4.6 ± 0.8[a]	0.8 ± 0.1[a]
Extruded oat fibre – 50 % MC - 300 rpm	5.6 ± 0.4[a]	0.4 ± 0.3[a]

Where MC = moisture content and rpm = revolutions per minute. Different letters mean significantly different within the same column ($p < 0.05$). Samples are listed in order of increasing SME.

4.3.2. Total soluble solids and water absorption index

There was no significant difference in the total soluble solids (TSS) and water absorption index (WAI) measured for a 1 % w/w dispersion made with non-extruded or extruded oat fibre ($p < 0.05$), as shown in Table 4.4. The similar TSS across treatments indicates a low degree of molecular fragmentation (Hagenimana et al., 2006; Xu et al., 2014), consistent with the similar molecular weight following extrusion (Table 4.3). While the similar WAI indicates a consistent bulk absorption of water and degree of swelling (Choi et al., 2012).

The similar TSS and WAI following extrusion are consistent with the high moisture content of the feed used here (50 % and 60 %) potentially reducing the impact of extrusion processing. Changes in water absorption index have previously been observed to decrease the WAI (22 %-14 % (w/w) for wheat-based materials and 20 %-12 % (w/w) for sour cassava starch (Ding et al., 2006; Mesquita et al., 2013) but these studies employed much drier feed materials; unfortunately the SME applied was also not cited preventing direct comparisons with the conditions used here. Large reductions in feed moisture during extrusion are thought to allow greater unfolding of the macromolecular structure of starch or fibre, resulting in greater penetration of water and a higher WAI and TSS (Moisio et al., 2015).

Table 4.4. Total soluble solids (TSS) and water absorption index (WAI) measured for a 1 % (w/w) aqueous dispersion of non-extruded or extruded oat fibre.

Sample	TSS (g /100 g)	WAI (g/g)
Non-extruded oat fibre	0.3 ± 0.0^{a}	6.5 ± 0.9^{a}
Extruded oat fibre – 60 % MC - 200 rpm	0.3 ± 0.0^{a}	7.0 ± 0.6^{a}
Extruded oat fibre – 60 % MC - 300 rpm	0.3 ± 0.0^{a}	7.0 ± 1.0^{a}
Extruded oat fibre – 50 % MC – 200 rpm	0.3 ± 0.1^{a}	7.6 ± 0.7^{a}
Extruded oat fibre – 50 % MC - 300 rpm	0.2 ± 0.0^{a}	7.4 ± 1.2^{a}

Where MC = moisture content and rpm = revolutions per minute. Different letters mean significantly different ($p < 0.05$) within the same column. Samples are listed in order of increasing SME.

4.3.3. Dynamic vapour sorption

Dynamic vapor sorption analysis was next used to assess the sorption of water, as this method allows for more sensitive measurement of the interactions between water molecules and the non-extruded and extruded oat fibre samples (Figure 4.2).

Oat fiber samples extruded with a 50 % barrel moisture content (either at 200 rpm or 300 rpm) appeared most effective at absorbing water vapor (Figure 4.2), likely due to the higher SME applied to these samples, which may have resulted in subtle physical changes to the structure that can be assessed by DVS analysis. More water was absorbed by samples with 50 % moisture content than non-extruded oat fibre samples at a relative humidity > 10 %, i.e. the isotherms appear higher, these samples also absorbed more water than oat fiber samples extruded with a 60 % barrel moisture content (either at 200 rpm or 300 rpm), which absorbed the least water, particularly at a low relative humidity (< 40%). These DVS measurements likely detect much smaller changes in the physical structure than can be determined by bulk characterization techniques, such as the water absorption index (WAI), which has high variability between replicate measures and did not detect significant changes in water absorption following extrusion.

The sorption isotherms were fitted with the Guggenheim-Anderson-De Boer (GAB) model in order to gain a greater understanding of the sorption behavior and implications for the extruded oat fiber product. Table 4.5 lists the GAB model parameters, including the monolayer water content, specific surface area and GAB constants determined for non-extruded and extruded oat fiber.

The monolayer value (g H_2O/100 g dry mass) determined positively correlated with SME ($p < 0.05$) (Figure 4.3.A), with a higher SME resulting in a great absorption of water

associated as a monolayer. This was also evident in ANOVA comparisons shown in Table 4.5. The high SME applied at 50 % barrel moisture content with the screw speed of 200 rpm or 300 rpm significantly altered the mass of associated water. This is possibly due to the greater breakdown of oat fiber molecules during extrusion under conditions of lower moisture, resulting in a larger specific surface area that can absorb more water molecules.

These insights appear new for extruded oat fiber materials and the limited application of DVS to study the absorption of water to fibrous product prevents further comparison with the literature.

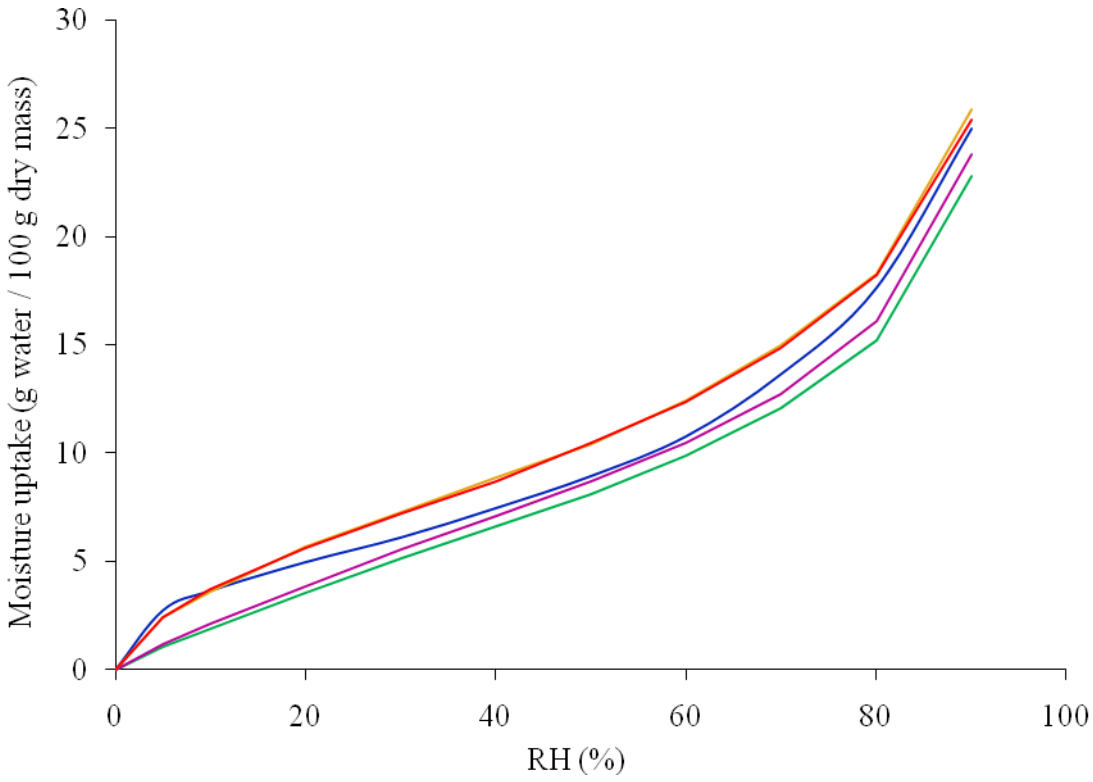
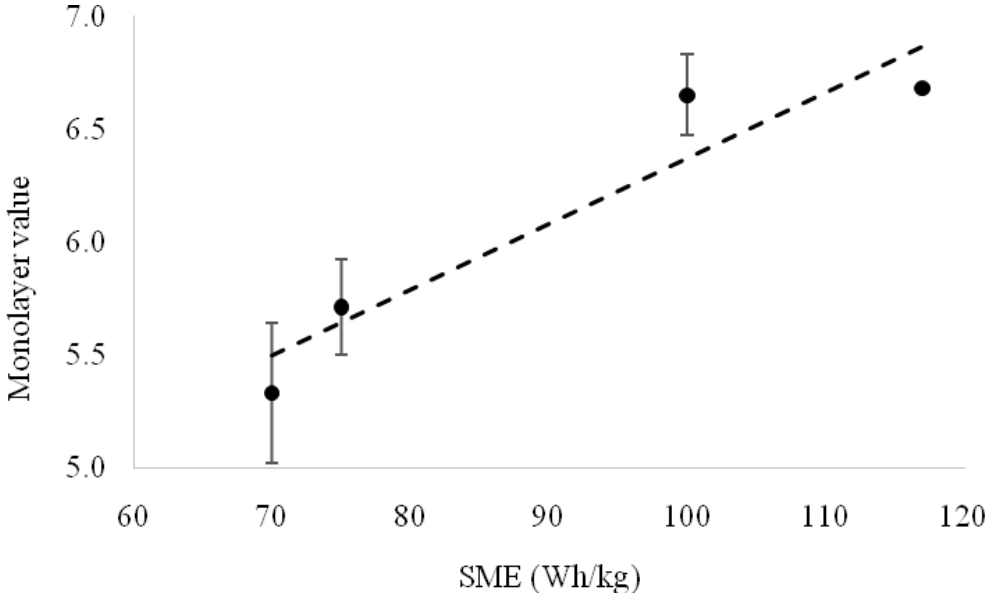


Figure 4.2. Dynamic vapor sorption isotherms of non-extruded oat fibre samples (blue), extruded oat fibre with 60 % moisture content - 200 rpm (purple), extruded oat fibre with 60 % moisture content - 300 rpm (green), extruded oat fibre with 50 % moisture content - 200 rpm and (orange) and extruded oat fibre with 50 % moisture content - 300 rpm (red).

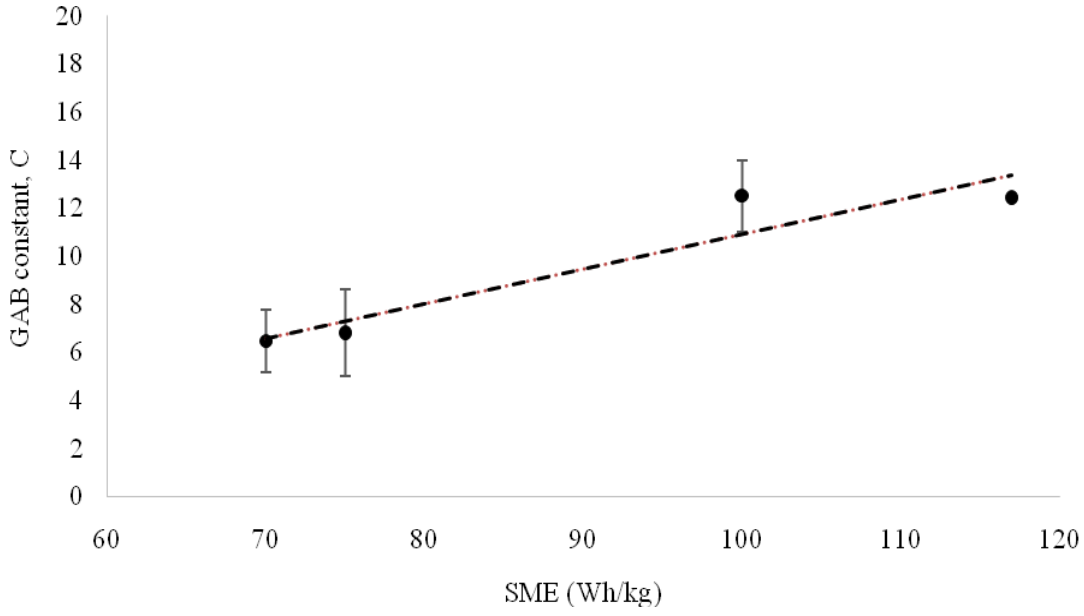
The GAB constant C, a measure water sorption energy or binding strength to the primary binding sites of the material (Quirijns et al., 2005), also positively correlated with the SME applied ($p < 0.05$) (Figure 4.3.B). An increase in SME during extrusion increased the sorption energy of the monolayer water i.e. the binding strength between the monolayer molecules and the material. These differences were again shown in the ANOVA comparisons between treatments in Table 4.5.

The GAB constant K, which relates to the sorption energy of water in multilayer on the surface (Fasina et al., 1997) negatively correlated ($p < 0.05$) with SME. An increase in SME during extrusion reduced the sorption energy of this multilayer water (Figure 4.3.C). This trend was again observed by ANOVA comparisons, as shown in Table 4.5. This constant K indicates that the water molecules were more structured and less closely resembled absorbed bulk liquid (Fasina et al., 1997) in samples with lower moisture content that experienced a higher SME during extrusion.

(A)


7.0
6.5
6.0
5.5
5.0
Monolayer value
60
70
80
90
100
110
120
SME (Wh/kg)

(B)


20
18
16
14
12
10
8
6
4
2
0
GAB constant, C
60
70
80
90
100
110
120
SME (Wh/kg)

(C)

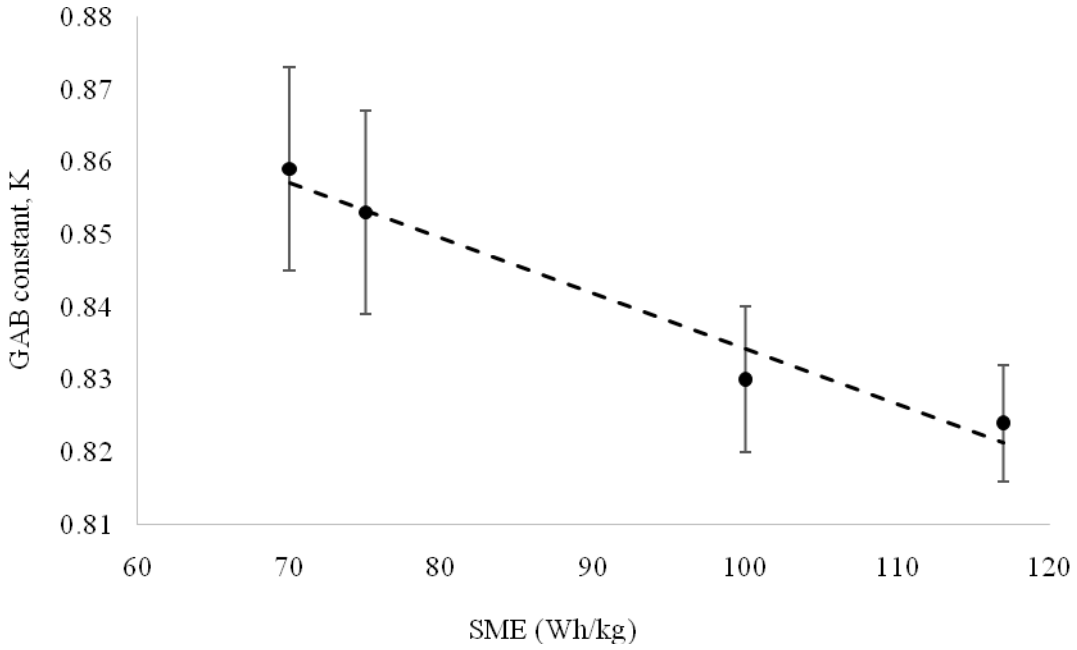


Figure 4.3. Correlation between SME and (a) monolayer value ($p < 0.05$), $R^2 = 0.895$, (b) GAB constant C ($p < 0.05$), $R^2 = 0.892$, and (c) GAB constant K ($p < 0.05$), $R^2 = 0.967$.

Table 4.5. Values determined for the Guggenheim-Anderson-De Boer (GAB) model parameters determined from the model fit to the dynamic vapour sorption (DVS) data obtained for non-extruded or extruded oat fibre.

Sample	Monolayer ($g\ H_2O/100\ g$ dry mass)	Specific surface area (m^2/g)	GAB constant (C)	GAB constant (K)
Non-extruded oat fibre	5.41 ± 0.07^a	196 ± 3.0^a	17.26 ± 1.45^a	0.87 ± 0.00^a
Extruded oat fibre - 60 % MC - 200 rpm	5.33 ± 0.30^a	193 ± 11.0^a	6.47 ± 1.30^b	0.85 ± 0.01^b
Extruded oat fibre - 60 % MC - 300 rpm	5.71 ± 0.30^a	206 ± 11.0^a	6.80 ± 1.32^b	0.85 ± 0.01^b
Extruded oat fibre - 50 % MC - 200 rpm	6.65 ± 0.21^b	240 ± 8.0^b	12.51 ± 1.82^c	0.83 ± 0.00^c
Extruded oat fibre - 50 % MC - 300 rpm	6.68 ± 0.18^b	241 ± 7.0^b	12.43 ± 1.48^c	0.82 ± 0.00^c

Where MC = moisture content and rpm = revolutions per minute. Different letters mean significantly different ($p < 0.05$) within the same column. Samples are listed in order of increasing SME.

4.3.4. Thermal properties

The thermal stability of the oat fibre samples was preserved after extrusion. All samples were found to be highly thermally stable, as the first exothermic peak observed was at a temperature of 200 °C, corresponding to the thermal degradation of oat fibre (Zhang et al., 2009).

The general thermal properties of the oat fibre samples did not appear to be affected by extrusion; the temperature of the onset the endothermic peak (T_0) was not systematically affected, nor was the glass transition temperature (T_g), significantly affected with the exception of the oat fibre extruded at 50 % moisture content and 200 rpm screw speed, where the T_g was significantly higher ($p < 0.05$) (Table 4.6).

Previous studies have linked molecular fragmentation to increases in the T_g, as smaller fragments are thought to form a network structure through inter-chain association (Lazaridoua, 2004; Li et al., 2006). This phenomenon has also been observed for extruded soluble dietary fibre (Zhang et al., 2011b). Although the SME applied in this study was not cited, preventing direct comparisons, it is likely that the SME applied here was not sufficient to induce large systematic changes in T_g.

Table 4.6. The onset temperature (T_0) and glass transition temperature (T_g) determined from differential scanning calorimetry analysis of non-extruded and extruded oat fibre samples.

Sample	T_0	Tg
Non-extruded oat fibre	49.8 ± 0.0a	100.8 ± 0.1a
Extruded oat fibre – 60 % MC – 300 rpm	69.1 ± 4.5^{b}	107.7 ± 2.1^{a}
Extruded oat fibre – 60 % MC – 200 rpm	56.6 ± 2.8^{c}	105.3 ± 3.6^{a}
Extruded oat fibre – 50 % MC – 300 rpm	$67.5 \pm 4.5^{d,c,a}$	104.5 ± 3.9^{a}
Extruded oat fibre – 50 % MC – 200 rpm	75.5 ± 1.7^{e}	116.6 ± 0.8^{b}

Where MC = moisture content and rpm = revolutions per minute. Different letters mean significantly different ($p < 0.05$) within the same column. Samples are listed in order of increasing SME.

4.3.5. Pasting property

The physical properties of the oat fibre samples do not appear to have been significantly altered by extrusion, indicating that the extrusion conditions applied in the current study have the potential to produce oat fibre extrudates without detrimental effect on the viscosity and gel forming properties. Only small changes were observed in the gradient of the rapid visoco analyser profile determined for the extruded samples (Figure 4.4). This is unlike more intensive extrusion processing, which can significantly reduce the pasting properties and final viscosity as a result of fragmentation (Yao et al., 2011; Zhang et al., 2009).

Among the extruded samples, the sample that experienced the lowest SME (70 Wh/Kg, extruded at 60 % barrel moisture content- 200 rpm screw speed) had the highest peak viscosity, as well as the highest final viscosity indicating the least fragmentation of the oat fibre molecules (Sharma & Gujral, 2013).

The retention of high viscosity of at least ~ 11000 mPa.s peak viscosity in all extruded samples is significant, as these properties influence processing and are also thought responsible for some of the health benefits attributed to oat fibre products. The data presented in Figure 4.4 indicate that these properties will be largely retained under the conditions of extrusion employed here.

The ability of extruded oat fibre samples to hydrate and contribute to the viscoelastic properties of the samples is also demonstrated by the peak in the viscosity observed at ~4-5 minutes. The subtle differences in peak intensity indicate potential small differences in molecular fragmentation between the treatments, as the intensity typically decreased with increasing SME. The slightly earlier appearance of the viscosity peak in extruded samples

compared to non-extruded samples is also indicative of a decrease in molecular weight (Lazaridou et al., 2003), although the small translation on the x axis for these peaks suggests that the degree of fragmentation is small, consistent with other measurements.

The final viscosity was highest for non-extruded oat fibre, possibly due to the intact oat fibre molecules. The viscosity of extruded products is mainly related to the extent of molecular breakdown during extrusion (Yao et al., 2011). Changes in final viscosity from 5500 cp to 2994 cp with β-glucan content of 3.2-8.6 % (dry basis) have been observed during conditions of intensive extrusion where the barrel moisture content was much lower (19.4 % (w/w)) but conditions otherwise approximately similar to those employed here (die temperature of 110 °C – 130 °C and screw speed of 200 rpm or 400 rpm) (Yao et al., 2011). Lower moisture and higher temperatures (7 - 18.7 % (w/w), 180 - 237 °C respectively) also reduced viscosity from 2900 mPa.s to 131 mPa.s in other oat fibre preparations containing lower amounts of β-glucan (~ 14 % w/w) (Tosh et al., 2010). In the current study, the high proportion of β-glucan and resulting high moisture content in the feed material leads to a product that not only has high soluble fibre but also retains many of the properties of the non-extruded oat fibre material.

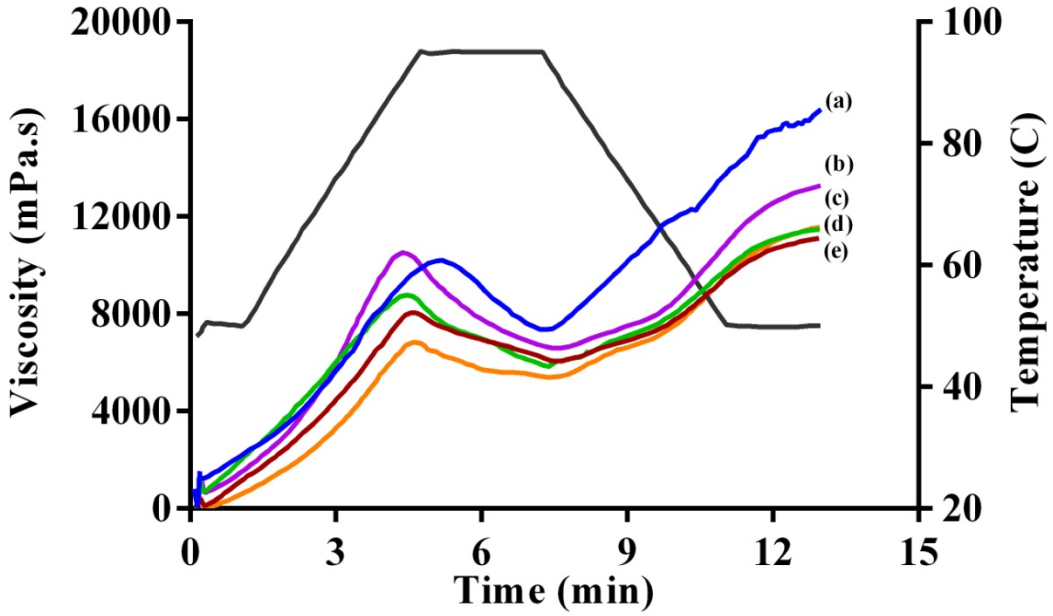


Figure 4.4. Viscosity profile of (a) non-extruded oat fibre and oat fibre extruded at (b) 60 % MC - 200 rpm, (c) 60 % MC - 300 rpm, (d) 50 % - 300 rpm, (e) 50 % - 200 rpm (MC – moisture content; rpm – revolutions per minute).

4.4. Conclusion

Oat fibre preparations containing high levels of β-glucan were extruded using a feed with high moisture (50 % or 60 %) and two screw speeds (200 or 300 rpm). The mild extrusion process did not appear to induce significant molecular fragmentation and many of physicochemical properties were preserved, including molecular weight, total soluble solids and thermal properties. A higher specific mechanical energy applied during extrusion lead to a higher specific surface area and subtle physical changes that increased the absorption of water vapour as a surface monolayer, the sorption energy of this layer and the structure of upper multilayer of water associated with the surface. These changes in water sorption were detected by the more sensitive dynamic vapour sorption analysis but not with bulk techniques, such as the water absorption index. The pasting properties of the extruded oat fibre were largely retained and while final viscosity was slightly reduced by extrusion, this change was much less than that previously observed for oat fibre extruded under more intense extrusion conditions, consistent with the low extent of molecular fragmentation observed here. These data show that mild extrusion conditions using high moisture can be applied to produce extruded products from commercially available oat fibre, with high β-glucan content, where the physico-chemical properties of oat fibre are preserved and the health benefits potentially enhanced by the presence of additional β-glucan.

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Chapter 5

Effect of curcuminoids addition and extrusion conditions on the properties of a curcumin-enriched oat fibre-corn based extruded product

5.1. Introduction

Ready-to-eat snacks are popular among consumers due to their convenience and attractive appearance and texture (Brennan et al., 2013b). One of the main technologies known to have a great potential for manufacturing of snack products is extrusion cooking. During extrusion, the raw materials are subjected to high temperature and shear resulting in many chemical and structural changes and the molecular transformation of biopolymers such as starch and proteins (Moscicki & Zuilichem, 2012; Wolf, 2010).

These snacks are regarded as high energy, nutritionally poor food products (Brennan et al., 2013a), therefore it is reasonable to add bioactive components to them in order to improve their nutritional value. However; the degradation of bioactive components during extrusion processing has been the subject of many studies (Brennan, 2011; Hirth et al., 2014; Ying et al., 2015).

Curcuminoids is a hydrophobic polyphenol derived from the rhizome of turmeric plants (Anand et al., 2007). There are 3 major curcuminoids including curcumin (curcumin I), demethoxycurcumin (curcumin II) and bisdemethoxycurcumin (curcumin III) (Anand et al., 2007; Schaffer et al., 2011). This ingredient has a broad range of biological and pharmacological activities (Anand et al., 2007) and can be incorporated into food substances

as a bioactive food component and natural food colorant for the development of health promoting functional foods. A minimum recommended dosage of 500 mg per day has been shown to decreased serum cholesterol and lipid peroxide levels (Pari et al., 2008).

Oat fibre, rich in soluble fibre such as β-glucan, is known for its beneficial effects, which include lowering blood cholesterol and reducing the risk of heart disease (Wood, 2007). The results from our previous study showed the potential for the curcuminoid carrying capacity of oat fibre to be used in the fortification of food with curcuminoids (Sayanjali et al., 2014). However; the incorporation of curcuminoids in an oat fibre based extruded products has not been known so far.

In current study, we aimed to produce extruded products made from an oat fibre-corn based matrix fortified with 1.5 % (w/w) curcuminoids which is equivalent to 600 mg per 40 g serving of extruded product. This study, will demonstrate the potential of extrusion technology to produce functional food products that incorporate the benefits of oat dietary fibre and curcuminoids. The effect of curcuminoids addition, extruder barrel moisture content and screw speed on the physical/structural properties of the extrudates and stability of curcuminoids during extrusion, drying and storage were also investigated.

5.2. Materials and Methods

5.2.1. Materials

Corn grit (Polenta No 1) was purchased from Scalzo Food Industries (West Melbourne, Victoria, Australia). Calcium carbonate ($CaCO_3$) from IMCD Ltd (Melbourne, Victoria, Australia) was used to supplement the corn grit to aid expansion. Oat fibre was purchased from CreaNutrition (Sumpfstrasse, Zug, Switzerland). All HPLC solvents were purchased from Merck (Melbourne, Victoria, Australia). Ethanol (EtOH, 100 %) was obtained from Sigma-Aldrich (Sydney, New South Wales, Australia). Sodium chloride was purchased from a local supermarket.

5.2.2. Gross compositions

Corn grit containing 79.5 % carbohydrate, 7.4 % protein, 3.1 % dietary fibre, 0.7 % fat and 10.2 % moisture (according to product specification) was used. The protein, β-glucan, total fat and total solids content in the oat fibre were determined. The protein content was analysed using a LECO FP-2000 Nitrogen Analyser (LECO Australia Pty Ltd., Castle Hill, New South Wales, Australia). The quantification of β-glucan, lipid and total solids was carried out based on the AOAC Official Method 995.16 (AOAC, 2005), Australian Standard Method (Standards Association of Australia, 1988) and AOAC official method 990.20 (AOAC, 1993) respectively. The oat fibre contained 27.11 ± 0.02 (% w/w) protein, 27.54 ± 1.12 (% w/w) β-glucan, 5.20 ± 0.02 (% w/w) total fat, 6.6 ± 0.2 (% w/w) moisture, and 33.5 ± 0.5 (% w/w) other components (including carbohydrates and other dietary fibre), as previously determined (Sayanjali et al., 2014). A powdered turmeric extract (Biocurcumin, BCM- 95CG, total curcuminoidsoids complex, purity: 95.7 %) was provided by Arjuna Natural Extracts Ltd (Aluva Kerala, India). Previous analysis in our laboratory indicated that this material consists

88 % (w/w) curcuminoids, (of 70 ± 0.5 % curcumin, 16.0 ± 0.2 % demethoxycurcumin, and 2 % ± 0.1 bisdemethoxycurcumin) (Fu et al., 2014).

5.3. Sample formulation

The composition of the pre-mixed dry powder formulation used for extrusion was 30.0 % (w/w) oat fibre, 68.7 % (w/w) corn grit, 0.3 % (w/w) NaCl and 1 % (w/w) $CaCO_3$ (as nucleating sites in the expansion process at the extruder die). This formulation was selected based on preliminary test. Two formulations were prepared for extrusion: one without curcuminoids, described as "(-) curcuminoids" and the other with curcuminoids added as a powder at 1.5 % w/w, described as "(+) curcuminoids".

5.4. Extrusion

The extrusion process was carried out in a co-rotating twin-screw extruder (MPF 19:25, APV Barker Ltd., Peterborough, East England, United Kingdom). The barrel diameter was 19 mm and the length to diameter ratio (L/D) was 25. The pre-mixed dry powder formulation was fed with a twin screw volumetric feeder (K-MV-KT20; K-Tron LLC, Niederlenz, Lenzburg, Switzerland). The melt pressure was monitored with a pressure transducer (Terwin 2076, Terwin Instrument Ltd., Bottesford, Nottinghamshire, United Kingdom) fitted into the die block. The barrel has 4 temperature zones set to 80 / 90 / 100 / 110 °C.

Deionized water was injected into the extruder barrel to achieve the desired barrel moisture content (21 %, 28 %, and 35 % w/w moisture). Extruded samples were made from duplicate runs for each treatment. The ratio of dry feed and water was adjusted so that a total materials feed rate (dry feed plus water) of ~ 4 kg/h was maintained. After extrusion, samples were dried at 50 °C for 4 h in an oven with air circulation (Thermotec 2000, Contherm, Lower

Hutt, Wellington, New Zealand). Samples were sealed in moisture barrier bags and kept at 4 °C prior to analysis.

For each treatment, the specific mechanical energy (SME) input was calculated using the following equation (4.1) (Ryu & Ng, 2001):

$$\mathrm{SME} = \frac{\mathrm{rpm\ (test)}}{\mathrm{rpm\ (rated)}} \times \frac{\mathrm{\%\ Motor\ load}}{100} \times \frac{\mathrm{Motor\ power\ (rated)}}{\mathrm{Feed\ rate}} \qquad (4.1)$$

The unit for SME is Wh/kg. The “rpm (test)” is the set screw speed during extrusion and the “rpm (rated)” is the rated screw speed of the drive motor for the extruder (500 rpm). The rated motor power is 2.0 kW, and the feed rate is total mass input of dry-feed and water injection rate (kg/h). The extrusion condition and corresponding SME and torque are presented in Table 5.1.

Table 5.1. Extrusion conditions and corresponding SME and torque during extrusion

Sample	Extrusion Condition	SME* (W·h/Kg)	Torque (N·m)
(+) curcuminoids	35 % MC* - 300 rpm*	65	10.3
	35 % MC - 200 rpm	48	11.5
	28 % MC - 300 rpm	87	14.5
	28 % MC - 200 rpm	78	17.2
	21 % MC - 300 rpm	129	22.5
	21 % MC - 200 rpm	109	27.5
(-) curcuminoids	35 % MC - 300 rpm	60	10.7
	35 % MC - 200 rpm	57	11.5
	28 % MC - 300 rpm	86	15.3
	28 % MC - 200 rpm	81	17.6
	21 % MC - 300 rpm	146	22.9
	21 % MC - 200 rpm	127	29.0

*SME - Specific mechanical energy; MC – moisture content; rpm – revolutions per minute.

(+) Curcuminoids formulation: 67.2 % (w/w) corn grit, 30 % (w/w) oat fibre, 0.3 % (w/w) NaCl, 1 % (w/w) $CaCO_3$, 1.5 % (w/w) Curcuminoids

(-) Curcuminoids formulation: 68.7 % (w/w) corn grit, 30 % (w/w) oat fibre, 0.3 (w/w %) NaCl, 1 % (w/w) $CaCO_3$

5.5. Curcuminoids stability

Samples of oat fibre-corn based mixture (+) curcuminoids were taken (1) before extrusion, (2) immediately after exiting the extruder and (3) after drying. The dried oat fibre-corn based extrudates (+) curcuminoids were stored at 25 °C for 80 days, and 6 g samples of each treatment were taken every 10 days and kept at -80 °C until ready for curcuminoids analysis. The curcuminoids analysis and quantification using HPLC are described below.

5.6. Physical analysis

5.6.1 Bulk Density

Bulk density (BD) (g/cm^3) was calculated according to the following equation (2) (Alvarez-Martinez et al., 1988):

$$BD = 4m/\pi d^2 L \qquad (4.2)$$

Where, m is the mass (g), L is length (cm) and d is diameter (cm) of the extrudates. The dimensions of an extrudate were measured using a vernier calliper and the apparent volume was calculated assuming the shape of the extrudate was cylindrical consistent with the moulded shape of the die. Four randomly selected extrudates were used for the measurement (diameter and length) and the values reported were the average ± standard deviation (sd).

5.6.2 Expansion ratio

The expansion ratio was defined as the ratio between the diameter of the extrudate and the diameter of the die (Alvarez-Martinez et al., 1988). Ten samples for each process condition were measured and the values reported were the average ± standard deviation (sd).

5.6.3 Hardness

The mechanical properties of the extrudates were measured using a Lloyd LRX/plus Universal Testing Machine (Lloyd Instruments Ltd., Bognor, West Sussex, United Kingdom) with a 2.5-kN load cell (Pitts et al., 2014). Four pieces of extrudates were chosen randomly, cut to approximately 8 cm in length and placed across the bottom of a Kramer Type Shear Cell. Samples were compressed perpendicular to the length of the extrudate at a compression speed of 60 mm/min. The compression force was recorded using the manufacturer's Nexygen V4.6 software. A minimum of ten replicate tests were carried out for each sample. The data presented in the average ± standard deviation (sd).

5.6.4. Colour

The colour of the samples was measured with a colorimeter (CR-300, Minolta, Osaka, Japan). The results were expressed as values L*, a* and b*. The L* value gives a measure of the lightness of the product from 100 for perfect white to zero for black. The redness/greenness and yellowness/blueness are presented by a* and b* values, respectively. The colour of the samples was measured after grinding the samples to a fine powder using a Breville grinder, CG2B (Sydney, Australia).

5.6.5. Cross sectional examination

Scanning electron microscopy (Quanta; Hillsboro, Oregon, United State of America) was used to analyse the cross-section of the extrudates (+) curcuminoids. For SEM examination, the extrudates were cut using a sharp blade, mounted on double sided carbon tape and then coated with gold. The SEM examination was carried out with a10 kV beam power.

5.7. Curcuminoids analysis and quantification

5.7.1. Extraction of Curcuminoids

Extrudates containing curcuminoids were ground using a grinder (CG2B, Breville, Sydney, New South Wales, Australia) and passed through a 250 μm sieve to remove large particles. Sieved samples (100 mg) were then mixed with 10 ml of ethanol (EtOH). Samples were sonicated in an ultrasonic bath (Unisonic Australia Pty, Sydney, New South Wales, Australia) at 20 kHz and 30 °C for 60 min followed by shaking at 30 °C for 4 h. Samples were then centrifuged at 1000 g for 5 min to obtain the supernatant. A second extraction of the pellet was carried out and the supernatants from the first and second extractions were combined, weighed and kept at 4 °C before analysis.

The concentration of curcuminoids from the extracts was analyzed by high-performance liquid chromatography (HPLC). The supernatants were filtered through a 0.45 μm syringe filter (Merck Millipore, Carrigtwohill, Cork, Ireland). The concentration of curcuminoids was quantified using a calibration curve of curcuminoids in EtOH at concentrations of 3.68-184 μg/mL. The standard solutions were kept at -18 °C before analysis. To evaluate the accuracy of the extraction method, spiking experiments with a known amount of curcuminoids were also conducted. The results showed a recovery of approximately 91 % of curcuminoids from the matrix.

5.7.2. High-performance liquid chromatography analysis (HPLC)

The curcuminoids content of extrudates was determined by a 1100 series HPLC (Agilent, Santa Clara, California, United States) consisting of a binary pump, an Agilent 1100 series diode-array detector, a ChemStation software, an 1100 well plate auto sampler and an X

Terra MS C18 column (4.6 mm × 250 mm; 5 µm, Waters Corporation, Milford, Massachusetts, United State of America). The mobile phase was composed of 2 % (v/v) acetic acid in MilliQ water as mobile phase A and 2 % (v/v) acetic acid, and 10 % (v/v) methanol in acetonitrile as mobile phase B. The gradient program was as follows: 0–10 min, 45 % B increasing to 50 % B; 10–25 min, decreasing to 45 % B (Fu et al., 2014).

5.8. Statistical Analysis

The experimental data were analysed using an analysis of variance (one-way ANOVA) using VassarStats. A probability of $p < 0.05$ was considered to be statistically significant. Results are presented as the average of duplicate analysis of each formulation produced in duplicated extrusion trials (n=4).

5.3. Results and Discussion

5.3.1. Bulk density and expansion ratio

Figure 5.1 shows the effect of barrel moisture content and screw speed on bulk density and expansion ratio of the dried extrudates. The bulk density of extrudates is significantly decreased ($p<0.05$) from ~ 1.0 to ~ 0.2 g/cm^3 by decreasing the barrel moisture content from 35 % to 21 %, while the expansion ratio significantly increased ($p<0.05$) from ~ 1.5 to ~ 2.8 g/cm^3. However, the bulk density and expansion ratio of extrudates with or without curcuminoids was not significantly affected ($p>0.05$) by the screw speed when the barrel moisture is maintained. Furthermore, the addition of 1.5 % curcuminoids (w/w dry basis) in the formulation did not affect the bulk density and expansion ratio of extrudates.

The dough viscosity and elastic force (i.e. the die swell) affects the expansion and density of extrudates (Moraru & Kokini, 2003). The bubbles inside the viscoelastic melt grow when the

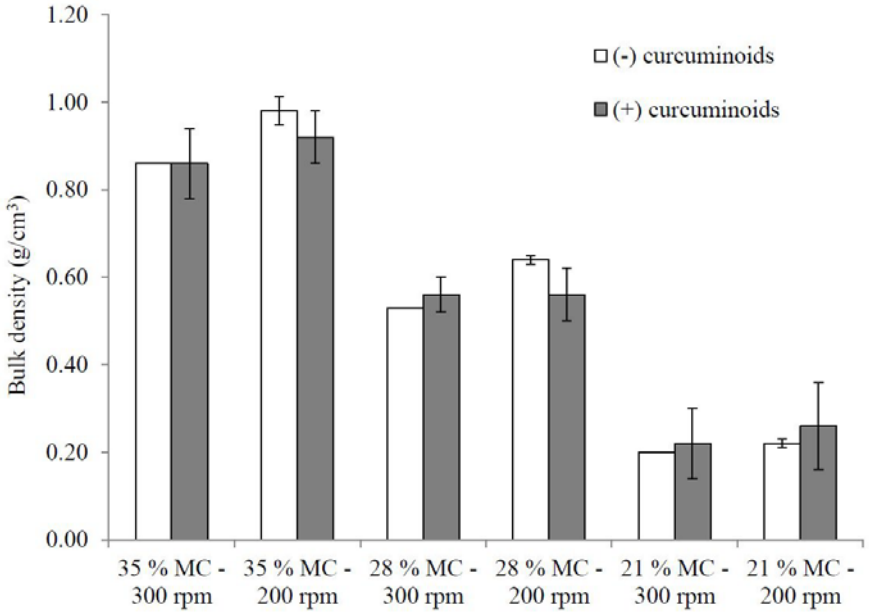
melt exits the extruder die due to moisture flash-off (Patil et al., 2007). The higher moisture content of feed materials reduces the elasticity of the dough due to the plasticization effect of water on the dough (Singh et al., 2015). The Reduction in the elasticity of the dough causes the air bubbles to collapse inside the dough, resulting in a lower expansion and higher density in high moisture treatment (Gulati et al., 2016). Reducing the moisture content inside the extruder barrel increases the melt viscosity and porosity that leads to enhanced expansion and a reduction of bulk density. However, it has been reported that there is an optimum moisture content for achieving the maximum expansion (Baik et al., 2004). At a moisture content below a certain level, the shear rate inside the barrel increases, resulting in molecular disruption that causes a reduction in the expansion ratio (Baik et al., 2004).

The effect of barrel moisture content on bulk density and expansion has been already reported in expanded snacks made of waxy and regular barley flour (Baik et al., 2004), which were extruded at a moisture content ranging from 17 – 21 %, screw speed of 50, 75, 100, 125 and 150 rpm. The expansion ratio increased from 1.81 to 2.68, but the bulk density decreased from 0.46 g/mL to 0.18 g/mL as the moisture content decreased from 21 % to 17 % (Baik et al., 2004). Similar trends have also been reported for changing bulk density (0.2 to 0.7 g/cm^3) and expansion ratio (2 to 4) for corn starch material which were extruded at a screw speed of 150 or 250 rpm, die temperature of 100 °C or 260 °C and feed moisture content of 12 or 25 kg/100 kg wet basis (Thymi et al., 2005).

In another study, the expansion ratio of expanded snacks made from rice flour, wheat bran and corn grits decreased up to 25 % due to the presence of tomato paste (containing ~ 2.5 % w/w lycopene, dry basis) (Dehghan-Shoar et al., 2010). The authors attributed the reduced expansion to the lubricating effect of tomato paste on the dough, leading to a reduction in

SME and die pressure. The tomato paste also contained fibres, resulting in higher water absorption, leading to a higher bulk density compared with snacks without tomato paste (Dehghan-Shoar et al., 2010). In the current study, the addition of powder curcuminoids into the feed material did not affect the bulk density and expansion ratio of extrudates. This may be in part because curcuminoids itself is hydrophobic and does not absorb water; hence it is unlikely that curcuminoids influence the properties of the dough. Whilst the interaction of curcuminoids with macromolecules in the feed materials may be altered as a result of extrusion, this did not appear to affect the properties of the extrudate. The lack of a marked influence of curcuminoids addition (1.5 % w/w) on the extrudability of the formulation means that this amount of curcuminoids can be readily added into ready-to-eat cereals/snacks without compromising the expansion and density of extrudates when the same processing conditions are used.

(a)



(b)

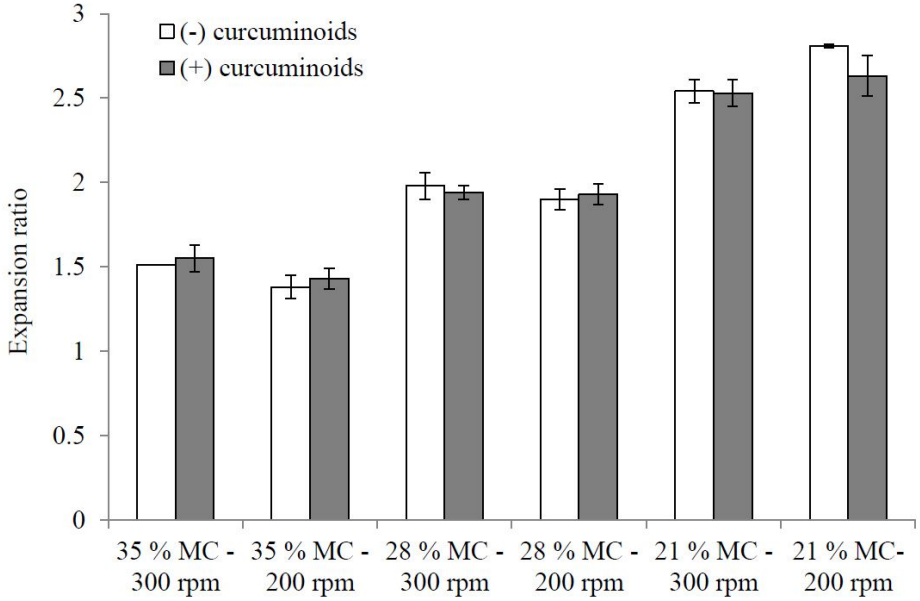


Figure 5.1. Bulk density (a) and expansion ratio (b) of dried extruded products with and without curcuminoids (MC – moisture content; rpm – revolutions per minute). The data is the average ± s.d. (n =4), $p < 0.05$.

5.3.2 Hardness

The hardness of extrudates was obtained by measuring the force required to break the dried extrudates (Figure 5.2). No consistent trend was observed for the effect of the extruder barrel moisture content and screw speed (Figure 5.2). The change in hardness is not significant ($p>0.05$) when the barrel moisture content was reduced from 35 % to 28 % in spite of the enhanced expansion ratio (from ~ 1.5 to ~ 2) (Figure 5.1b). Increasing the expansion ratio from 1.5 to 2 is not sufficient to significantly reduce the hardness of the extrudates ($p<0.05$). However, when the barrel moisture content was further reduced to 21 %, the hardness of extrudates was reduced significantly ($p<0.05$).

The effect of extruder barrel moisture content on the hardness of extrudates has been widely studied (Liu et al., 2000; Seth et al., 2015; Zarzycki et al., 2015). At a higher feed moisture content, the viscosity of the dough and mechanical energy in the extruder decreased, stopping the bubble growth at temperatures below the glass transition temperature (Kristiawan et al., 2016). Under these conditions, vapour condensation occurred and extrudates became more dense and hard (Ding et al., 2005; Kristiawan et al., 2016). In one study, where the hardness of corn-oat extrudate was investigated, the hardness of the extrudate increased as the barrel moisture content increased from 18 % to 21 %, whereas an increase in the screw speed from 200 rpm to 400 rpm lead to a decrease in hardness (Liu et al., 2000). However, in our study no consistent trend was observed for hardness of the different extrudates produced at different screw speeds between 200 and 300 rpm.

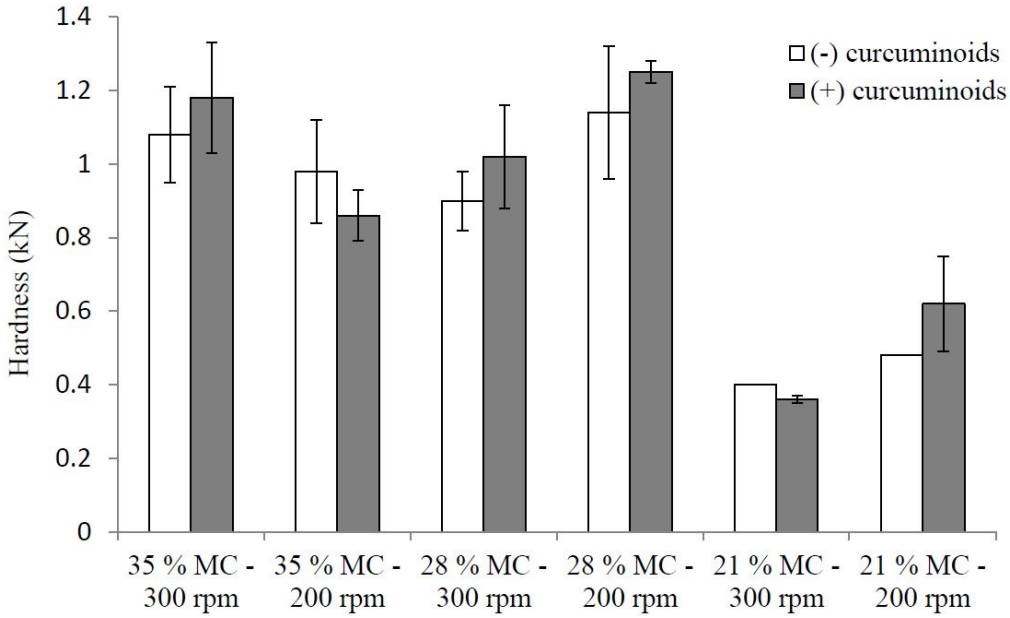


Figure 5.2. Hardness of dried extruded products (MC – moisture content; rpm – revolutions per minute). The data is the average ± s.d. (n =4), $p < 0.05$.

5.3.3 Cross sectional examination

The expansion, bulk density and hardness of the extrudates were not significantly affected by the addition of curcuminoids ($p > 0.05$) (Figure 5.1 and 5.2). Therefore, examination of the cross-sectional structure was only carried out on the extrudate with curcuminoids. The SEM images of extrudates (+) curcuminoids are shown in Figure 5.3. The moisture content had a considerable effect on structure of extrudates. In extrudates produced at 35 % moisture air bubbles were surrounded by thick walls of extrudates materials, which resulted in harder and denser extrudates. Decreasing the moisture content from 35 to 28 % resulted in a structure where the pores appeared more numerous and the walls thinner, although these pores remained discrete. Further reducing the moisture to 21 % resulted in larger bubbles and thinner walls due to the greater expansion of the extrudates. Under these conditions, the

thinner walls also appear more susceptible to mechanical damages during sample preparation. Similar trends have been found for rye snacks (Saeleaw et al., 2012) that were extruded under two barrel temperature profiles (150 °C and 190 °C) and feed moisture content (12 % and 16 %). The higher feed moisture content (16 %) with low barrel temperature (150 °C) resulted in thicker cell walls (Saeleaw et al., 2012). Furthermore, increasing the feed moisture content from 13 % to 19 %, in corn-lentil extrudates, resulted in thicker cell walls and reduction in the number of air cells (Lazou & Krokida, 2010). The microstructure of extrudates (+) curcuminoids did not appear to be significantly ($p>0.05$) affected by the screw speed under conditions with the same barrel moisture.

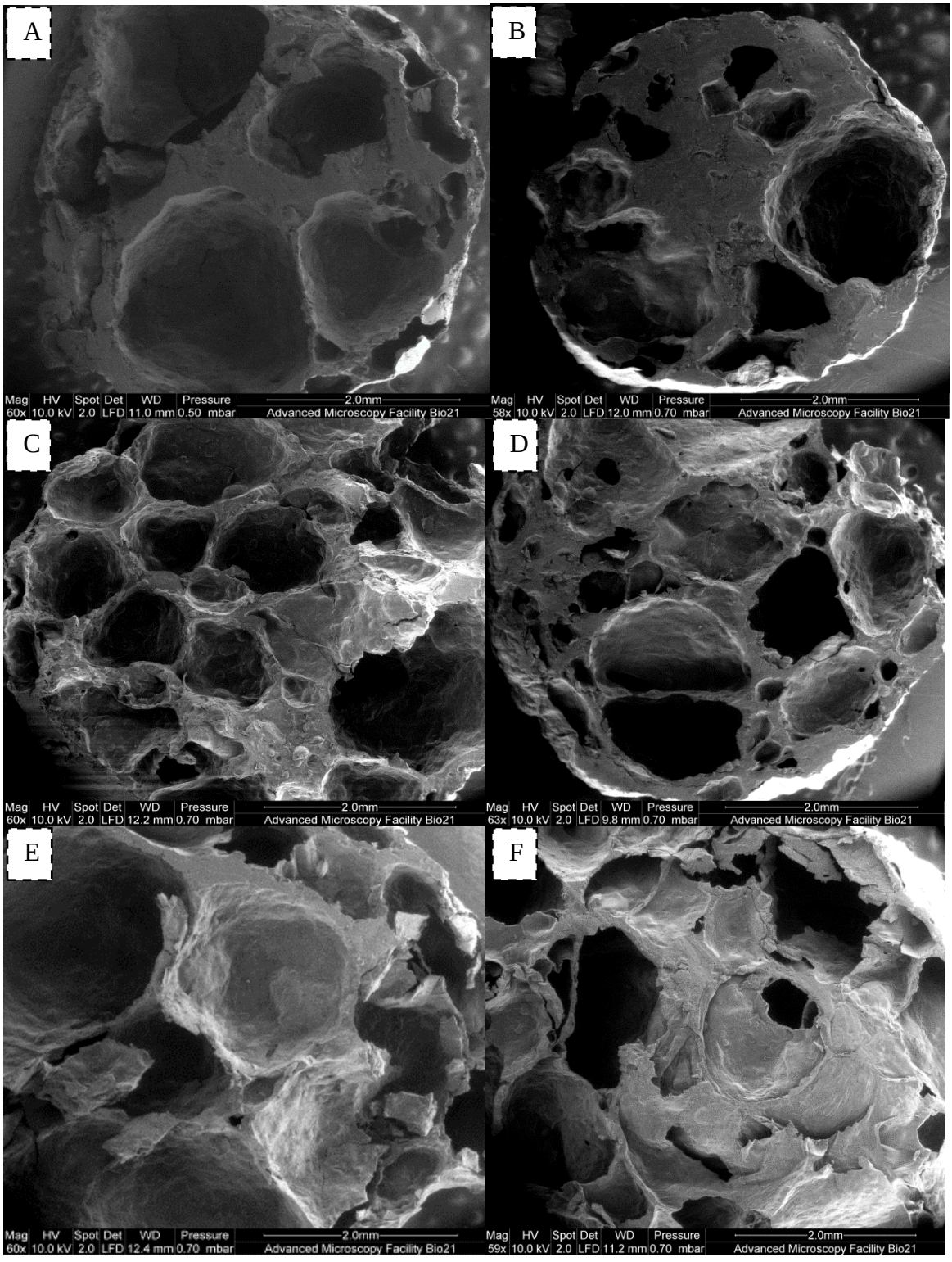


Figure 5.3. Scanning electron microscopy (SEM) images of the cross section of the extrudates containing curcuminoids extruded at (A) 35 % MC-300 rpm, (B) 35 % MC-200

rpm, (C) 28 % MC-300 rpm, (D) 28 % MC-200 rpm, (E) 21 % MC-300 rpm and (F) 21 % MC-200 rpm (MC – moisture content; rpm – revolutions per minute). The scale bars are 2.0 mm in length in A-F figures.

5.3.4 Colour

Colour is an important physical characteristic of extrudates that affect the acceptability of food products by customers. The visual observations of samples (Figure 5.4) showed that extrudates containing curcuminoids have a bright orange colour (lighter colour) when the barrel moisture was reduced without any noticeable colour difference between screw speed. Samples without curcuminoids also showed a similar trend displaying a lighter colour as the barrel moisture decreased from 35 % to 21 %. The lighter colour is due to the enhanced expansion of the samples when extruded at lower barrel moisture (Figure 5.1b). The colour parameters of dry extrudates at different conditions are also shown in Table 5.2. Decreasing in barrel moisture from 35 % to 21 % led to an increase in lightness (L*) for extrudates without curuminoids, while it slightly decreased for that containing curcuminoids. No systematic changes was observed for a* and b* amongst the extrudates with curcuminoids. A similar trend was also observed for extrudates without curcuminoids. The difference between the values of a* and b*, however, was significant for samples containing curcumin and without curcuminoids (Table 5.2).

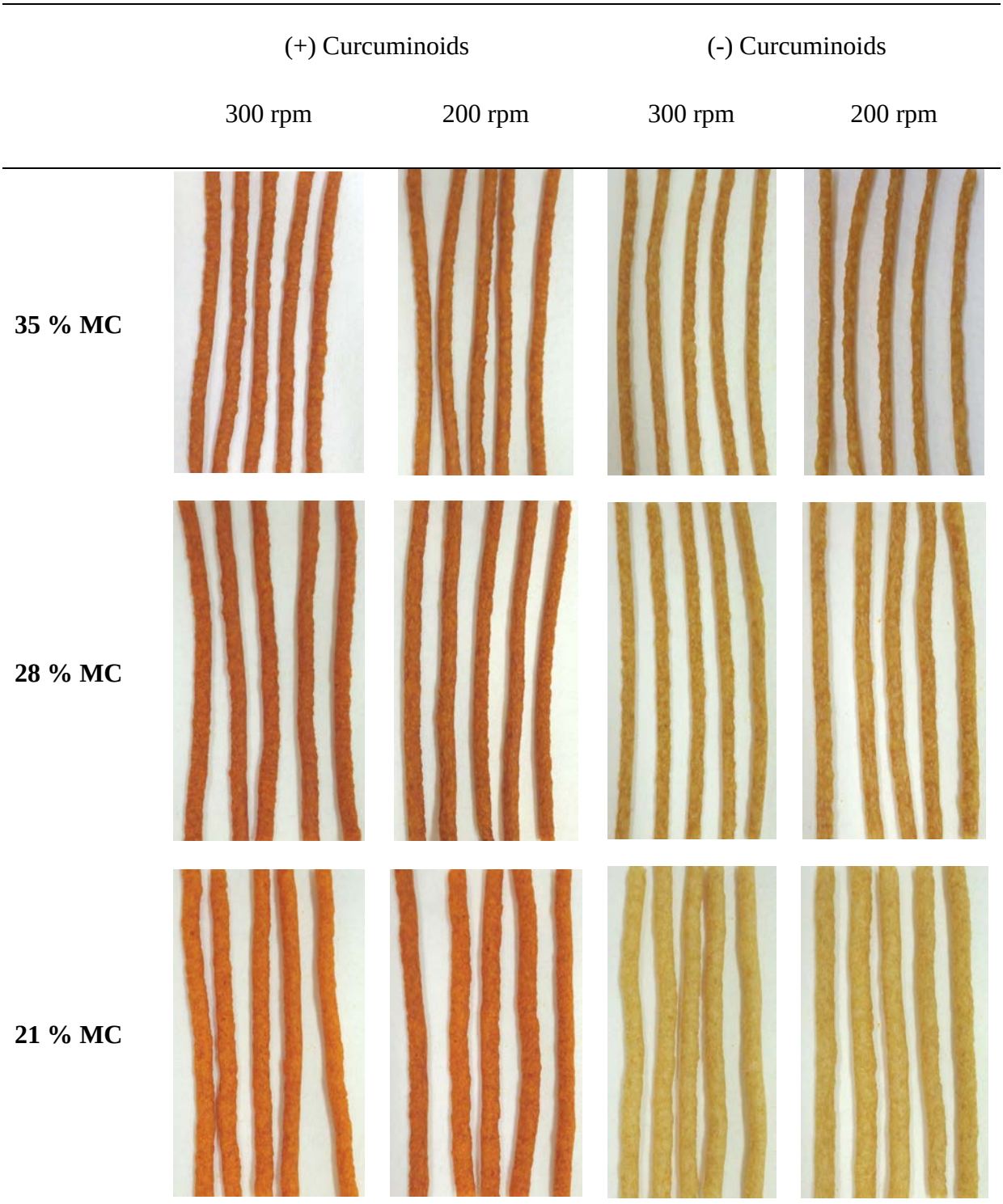


Figure 5.4. Dried extrudates produced at 21 %, 28 % or 35 % moisture content (MC) and 200 or 300 rpm screw speed.

Table 5.2. Colour characteristics of dried extrudates produced at 21 %, 28 % or 35 % moisture content (MC) and 200 or 300 rpm screw speed.

Sample	Extrusion conditions	Colour		
		L*	a*	b*
(-) Curcuminoids	35 % MC-300 rpm	69.7 ± 0.2	4.9 ± 0.0	35.2 ± 1.8
	35 % MC -200 rpm	70.3 ± 0.1	4.2 ± 0.0	34.7 ± 0.5
	28 % MC -300 rpm	67.9 ± 0.2	4.9 ± 0.1	35.1 ± 0.5
	28 % MC -200 rpm	69.8 ± 0.3	4.3 ± 0.3	30.8 ± 1.1
	21 % MC -300 rpm	73.5 ± 2.4	3.2 ± 0.8	34.3 ± 2.2
	21 % MC -200 rpm	73.6 ± 1.4	2.4 ± 0.6	32.1 ± 0.0
(+) curcuminoids	35 % MC -300 rpm	60.5 ± 2.9	14.9 ± 1.4	55.7 ± 2.8
	35 % MC -200 rpm	60.8 ± 1.1	15.1 ± 0.1	55.4 ± 1.1
	28 % MC -300 rpm	58.3 ± 1.5	20.0 ± 0.4	54.7 ± 2.1
	28 % MC -200 rpm	58.4 ± 0.7	17.3 ± 1.4	54.8 ± 1.1
	21 % MC -300 rpm	59.5 ± 0.3	22.8 ± 0.4	57.5 ± 0.9
	21 % MC -200 rpm	56.7 ± 1.4	21.4 ± 0.0	57.1 ± 1.7

5.3.5 Curcuminoids stability

5.3.5.1 Curcuminoids stability during processing

Curcuminoids loss during extrusion, drying and storage was analysed by measuring the curcuminoids content in the extrudates produced for each treatment (Figure 5.5). Curcuminoids loss in extrudates produced at 21 % barrel moisture content was much higher (~ 80 %) than the loss observed during extrusion at 28 % or 35 % moisture content (~ 32 % and ~ 10 %), respectively. However, curcuminoids loss was not affected by screw speed under conditions with the same barrel moisture.

It has been reported that thermally sensitive substances, such as polyphenolic compounds, are stabilized to a greater extent when the moisture content of extruder barrel is increased (Hirth et al., 2015; Ozer et al., 2006). A higher barrel moisture results in a reduction in dough viscosity and SME (i.e. effectively produced at milder extrusion conditions), reducing the destruction of polyphenolic compounds (Leyva-Corral et al., 2016; Ozer et al., 2006). At lower barrel moisture content, the thermal energy that is generated by friction is higher (Hirth et al., 2015). Therefore, the combined effects of lower barrel moisture content, higher SME and higher melt temperature result in greater degradation of phenolic compounds, leading to the reduction in their chemical activity or extractability (Obradovic et al., 2015; Sarawong et al., 2014). One study reported that the retention of cyanidin glycosides improved from 25 % to 65 % when the barrel moisture content increased from 15 g/100 g to 22 g/100 g using a constant barrel temperature of 100 °C and screw speed of 300 rpm (Hirth et al., 2015). Another study reported that a higher feed moisture content (45 %) could prevent anthocyanins from extensive degradation (Khanal et al., 2009).

The data presented here suggest that the conditions with higher barrel moisture content facilitate more interactions between curcuminoids and the oat fibre or corn grit (including protein, fibre, lipids), resulting in the greater protection of curcuminoids against thermal and oxidative degradation. Such interactions were previously observed between curcuminoids and oat fibre (Sayanjali et al., 2014) and may be enhanced under controlled conditions during extrusion.

After extrusion, the extrudates were dried at 50 °C for 4 hours. The trend of curcuminoids loss during drying was inversely related with curcuminoids loss during extrusion where the extrudates which lost more curcuminoids during extrusion, showed the highest retention during drying. The lowest curcuminoids loss during drying was observed for extrudates produced a 21 % barrel moisture content following the extrudates produced at 28 % and 35 % barrel moisture contents.

Drying process, however, has a significant effect on the content of polyphenolic compounds in food products (Chong et al., 2013). For example, it has been reported that 80% of total polyphenolic compounds of blackcurrant pomace degraded when the drying temperature increased from 50°C to 90 °C (Michalska et al., 2017) due to the acceleration of polyphenols oxidation (Skrede et al., 2000). It is therefore expected that curcuminoids are affected by drying processing.

In current study, as drying process was carried out after extrusion which already applied high temperature, therefore the direct analysis of effect of drying on curcuminoids content was not possible. The lowest rate of curcuminoids degradation in 21% feed moisture content extrudates (200 rpm or 300 rpm screw speed) is probably related to the highest degradation of cucuminoids in this condition of extrusion.

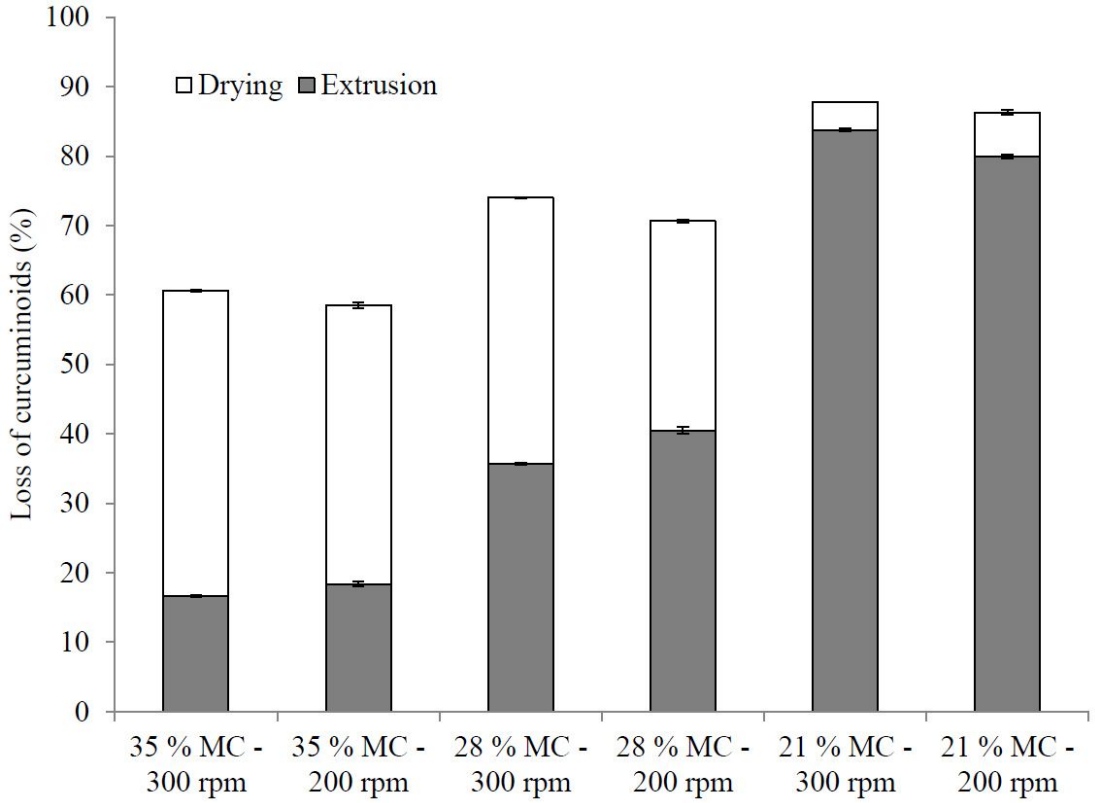


Figure 5.5. Percentage of curcuminoids loss during extrusion processing and drying of extruded products produced under different extrusion conditions (MC – moisture content; rpm – revolutions per minute). The data is the average ± s.d. (n = 4), $p < 0.05$.

5.3.5.2 Curcuminoids stability during storage

Figure 5.6 shows the stability of curcuminoids in dried extrudates stored at 25 °C under natural fluorescent light in open containers for 80 days. The initial amount of curcuminoids was different for each sample at day zero, which corresponds to the amount remaining after extrusion and drying. The residual curcuminoids percentage in samples extruded at 35% feed moisture content was higher than that extruded at 28% and 21% feed moisture content respectively regardless of screw speed. Previously it has been reported that encapsulation of curcuminoids with β- cyclodextrins could not improve curcuminoids stability stored at 25 °C

under natural light for 90 days (Mangolim et al., 2014). However, in our study curcuminoids remained stable in all samples during 80 days of storage at concentration ranging from around 0.10 - 0.4 % of initial curcuminoid concentration in feed materials (Figure 5.6). No significant difference was observed between the percentages of residual curcuminoid in products extruded at constant feed moisture content with different screw speed. The stability of curcuminoids in dried extrudates stored for 80 days can be attributed to the limited molecular mobility and diffusion rates associated with the low moisture content of the dried extrudates (Galmarini et al., 2012).

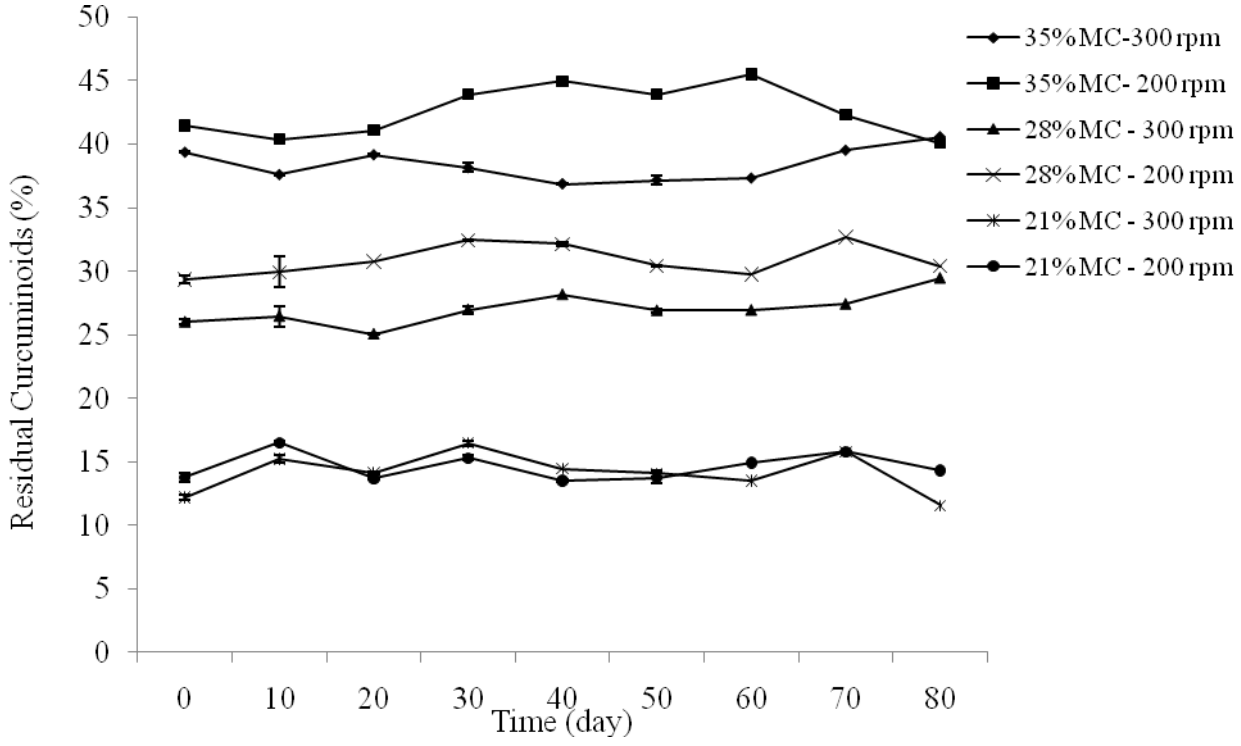


Figure 5.6. Stability of residual curcuminoids in dried extrudates during storage at 25 °C in open container under light for 80 days (MC – moisture content; rpm – revolutions per minute). The data is the average ± s.d. (n = 4), $p < 0.05$.

5.4. Conclusions

This study showed that curcuminoids degradation during extrusion processing is mostly affected by the barrel moisture content and is independent of screw speed. However, the drying process significantly affected curcuminoids degradation especially for extrudates produced with higher barrel moisture content. The maximum curcuminoids loss during extrusion occurred at 21 % barrel moisture content. However, between 15-40 % curcuminoids could be retained after extrusion and drying. Curcuminoids did not degrade in dried extrudates during storage at 25 °C for 80 days under condition of natural light in an ambient atmosphere. The addition of curcuminoids in the formulation at 1.5 % (w/w) did not influence expansion, bulk density and hardness of the extrudates. A barrel moisture content of 21 % resulted in higher expansion and lower hardness and density of extrudates which are favourable properties in extruded products for better consumer acceptance. This study illustrates the potential of using an oat fibre-corn based matrix to produce curcuminoids-enriched extruded products. Further investigations on optimising the drying process after extrusion are needed.

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Chapter 6

Conclusion and recommendations

6.1. Conclusion

Curcuminoids are hydrophobic polyphenols, derived from the rhizome of turmeric plants (Anand et al., 2007), that offer great potential for inclusion in snacks to create fortified extruded food products. Curcuminoids are poorly soluble and unstable in aqueous environments (Mehanny et al., 2016); therefore a carrier material could be an effective way to improve stability and bioactivity. The objective of this thesis was to develop a curcuminoid-enriched oat fibre and corn based product that could be produced by extrusion.

The project was divided into three experimental parts: 1) an investigation of the interaction of curcuminoids with oat fibre ingredients, 2) the evaluation of the effect of extrusion processing on the functional properties of oat fibre and finally 3) an investigation of the physical properties of curcuminoid-enriched oat fibre-corn based extruded products including the study of the stability of curcuminoids during extrusion, drying and storage. These three areas were the basis of three results chapters (Chapters 3-5) of this thesis.

In Chapter 3, the potential of oat fibre to act as a new carrier material for curcuminoids was examined as a potential route to overcome the low aqueous solubility of curcuminoids. The incorporation of the curcuminoids into a mixture with oat fibre ingredients resulted in an increase in the solubility of curcuminoids and protection of curcuminoids against degradation. Curcuminoid solubility increased to 88 μg/ml in the presence of 1 % w/w oat fibre dispersion in 2 % v/v EtoH, in comparison with the solubility of 4.1 μg/mL curcuminoids in 2 % v/v ethanol and 11 ng/mL in aqueous media without ethanol at pH 5.

The interaction between curcuminoids and oat fibre ingredients, which was confirmed by fluorescence spectroscopy analysis, leads to a positive increase in curcuminoid solubility. The fluorescence intensity of curcuminoids in the presence of 1 % w/w oat fibre dispersion in 2 % v/v ethanol was significantly increased. In addition, a blue shift occurred in the maximum emission wavelength of curcuminoids from ~ 566 nm for curcuminoids in 2 % v/v EtOH to 490 nm in 1 % w/w oat fibre dispersions with 2 % EtOH, indicating a change in the polarity of environment of the curcuminoid to a lower polar environment.

Partitioning experiments showed that oat β-glucan and protein are the main components of the oat fibre mixture that interact with the curcuminoids. Of the total protein in the oat fibre dispersion, 38 % partitioned into the supernatant and 61 % of the total β-glucan partitioned into the supernatant, indicating that these two components are mainly responsible for the interaction with the curcumioids and likely increase the solubility of curcuminoids. The combined effects of hydrophobic and hydrogen bonding between curcuminoids and oat protein/oat β-glucan are possibly responsible for the interactions with oat fibre and the increased the solubility of curcumin.

The results of an X-ray powder diffraction experiment, in Chapter 3 indicate that curcuminoids converted from a crystalline form to an amorphous state in the presence of oat fibre. This structural change is beneficial in terms of the bioavailability of curcuminoids following consumption. In addition, the amorphous state of curcuminoids in the precipitate fraction of the 1 % w/w oat fibre dispersion resulted in greater stability of the curcuminoids in the precipitate compared to the supernatant of the 1 % w/w oat fibre dispersion at 25 °C for 11 days. This chapter demonstrates the potential of oat fibre to act as a suitable carrier for curcuminoids in functional foods.

In Chapters 4 and 5, extrusion technology was explored as a method to deliver curcuminoids in an oat fibre based product. Extrusion processing involves high temperatures, high pressures and mechanical forces, resulting in chemical changes to the oat fibre (Lazaridoua, 2004). These changes can modify the physico-chemical properties of oat fibre, potentially improving the functional and physiological properties of oat fibre, although polyphenolic compounds, such as curcuminoids, could be degraded during extrusion and this should be monitored and the conditions selected to optimise retention of bioactivity (Brennan, 2011).

The Focus of chapter 4 was to examine the effect of extrusion technology on the functional properties of oat fibre containing 28 % β-glucan, prior to mixing with curcuminoids. This chapter demonstrates the potential to produce extruded products from commercially available oat fibre high in β-glucan under mild extrusion conditions with high feed moisture, where the product properties are retained and the health benefits are expected to be preserved and potentially enhanced by the high levels of β-glucan incorporated in the extrudates.

Extrusion of oat fibre at a screw speed of 200 rpm or 300 rpm and feed moisture content of 50 % or 60 % (equivalent to a range of SME from 70 to 117 Wh/Kg) did not change the functional properties of oat fibre significantly. The molecular weight of soluble components of oat fibre did not change significantly, this was likely due to the reduced specific mechanical energy employed in this study compared to previous studies (Gutkoski & Eldash, 1999). The total soluble solids content of the extruded oat fibre at all conditions was not significantly different from the soluble solids content of non-extruded oat fibre. As the average molecular weight of soluble fractions of oat fibre did not significantly change after extrusion, this lack of change was expected.

The difference between water absorption index of the samples following extrusion was not significant. Application of more sensitive techniques such as dynamic vapour sorption to measure the water absorption ability of oat fibre, however, showed that a positive correlation existed between the SME applied and the sorption capacity of extruded oat fibre. Increasing the SME from 70 to 117 Wh/Kg resulted in increase in specific surface area and monolayer from 193 m^2/g - 5.33 (g H_2O/100g dry mass) to 241 m^2/g - 6.68 (g H_2O/100g dry mass) respectively, also leading to increase in the sorption energy of monolayer.

Extrusion caused a small decrease in the final viscosity of the extruded oat fibre samples. The ability of extruded oat fibre to form a gel, however, was preserved. This observation showed that the extrusion conditions applied have the potential to produce oat fibre extrudates without a detrimental effect on the visco-elastic properties.

The potential inclusion of curcuminoids into an oat fibre based extruded products has not been examined prior to this work. In addition, the effect of curcuminoids on the physical properties of oat-fibre based snack foods has not been assessed. Therefore in Chapter 5, extruded products made from an oat fibre-corn based matrix fortified with 1.5 % (w/w) curcuminoids were produced in a co-rotating twin-screw extruder. The addition of curcuminoids in the formulation of oat fibre-corn based extruded products did not affect the physical properties of extruded products, indicating that this mass of curcuminoids, which is relevant for a biological effect (Pari et al., 2008), can be readily added into ready-to-eat cereals or snacks without compromising the physical properties of the extrudates.

The bulk density of the extrudates significantly decreased from ~ 1.0 g/cm^3 to ~ 0.2 g/cm^3 by decreasing the barrel moisture content from 35 % to 21 % while the expansion ratio significantly increased from ~ 1.5 g/cm^3 to ~ 2.8 g/cm^3. Curcuminoid addition did not

significantly affect these properties, demonstrating that these conditions can be used to generate snack products with a range of physico-chemical properties by altering the screw speed while the barrel moisture is maintained.

Although the expansion ratio of extrudates increased from ~ 1.5 to ~ 2 by decreasing the barrel moisture content from 35 % to 28 %, the hardness of extrudates was not significantly changed. However, when the barrel moisture content was further reduced to 21 %, the hardness of extrudates was reduced significantly, indicating that an increase in the expansion ratio of extrudates does not necessarily lead to a suitable hardness of extrudates. In addition, no consistent trend was observed for hardness of the different extrudates produced at different screw speeds between 200 and 300 rpm. These results indicate that the barrel moisture content has a greater effect on the physical properties of curcuminoid-oat fibre based extruded products than the screw speed.

Extrudates containing curcuminoids have a bright orange colour (lighter colour) when the barrel moisture was reduced without any noticeable colour difference between screw speed. Decreasing the barrel moisture content from 35 % to 21 % led to an increase in lightness (L*) of extrudates without curcuminoids while it slightly decreased for extrudates containing curcuminoids. Samples without curcuminoids also showed a similar trend displaying a lighter colour as the barrel moisture decreased from 35 % to 21 %. No systematic changes was observed for a* and b* for extrudates either with or without curcuminoids.

Between 10-40 % of initial curcuminoids were retained after extrusion and drying steps. The maximum curcuminoid retention (~ 90 %) during extrusion occurred at 35 % barrel moisture content. The curcuminoids did not degrade in dried extrudates during storage at 25 °C for 80

days under conditions of natural light in an ambient atmosphere, illustrating the potential of using an oat fibre-corn based matrix to produce curcuminoid-enriched extruded products.

This thesis contributes to a better understanding of the behaviour of bioactive curcuminoids when incorporated into oat fibre matrix before and after extrusion processing. These findings show that oat fibre has potential to be a carrier for curcuminoids for use in the fortification of extruded food products. The extrusion conditions used in the current study can be applied to produce extruded products from commercially available oat fibre preparations high in β-glucan. Such extrudates are expected to have similar physico-chemical properties and the health benefits of oat dietary fibre.

6.2. Recommendations

The application of curcuminoids in food and pharmaceutical products is limited due to the low solubility and stability of curcuminoids in aqueous environments at neutral and alkaline pH (Tønnesen, 2002). The solubility and stability of curcuminoids may be increased when they are incorporated in variables carriers such as modified starch (Yu & Huang, 2010), milk proteins (Rahimi Yazdi & Corredig, 2012) and buttermilk (Fu et al., 2014). The bioavailability of curcuminoids may also be increased when formulated in appropriate delivery systems.

This thesis demonstrates the potential of oat fibre components to act as a novel food grade carrier for curcuminoids, in order to enhance both solubility and stability of these polyphenolic compounds. Similar to previous studies (Fu et al., 2015; Yu & Huang, 2010); the simulated digestion model can be used in order to examine the *in vitro* bioaccessibility of curcuminoids delivered together with oat fibre. The simulated digestion model can be designed with the fasted state to simulate a digestion condition before meals and the fed state

to simulate the condition after meals. The stability of curcuminoids after and before simulated gastrointestinal digestion could then be determined by extracting the curcuminoids from digested sample. Such i*n-vitro* experiments could lead to a better understanding of curcuminoid stability and availability for absorption when released from an oat matrix into the gastrointestinal tract (Fernandez-Garcia et al., 2009).

In this study curcuminoids were mostly degraded during extrusion at 21 % barrel moisture, either at a screw speed of 200 rpm or 300 rpm. Yet these conditions give a higher expansion, lower hardness and lower density for the extrudate, which are favourable properties for consumer acceptance. In order to better protect curcuminoids against degradation at lower barrel moisture content, testing of other delivery formats of curcuminoid-oat fibre complexes during extrusion is suggested. For instance, curcuminoid-oat fibre complexes can be delivered to the extruder as dispersion rather than a powder mixture. Furthermore, the application of an emulsifier/stabilizer matrix in the feed material during extrusion can also protect phenolic compounds during extrusion, as has been previously demonstrated with β-carotene in extruded corn-based formulations (Ying et al., 2015). In these studies, β-carotene was retained during extrusion (~90 %) when it was delivered as an oil-in-water emulsion stabilized by a heated protein-carbohydrate matrix, compared to the 70-85 % and 65-80 % retention in oil-in-water emulsion stabilized by Tween 80 or solubilised in oil, respectively (Ying et al., 2015), illustrating the potential for this approach to stabilise curcuminoids.

This thesis provides new insights into oat fibre, another nutritional component in extruded snacks. The changes in the ratio of insoluble and soluble dietary fibre after extrusion may help establish the nutritional value of these extruded products. The provision of the nutritional information for this product will affect the formulation of future oat fibre products and potentially affect consumer choices. It is also suggested that further studies are required

to examine the consumer acceptability of the current curcuminoid-enriched oat fibre-corn based extruded products, to determine the feasibility and acceptability of this new functional product.

Finally, *in-vivo* studies are required to support the methods developed for the production of curcuminoid-enriched oat fibre-corn based extruded products and to confirm the actual bioavailability of curcuminoid. Such studies have been already conducted for curcuminoid as an amorphous solid dispersion in a matrix consisting of hydroxypropyl methyl cellulose (HPMC), lecithin and isomalt using hot melt extrusion (Chuah et al., 2014). Similar approaches could be taken when curcuminoid is incorporated in oat fibre-corn based extruded products; involving the clinical studies of curcuminoid enriched oat fibre-corn based extruded products.

This thesis has shown new steps towards the development of a new functional extruded product. Together, the additional research directions suggested could further improve our understanding, development and evaluation of functional extruded products, assisting the introduction of extruded curcuminoid-oat fibre products into the food industry.

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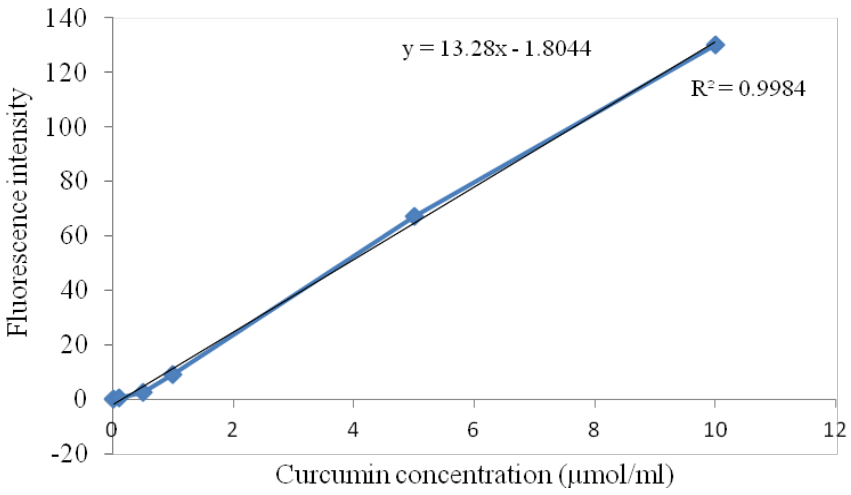
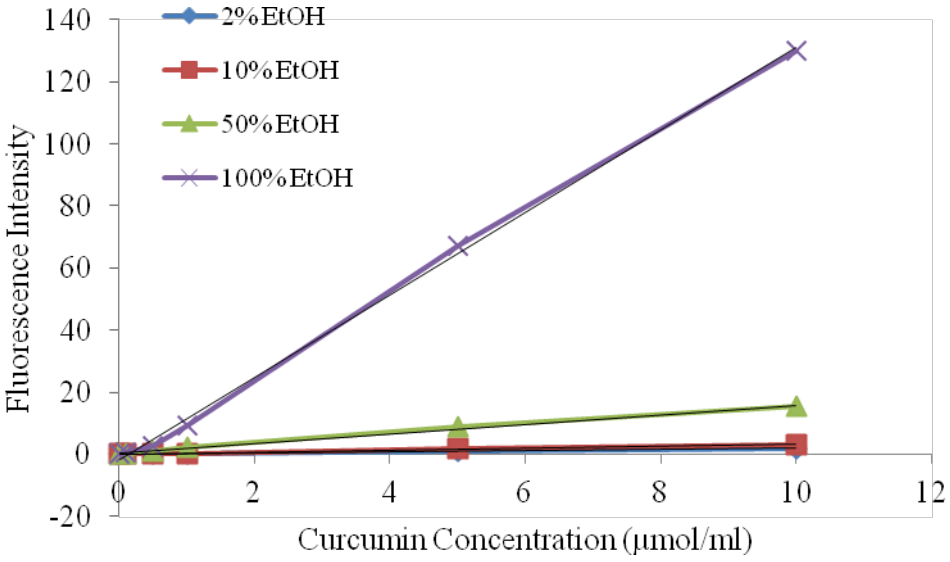
Appendices

Appendix A

The followings are additional Figures and Tables relating to Chapter 3.



Appendix A. Figure 1. Standard curve of curcuminoids in 100 % EtOH



Appendix A. Figure 2. Standard Curve of curcuminoids in different EtOH concentration

Appendix A. Table 1. Summary of previous studies regarding curcuminoid interaction with variables carrier.

Carrier matrix	Bioactive component	Other ingredient	Method	Solubility	Particle size	Other properties	Instrument
hydrophobically modified starch (HMS)	Curcumin 85% purity	chloroform	Dissolving in HMS solution with high speed homogeniser and lyophilisation. Curcumin Concentration in HMS solution: 18.4µg/mL.	Increased by 1670-folds	-	Anticancer (Max 10µg)	Fluorescence spectrophotometer. Synchrotron small-angle X-ray scattering (SAXS). Infrared (IR) spectra of lyophilised curcumin HMS powder and HMS powder alone.
β-cyclodextrin	Curcumin	Ethanol methanol	Dissolving Curcumin/ βcyclodextrin aqueous buffer solution (pH 5) 0.18/1.18g	-	-	Light Stability: 87% retention after 30 days. Storage Stability Highest lost of curcumin: RH>75% Thermal Stability: Yeast Capsulation had the highest heat stability.	Fluorescence microscopy Fourier-transform infrared spectroscopy (FT-IR) Differential scanning calorimetry (DSC)
Modified starch	Curcumin	Tween 80	Dissolving (homogenizer & spray dry) 1gr/20gr in 50 ml deionised water	-	-		
Saccharomyces cerevisiae	Curcumin	Ethanol	Dissolving Mass ratio of Curcumin/Cells (0.2-2)	-	-		
β-lactoglubolin	Curcumin	Methanol	Dissolving	Increased to 625µM	142nm	In Vitro release 16% release under pH:2, 24 h.	Spectrofluorimeter
Camel β-casein	Curcumin	Ethanol Phosphate buffer NaCl	Dissolving (final concentration of curcumin: 370 µmol/L)	Aqueous and micellar solubilities of curcumin were 2.99×10^{-8} and 7.7×10^{-5} molL	-	In vitro cytotoxicity antioxidant activity	Fluorescence spectroscopy UV/Vis spectroscopy
fenugreek soluble dietary fibre	Curcumin 90% purity	hydroxypropylmethyl cellulose glycerine	Dispersion of curcumin with application of ultrasound. 1 g curcumin powder suspended in 100 ml of water containing 0.1% weight of hydroxypropylmethyl cellulose and 10% (w/v) glycerine under sonication.		150nm	In Vitro	Sonicator UV–vis–NIR spectrophotometer Fourier-transformed infrared spectra (FTIR) powder X-ray diffraction (PXRD)

Appendix A. Table 2. Summary of previous studies regarding curcuminoid interaction with variables carrier.

Capsule matrix	Bioactive component	Other ingredient	Method	Solubility	Particle size	Other properties	Instrument
Calcium alginate	Curcumin	Pluronic F127 0.1% (w/v)	Curcumin in nanoparticle formulation	-	100 nm	Cytotoxicity test (500µg/ml nanoparticles is safe for cell study)	Scanning Electron Microscopy Atomic force microscopy (AFM) Fourier transform infrared (FT-IR)
Corn oil	Curcumin	β lactoglubolin as an emulsifier	Emulsion of 0.15wt % curcumin in oil phase	Max amount of curcumin which is soluble:2.98 wt% in SCT	174nm	-	UV–VIS spectrophotometer Dynamic light scattering (measuring particle size)
Methoxy poly ethylene glycol and palmitic acid as the hydrophobic segment	Curcumin		Curcumin solution in methanol was added to the solution of mPEG–PA in chloroform to obtain different drug: (Donsì, 2010)polymer ratios ranging from 1:20 to 1:10		47.36 nm	cytotoxicity studies encapsulated curcumin (IC_{50} = 15.58 µM) inhibited cell proliferation comparably to free curcumin (IC_{50} = 14.32 µM)	Dynamic light scattering (DLS) Atomic force microscopy (AFM) UV–Vis spectrophotometer
Maltodextrin (MD)	Curcumin 85%purity	-	Preparation of curcumin suspension (1% w/w) at MD solution in accordance with High Pressure homogenization (HPH) (150Mpas, 25°C) and finally encapsulated with spray drying.	After 10 cycles of HPH the increasing rate of the curcumin concentration reached a constant (5mg/L)	600nm	-	Photon correlation spectroscopy (PCS) UV/vis spectrophotometer Atomic Force Microscopy (AFM). Differential Scanning Calorimetry (DSC) X-ray Diffraction (XRD)
Chitosan	Curcumin 85%purity	Tween 80 0-0.05w/v% Acetic Acid	spray drying of 50mg curcumin in 1% acetic acid and chitosan (0.05w/v %) dispersion	Curcumin saturation point increases with an upper limit of 294 µM curcumin with 0.05 w/v% Tween 20. (12.7-fold increase).	385nm		Scanning Electron Microscopy Confocal microscope

Appendix B

The followings are additional Figures and Tables relating to Chapter 4.

Appendix B. Table 1. Moisture content of oat fibre extrudates

Sample	**Moisture content (%)**		
	Dry Feed	Exit extruder	Dried samples
High moisture - High screw speed	5.21 ± 0.12	47.23 ± 0.29	10.29 ± 1.82
High moisture - Low screw speed	5.21 ± 0.12	47.00 ± 1.45	5.62 ± 0.86
Low moisture - High screw speed	5.21 ± 0.12	36.30 ± 0.18	8.47 ± 2.46
Low moisture - Low screw speed	5.21 ± 0.12	37.50 ± 0.23	8.39 ± 0.78

Appendix B. Table 2. Compositions of different extruded oat fibre

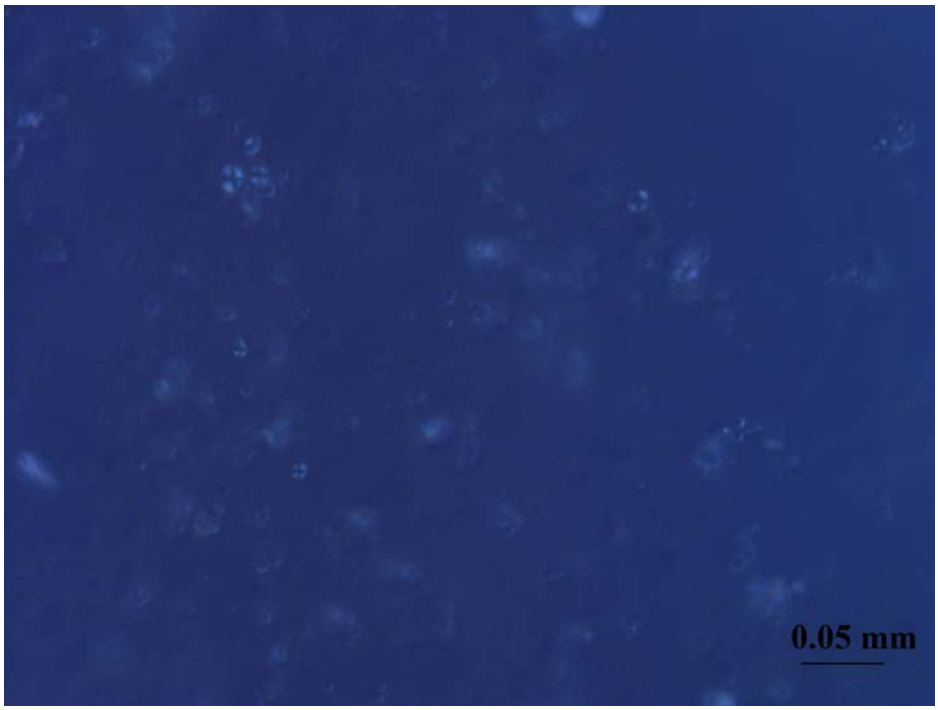
Sample	**Moisture content (%)**	**Protein content (%)**	**B-glucan content (%)**
Non-extruded oat fibre	6.60 ± 0.36	21.89 ±0.02	27.54 ± 1.12
60 % MC-300 rpm	12.26 ± 0.67	20.10 ± 0.0.3	27.34 ± 0.19
60 % MC- 200 rpm	8.47 ± 0.027	20.71 ±0.14	24.87 ± 0.96
50 % MC- 300 rpm	9.59 ± 0.89	20.71 ±0.05	25.55 ± 2.66
50 % MC-200 rpm	10.49 ± 0.29	20.87 ± 0.16	26.97 ± 0.73

(MC – moisture content; rpm – revolutions per minute)

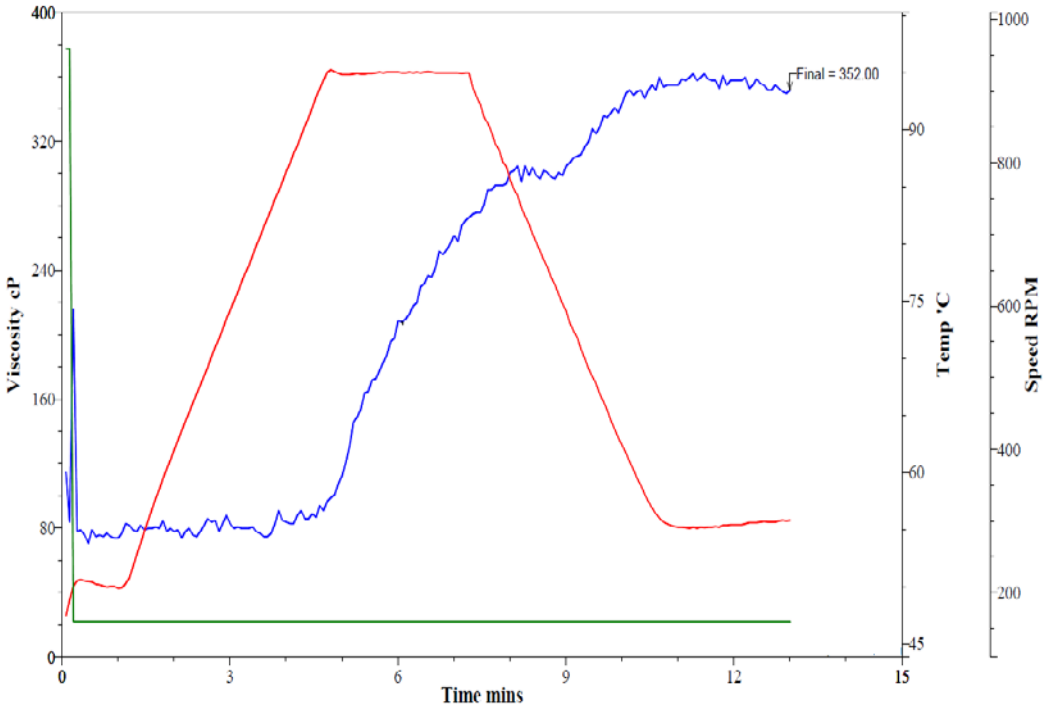
Appendix B. Table 3. curcuminoid concentration (μg/mL) in the supernatant of 1% w/w oat fibre- 220 μg/ml curcuminoids dispersion.

Samples	Fraction	Weight (g)	Total solid (g/100 g of dispersion)	Protein (g/100 g)	β-glucan (g/100g)	Curcuminoid (%)
Dispersion of 1% w/w non-extruded oat fibre-220 μg/ml curcuminoids	Supernatant	26.2 ± 0.17	0.22 ± 0.027	0.0076 ± 0.00	0.11 ± 0.012	23.02
	Pellet	5.50 ± 0.13	0.86 ± 0.034	0.23 ± 0.029	0.17 ± 0.00	63.57
Dispersion of 1 % w/w extruded oat fibre - 220 μg/mlCurcumin (50% MC-300 rpm)	Supernatant	26.36 ± 0.02	0.27 ± 0.03	0.0052 ± 0.00	0.12 ± 0.00	21.03
	Pellet	5.14 ± 0.09	0.78 ± 0.08	0.21 ± 0.10	0.17 ± 0.035	65.56
Dispersion of 1 % w/ extruded oat fibre- 220 μg/mlCurcumin (50% MC- 200 rpm)	Supernatant	26.40 ± 0.16	0.19 ± 0.11	0.003 ± 0.00	0.11 ± 0.026	23.27
	Pellet	5.09 ± 0.20	0.80 ± 0.04	0.23 ± 0.03	0.18 ± 0.015	63.32
Dispersion of 1% w/w extruded oat fibre- 220 μg/mlCurcumin (40% MC- 300 rpm)	Supernatant	25.76 ± 0.23	0.17 ±0.08	0.002 ± 0.00	0.11 ± 0.00	24.65
	Pellet	5.35 ± 0.70	0.83 ± 0.18	0.20 ± 0.03	0.15 ± 0.00	61.95
Dispersion of 1% w/w extruded oat fibre- 220 μg/mlCurcumin (40%- 200 rpm)	Supernatant	26.46 ± 0.51	0.16 ± 0.15	0.0045 ± 0.00	0.10 ± 0.00	27.31
	Pellet	6.22 ± 0.85	0.88 ± 0.05	0.20 ± 0.02	0.19 ± 0.00	59.3

(MC- Moisture content, rpm –revolution per minute)



Appendix B. Figure1. Birefringence characteristic of starch granules in 1% w/w oat fibre dispersion



Appendix B. Figure2. Viscosity profile of extracted starch from oat fibre.

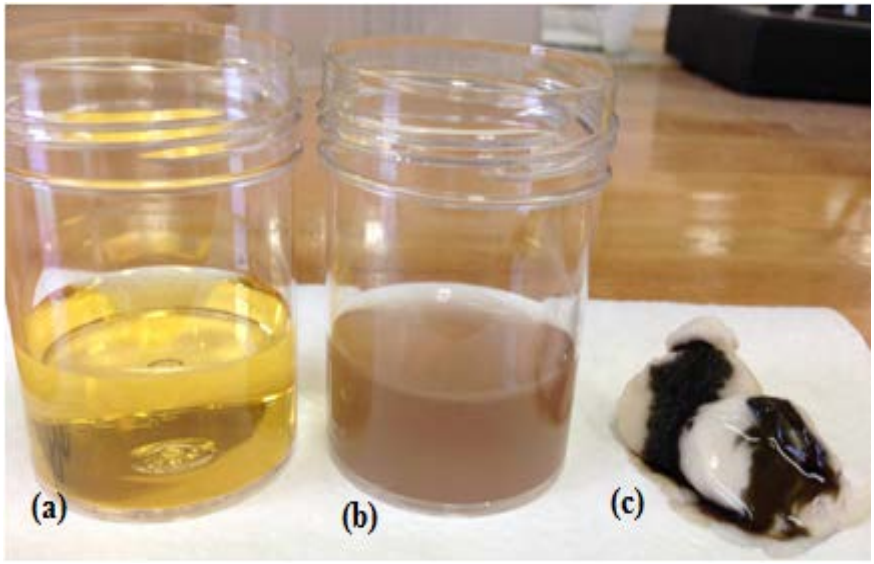
Appendix C

The followings are additional Figures and Tables relating to Chapter 5.

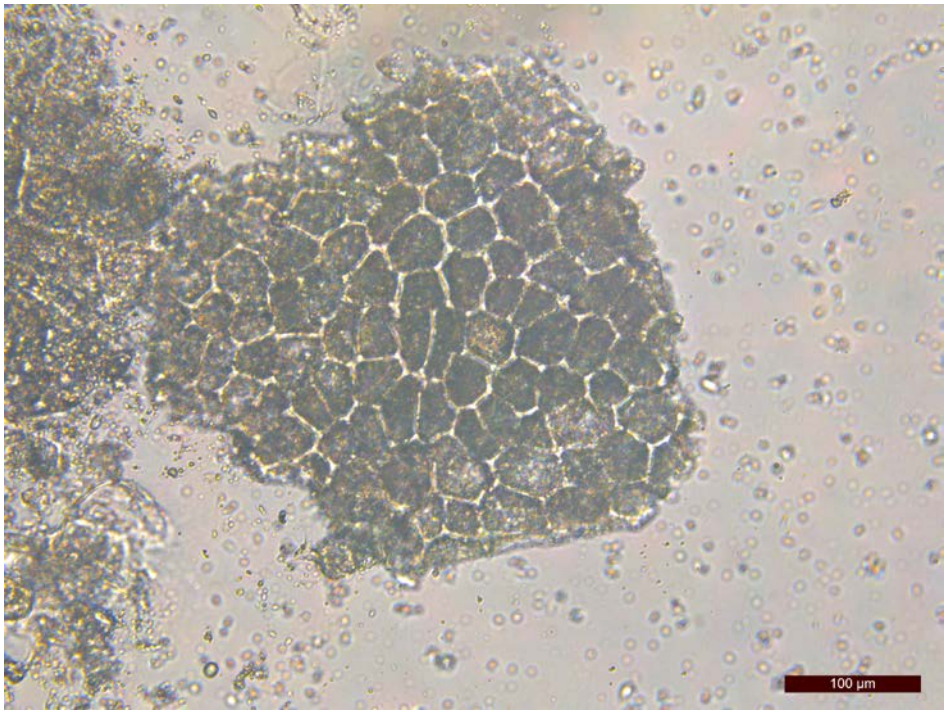
Appendix C. Table 1. Moisture content of curcuminoids-enriched oat fibre-corn extrudates at different stages of extrusion.

Extruded Samples	Moisture content (%)		
	Dry Feed	**Exit extruder**	**Dried samples**
35 % MC-300 rpm	10.43 ± 0.05	27.81 ± 0.51	7.33 ± 0.16
35 % MC- 200 rpm	10.43 ± 0.05	27.46 ± 0.95	7.63 ± 0.63
28 % MC- 300 rpm	10.43 ± 0.05	22.23 ± 0.58	7.38 ± 0.20
28 % MC-200 rpm	10.43 ± 0.05	21.32 ± 1.33	7.10 ± 1.06
21 % MC-200 rpm	10.43 ± 0.05	12.16 ± 0.5	5.88 ± 0.37
21 % MC-200 rpm	10.43 ± 0.05	14.14 ± 0.42	5.81 ± 0.39

(MC – moisture content; rpm – revolutions per minute)



Appendix C. Figure 1. One drop of 0.2 % (v/v) iodine solution was added to (a) water (b) supernatant of 1% (w/w) oat fibre dispersion (c) Precipitate of 1% oat fibre dispersion.



Appendix C. Figure 2. Microscopic picture of oat fibre captured by polarized microscopy.

Author/s:
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Title:
The incorporation of Curcuminoids in oat fibre extruded products

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2016

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