COMPOSITIONAL ANALYSIS OF LOCALLY CULTIVATED CAROB (*Ceratonia siliqua*) CULTIVARS AND DEVELOPMENT OF NUTRITIONAL FOOD PRODUCTS FOR A RANGE OF MARKET SECTORS

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Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the owner of the copyright thereof (unless to the extent explicitly otherwise stated) and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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ABSTRACT

Carob (*Ceratonia silliqua*) is an evergreen, drought resistant tree of Mediterranean origin. Popularly known as St John's bread, the carob pod has a long history of use in food (over 4 000 years). Carob has a good nutritional value, a long shelf-life (2-3 years) and it is relatively cheap. Due to its high sugar content, carob is naturally sweet. It also has a nutty chocolate-like flavour, but unlike chocolate or cocoa, carob does not contain any caffeine, thiobromine or oxalic acid. In addition, carob is normally regarded as a healthy food because of its low fat content (0.2 – 2.3%). Carob trees are also found in South Africa, especially in the Western Cape Province. Locally, carob trees have been used mainly ornamentally or as a source of animal fodder, with minimal use of the pods as a nutritious food source. Knowledge of the nutritional composition and the overall nutritional potential of locally (South African) grown carob cultivars is also limited. Carob could potentially be used as an alternative food source in South Africa as currently, most of this nutritious product goes to waste each year.

In this study, the feasibility of using carob pods as an alternative source of food in South Africa was investigated. This was done by firstly, analysing the cultivars for proximate composition (moisture, carbohydrates, sugars, dietary fibre, protein, polyphenols, fat and ash) as well as for amino acids, fatty acids and minerals, in order to determine and compare their nutritional contents. Five cultivars (Tylliria, SFax, Aaronsohn, Santa Fe and an "Unknown" cultivar) were examined. The average proximate composition of raw carob pods was 8.17 – 9.56% moisture, 89.57 – 91.12% carbohydrates, 40.69 – 54.74% total sugars (33.70 – 45.09% sucrose, 1.79 – 4.95% glucose and 1.80 – 5.19% fructose), 29.88 – 36.07% dietary fibre, 3.07 – 4.42% protein, 2.58 – 3.08% polyphenols, 0.45 – 0.86% fat and 2.13 – 2.69% ash. Seven essential amino acids were present in all the cultivars, except for methionine which was not detected in the Single unknown cultivar. This study has shown that all the cultivars had good long-chain fatty acid (LCFA) proportions in terms of the saturated to polyunsaturated fatty acid (SFA: PUFA) and *n*-6 to *n*-3 ratios. The short-chain fatty acid content of the cultivars was low. All nine minerals (calcium, phosphorus, potassium, magnesium, sodium, manganese, iron, copper and zinc) analysed for in this study were detected in all five carob cultivars and all cultivars were very low in sodium.

The impact of various roasting times (45, 60 and 75 min) at 150ºC, on the temperature sensitive components such as sugars, protein and fat, was also examined. Roasting had no significant (P>0.05) effect on the fat content. Although roasting

significantly (P<0.05) reduced the sugar and protein content from 54.74 to 32.53% and 3.59 to 3.18%, respectively, levels in both raw and roasted carob still represented a potentially nutritious food source and alternative to cocoa.

A variety of food products targeted at the various food market sectors were developed with carob as an ingredient. The formulations for five new food products (bread, porridge, breakfast cereal, mousse and milk-based drink) were developed where carob had successfully been incorporated as an ingredient. Microbiological and consumer sensory analyses carried out showed that all products developed were safe and acceptable. The findings of this study provide useful scientific evidence towards the fact that carob could potentially be used as an alternative food source in South Africa.

UITTREKSEL

Karob (*Ceratonia siliqua*) is ’n immergroen, droogte bestande boom van Mediterreense oorsprong. Die karob peul, algemeen bekend as Johannesbrood, het ’n lang geskiedenis van gebruik in voedsel (meer as 4 000 jaar). Karob het ’n goeie voedingswaarde, lang raklewe (2-3 jaar) en is relatief goedkoop. Karob is van nature soet as gevolg van die hoë suikerinhoud. Karob het ook ’n neutagtige sjokolade smaak, maar anders as sjokolade of kakao bevat karob geen kaffeïene, teobromien of oksaalsuur nie. Verder word karob gewoonlik beskou as ’n gesonde voedsel as gevolg van die lae vetinhoud (0.2 – 2.3%). Karob bome kom ook in Suid-Afrika voor, veral in the Wes-Kaap. Plaaslik word karob bome gewoonlik gebruik as versiering of as bron van veevoer met minimale gebruik van die peule as voedselbron. Kennis van die voedingswaarde en die algehele voedings-potensiaal van plaaslik (Suid-Afrikaanse) verboude karob kultivars is beperk. Potensieël kan karob dien as ’n alternatiewe voedselbron in Suid-Afrika aangesien die meeste van hierdie voedsame produk jaarliks tot niet gaan.

Tydens hierdie studie is die moontlikheid ondersoek om karob peule as ’n alternatiewe voedselbron in Suid-Afrika te gebruik. Dit is gedoen deur eerstens die kultivars se proksimale samestelling (vog, koolhidrate, suikers, dieetvesel, proteiene, polifenole, vet en as) so wel as aminosure, vetsure en minerale te analiseer, om sodoende hul voedingsinhoud te bepaal en te vergelyk. Vyf kultivars (Tylliria, Sfax, Aaronsohn, Santa Fe en ’n onbekende kultivar) is ondersoek. Die gemiddelde proksimale samestelling van rou karob peule was 8.17 – 9.56% vog, 89.57 – 91.12% koolhidrate, 40.69 – 54.74% totale suikers (33.70 – 45.09% sukrose, 1.79 – 4.95% glukose en 1.80 – 5.19% fruktose), 29.88 – 36.07% dieetvesel, 3.07 – 4.42% proteïene, 2.58 – 3.08% polifenole, 0.45 – 0.86% vet en 2.13 – 2.69% as. Sewe essensiële aminosure het in al die kultivars voorgekom, behalwe metionien wat nie in die enkele onbekende kultivar gevind is nie. Hierdie studie het gewys dat al die kultivars goeie lang-ketting vetsuur (LKVS) verhoudings in terme van versadigde tot poli-onversadigde vetsure (VVS:POVS) en n-6 tot n-3 verhoudings getoon het. Die kort-ketting vetsuurinhoud van die kultivars was laag. Al nege minerale (kalsium, fosfor, kalium, magnesium, natrium, mangaan, yster, koper en sink) waarvoor tydens hierdie studie getoets is, is in al vyf karob kultivars gevind en al die kultivars was laag in natrium.

Die impak van verskeie roostertye (45, 60 en 75 min) teen 150°C op die hitte sensitiewe komponente soos suikers, proteïene and vet is ook ondersoek. Daar was geen beduidende (P>0.05) effek op vet inhoud nie, maar die suiker en proteïeninhoud is wel

beduidend (P<0.05) verlaag van 54.74 tot 32.53% en 3.59 tot 3.18%, onderskeidelik. Vlakke in beide rou en geroosterde karob het steeds ŉ potensieël voedsame voedselbron en alternatief tot kakao verteenwoordig.

ŉ Verskeidenheid voedselprodukte gerig op verskeie marksektors is met karob as bestanddeel ontwikkel. Die formulasies vir vyf nuwe voedselprodukte (brood, pap, ontbytgraan, mousse en ŉ melk-basis drankie) waarin karob suksesvol as bestanddeel ingesluit is, is ontwikkel. Mikrobiologiese en sensoriese verbruikerstoetse van die produkte het getoon dat alle ontwikkelde produkte veilig en aanvaarbaar is. Die bevindinge van hierdie studie lewer handige wetenskaplike bewyse dat karob potensieël as alternatiewe voedsebron in Suid-Afrika gebruik kan word.

To my family

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CONTENTS

Language and style used in this thesis are in accordance with the requirements of the *International Journal of Food Science and Technology.* This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has, therefore, been unavoidable.

CHAPTER 1

INTRODUCTION

South Africa, like most developing countries, is characterised by a large population combined with a poor distribution of food (Monde, 2003; Tsubo *et al.*, 2003). The income of most households in rural areas falls well-below the poverty line. In some highly populated, poorly developed areas, a considerable number of people (especially children), suffer from malnutrition because of too little food and/or an imbalanced diet (Monde, 2003). Energy deficiency and micro-nutrient deficiency are the major concerns in this regard. For instance, it is estimated that some 2.3 million people are undernourished (diets not sufficient to meet daily energy requirements) and therefore need food aid (Naidoo *et al*., 1992). Generally, malnutrition contributes to child mortality, impaired scholastic ability and low productivity in adults (Jones, 1998). Efforts should, therefore, be exerted to reduce malnutrition.

In most rural areas of South Africa, as a result of the dry conditions, it is difficult to cultivate crops to produce food for human consumption (Tsubo *et al.,* 2005). The lack of rain during summer makes it difficult and uneconomical to cultivate most summer crops as large volumes of water are needed for irrigation (Van Zyl & Kirsten, 1992). Similarly, the seasonal droughts experienced during winter also limit production, while a relatively large area is needed to produce enough grain to sustain a family. This is especially the case in the North-Western part of the country. The use of alternative crops that are well adapted to the winter rainfall climatic conditions of the Western Cape should be considered as food sources to ensure a minimum level of food security in poor households. It is, therefore, important that a crop be used that could tap underground water while also producing a substantial amount of utilizable products for direct or indirect human consumption. One such a crop is the carob tree (*Ceratonia Siliqua*) found in Mediterranean countries such as Spain and Syria.

The ancient Greeks took carob from the Mediterranean region to Greece and Italy (Batlle & Tous, 1997; Zografakis & Dasenakis, 2000). The Arabs distributed it further to Israel, Jordan, Egypt, Tunisia, Libya, Morocco, Algeria, Spain, Portugal and France (Batlle & Tous, 1997). The current world production of carob extracts is estimated at 315 000 tonnes per year, with Spain being the main producer and exporter (42%), followed by Italy (16%), Portugal (10%), Morocco (8%), Greece (7%), Cyprus and Turkey (5%) (Biner *et al.,* 2007).

Even though it is more abundant in Mediterranean areas owing to its adaptability to harsh conditions such as drought, barren, rocky, dry or generally poor soils, the species (*Ceratonia siliqua*) is well distributed in many parts of the world, especially in areas that have climates similar to the Mediterranean climate (Batlle & Tous, 1997; Yousif & Alghzawi, 2000). This includes South Africa, especially the Western Cape Province, where carob trees are mostly planted as ornamental trees in public parks, gardens, parking areas and the side walks (Muller, 2005).

The carob tree is also a xerophyte in nature, making it well adapted to low rainfall areas (Rizzo *et al.*, 2004). Once budded, carob trees require minimal attention and have a relatively long life-span of up to 150 years (Marakis, 1996). The trees produce a large amount of pods (up to 800 kg per harvest, once peak production is attained) that have a good nutritional value for both human and animal consumption (Marakis, 1996).

For many centuries, carob pods have been used in many countries for both human and animal nutrition. The use of carob pods in food dates back to ancient times, where the pods are reported to have been consumed in raw form (Brandt, 2002; Haber, 2002; Owen *et al.*, 2003). In modern society, carob pods are ground into a nutritious powder which is incorporated as an ingredient into a variety of food products such as confectioneries, beverages, sweet bars and ice creams (Binder *et al.,* 1958; Collins, 1978; Bravo *et al.*, 1994).

Carob pods are naturally sweet since they contain as much as 60% sugar (mainly sucrose) and have substantial amounts of protein, up to 7.6% (Zografakis & Dasenakis, 2000; Owen *et al.*, 2003; Biner *et al.*, 2007). Carob is also high in dietary fibre (as high as 39.8%) and polyphenols (up to 20.0%) as well as containing some minerals such as calcium, phosphorous and potassium (Makris & Kefalas, 2004; Shawakfeh & Erefej, 2005; USDA, 2006). Once roasted (normally at 150°C), carob pods can be ground into a powder which imparts sensory characteristics (flavour and colour) similar to those of cocoa powder, but unlike cocoa, carob does not contain either caffeine, thiobromine (stimulants) or oxalic acid (toxic when consumed in large amounts) (Cantalejo, 1997; Marakis, 1996; Biner *et al*., 2007). Carob also has a much lower fat and sodium content making it a healthy food source (Bravo *et al.,* 1994; Petit & Pinilla, 1995; Makris & Kefalas, 2004). Carob could, therefore, also be used as a cocoa replacer or extender (Fadel *et al.,* 2006; Yousif & Alghzawi, 2000).

Presently, carob's application in the food industry is mainly focused on the extraction of carob bean gum (locust bean gum). This is added to a variety of products as a thickener, stabiliser or flavourant (Curtis & Race, 1998; Bouzouita *et al.*, 2006). The use

of the deseeded pod in food is, however, minimal and thus carob's economical market value is low. However, in the health food market, carob has been well established (Marakis, 1992; Avallone *et al.*, 1997).

It is rather disappointing to note that most people, even in places where carob trees are abundant such as the Western Cape Province, are unaware of its nutritional potential. Consequently, most of this highly nutritious product (carob pods) goes to waste every year. This happens in the midst of a struggle to feed the population, especially in the low-income rural communities. Efforts should, therefore, be exerted towards promoting the use of carob pods as a valuable food source, especially to local inhabitants, community upliftment organisations and governments.

Active food research and new product development could contribute greatly to the promotion of carob as a food source and hence towards its commercial value. One of the possibilities is the incorporation of carob powder into various foods (Dakia *et al.,* 2007). Additionally, the possibility of using carob as an ingredient in a variety of low technology food products that could be targeted at the low-income community market is worth exploring. This would diversify and promote carob's applications in human nutrition and its direct consumption by inhabitants in areas where it grows. Promotion of carob as a food source could contribute towards efforts aimed at addressing national nutritional problems and also directly serve as a food source for the low income rural communities. The pods could also be milled into a nutritious powder for use as a raw material for the food industry.

Generally, knowledge about the currently existing carob cultivars is still limiting, but it is believed that different cultivars may vary in terms of composition and thus in their nutrition potential. Knowledge about nutritional potential of the various cultivars would aid researchers in improving pod and seed yield. Similarly, this would enable sound and informed selection of specific cultivars for use in food.

For the roasting of carob, high temperature/long time combinations are normally applied. The possible impacts of roasting on nutritionally important, temperature sensitive components (for example protein, sugar and fat) must therefore not be overlooked. It is important that the roasting conditions be optimised so that the protein, sugar and fat contents in the final product will not be severely affected, while still producing organoleptically desirable products.

The main objective of this study was to investigate the feasibility of using carob pods as an alternative nutritious source of food. This was done by firstly, determining the composition of various South African grown carob cultivars in order to compare their nutritional contents. Secondly, the effect of roasting time (at 150°C) on temperature

sensitive components such as protein, fat and sugars was also be evaluated. Thirdly, a variety of new food products targeted at various food market sectors were developed using carob as a main ingredient. Consumer sensory studies on the products developed were carried out to determine consumer acceptability and degree of liking.

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CHAPTER 2

LITERATURE REVIEW

A. NUTRITIONAL STATUS OF SOUTH AFRICAN RURAL COMMUNITIES

A drastic growth of the global human population is a worldwide concern, and so is the consequent need for food. Humanity relies on a diverse range of cultivated and wild crops. A wide-range of edible, nutritious crops are grown each year but disappointingly, only a small portion of such produce reaches the end of the food supply chain (Crews & Peoples, 2004).

Food security refers to accessibility of adequate and nutritious diets by individuals and/or households in order to maintain a decent livelihood (Van Zyl & Kirsten, 1992). Food security is a very important concept in Southern Africa. This is mainly because these countries are periodically faced with droughts. A large proportion of the population in many developing countries is faced with poverty and malnutrition, and South Africa is no exception (Bayley, 1995; Walker *et al*., 1996). Sen (1981) clearly stated that there is a direct relationship between poverty and malnutrition. In developing countries, most foods consumed are generally deficient of many essential nutrients (Dashak *et al.,* 2001). Dashak *et al.* (2001) further emphasised that some of the food, however, is a good source of various nutrients, but these foods are not consumed in sufficient amounts.

Governments in Southern Africa are giving priority to nutrition by committing themselves to improving food security for their people as a basic objective (McLachlan & Kuzwayo, 1996). In an attempt to fight food insecurity, many governments have adopted various food policies. South Africa in particular, has opted for the policy of self-sufficiency (Monde, 2003). This policy was criticised by Van Zyl & Kirsten (1992), who argued that the policy was only concerned with food security at national level, rather than at household or even at individual level. As a result, poverty and malnutrition still prevails among South Africans in spite of a large increase in food exports (Van Zyl & Kirsten, 1992; Monde, 2003).

In recent years, South Africa's food production has constantly been increasing in line with population growth, but this is not reflective of the overall nutritional status of the population (Van Zyl & Kirsten, 1992). This is mainly because nutritional well-being of the population is also affected by factors other than the country's food production. Such factors may include, but are not limited to, geographical distribution of food, and food

prices (Duncan, 1998). Monde (2003) stressed that diets of many households (including those with a stable monthly income), may still be heavily deficient due to either ignorance or inadequate knowledge of human nutrition.

Good nutrition is a human right, but unfortunately, many people are deprived of this right. In South Africa specifically, poverty is viewed to be one of the major reasons for food insecurity in many households (McLachlan & Van Twisk, 1995; Van Zyl & Kirsten, 1992). Duncan (1998) further stressed that poverty prevents households from accessing food. This is well illustrated and supported by findings of various nutrition related research studies conducted in recent years. In the study on "Household food security in rural areas in Central Eastern Cape", Monde (2003) reported that 75 to 90% of households in areas of rural South Africa fall below the poverty line and therefore, many people are malnourished. The study also found that the majority of households in South Africa's rural areas obtained their food by purchasing from the markets rather than producing their own. Furthermore, many households (59.4%) belong to the ultra-poor poverty class and therefore rely on state (government) handouts alone, for survival (Monde, 2003).

In South Africa, various nutritional related studies have been done in recent years. A survey conducted in the Bloemfontein district on the nutritional status of pre-school age children showed that 19% of the study subjects were underweight, 18% were stunted and 7% were wasted (Van Rooyen & Sigwele, 1998). This study also indicated that most of the children had insufficient (less than 67% recommended daily allowance) intake of essential nutrients. Additionally, it was found that a large number (more than 80%) of rural households are failing to obtain the minimum nutritional requirements (Monde, 2003).

In the literature, it was also reported that among the various nutrient deficiencies, energy-deficiency and micronutrient (iron, calcium, vitamins A, B_6, B_{12} and C, folate, niacin, riboflavin) deficiencies could be highlighted (Vorster *et al*., 1995; Jooste *et al.*, 1996; Van Rooyen & Sigwele, 1998). Steyn *et al.* (1996) reported folate deficiency among eleven year-old children in the Western Cape. Folate deficiency has also been reported among black women (Ubbink *et al.,* 1999). Labadarios (1996) reported the prevalence of vitamin B_{12} deficiency among 6 – 71 month old black children in the rural areas of South Africa.

Malnutrition contributes to a high level of mortality in children, lowers scholastic ability in school going children, and lowers productivity in adults and therefore, efforts must be made to address it (Walker *et al.*, 1996). Duncan (1998) listed a number of recommendations to governments, households and individuals, concerning malnutrition and the fight to reach a sustainable food security level. To achieve food security, mainstream development strategies with strongly defined anti-poverty objectives must be

put in place. The eradication or reduction of poverty will lead to affordable food and hence household accessibility to food. Promotion of nutritional education in rural areas would enable individuals to make correct food choices (Duncan, 1998). McLachlan & Van Twisk (1995) suggested that the food industry also has a role to play in the fight against malnutrition and in improving food security in general. The food industry can contribute by developing cheap, basic (but relatively nutritious) food products, and in making such products accessible.

Another approach towards improving the nutritional situation in developing countries is by teaching the communities how they could put what is available in their localities to good (and sustainable) use. For South Africa, a good opportunity could be the use of carob pods. Carob (*Ceratonia siliqua*) of Mediterranean origin, has been used as a food source for more than 4 000 years in many countries (Batlle & Tous, 1997). Carob trees grow abundantly (wild and cultivated) in South Africa more especially in the Western Cape Province but disappointingly, these trees produce a highly nutritious food source (carob pods) which goes to waste each year (Muller, 2005). Low-income groups, especially in rural communities could collect carob pods and consume them raw as a cheap, but highly nutritious food source. Alternatively, the pods could be collected and sold to a community-based processing plant. Earnings from sales of these pods and the overall profit from the plant may then be used to purchase other food commodities and thus to improve the nutritional status of low-income groups. Muller (2005) clearly pointed out that even though some studies have been carried out on carob's potential in animal nutrition, no studies in South Africa have been done with regard to human nutrition.

B. BACKGROUND ON CAROB

Origin and geographical distribution

The carob tree (*Ceratonia siliqua*) has been grown and cultivated since ancient times (Batlle & Tous, 1997; Zografakis & Dasenakis, 2000). Even though there seems to be a lack of clarity on the exact origin of this evergreen, pod-bearing tree, many researchers have suggested that the species may have originated in eastern Mediterranean countries such as Syria (Marakis, 1996; Batlle & Tous, 1997; Yousif & Alghzawi, 2000; Shawakfeh & Erefej, 2005). In Israel, early archaeo-botanical findings (charred wood and seeds) have provided tangible evidence that the carob tree existed in eastern Mediterranean long before 4 000 B.C. (Zografakis & Dasenakis, 2000). The species is believed to have been introduced to Greece and Italy by the ancient Greeks (Batlle & Tous, 1996; Zografakis &

Dasenakis, 2000). Arabs spread it south along the North African coasts such as Jordan, Egypt, Tunisia, Libya, Morocco and Algeria and northwards into Spain and then eventually, Portugal and France.

Today, the species (*Ceratonia siliqua*) may be found in many parts of the world, especially ones with a climate similar to the Mediterranean climate, including South Africa, USA and Australia. However, the species is more widely distributed in the Mediterranean basin (Batlle & Tous, 1997; Yousif & Alghzawi, 2000). This vast distribution can be the result of the carob tree's adaptability to harsher environmental conditions such as drought, barren, rocky, dry or generally poor soils (Marakis, 1996).

Carob as a nutritious product might have received little research and development attention in the past, but it has recently attracted a lot of interest as an alternative use in reforestation and coastal agricultural development, especially in tropical and subtropical regions (Zografakis & Dasenakis, 2000; Biner *et al.*, 2007). Furthermore, recent interests are based on the nutritional potential of the pods and a host of industrial and agricultural uses associated with carob. This is further made evident by the recent distribution into countries such as South Africa and Australia (Batlle & Tous, 1996).

Yousif & Alghzawi (2000) reported the increase in carob plantations over recent years. Global carob production was estimated at 310 000 tons per year in 2002, with Spain being the leading carob producer, with an average of 135 tons per year (Zografakis & Dasenakis, 2000). The main producers and exporters of carob are thus, Spain (42%), Italy (16%), Portugal (10%), Morocco (8%), Greece (7%), Cyprus (6%) and Turkey (5%) (Biner *et al.*, 2007).

Biodiversity and botanical information

Nomenclature and taxonomy – The species *Ceratonia siliqua* belongs to the subfamily *Caesalpinioideae* of the *Leguminosae* family (Baumgartner *et al*., 1986; Biner *et al.*, 2007). Until some 25 years ago, the genus *Ceratonia* was believed to consist of only one species, *Ceratonia siliqua.* Batlle & Tous (1997) and Marakis (1996) described another species closely related to *Ceratonia siliqua*. The species, *Ceratonia oreothauma*, consists of two subspecies: *C. oreothauma*, which is native to Arabia; and *C. somalensis* from Somalia. Marakis (1996) also suggested that *Ceratonia oreothauma* might be the wild ancestor of the carob cultivars being cultivated today.

In different places and languages, carob is known by different names. In Table 1 some of the names by which carob is known, according to their respective places or

Table 1 List of names by which carob is known (Zografakis & Dasenakis, 2000)

Language	Name
English	Carob / locust bean
Hebrew	Kharuv
Arabic	Kharrub/ algarrobo
Spanish	Garrofero
Italy	Carrubo
French	Caroubier
German	Karubenbaum
Portuguese	Alfarrobeira
Greek	Charaoupi
Turkish	Charnup
Chinese	Chiao-tou-shu
Thailand	Chum het tai

languages are listed. Due to their consistence in size and weight, jewellers have used carob seeds as a gauge to measure the “carat” value of diamonds, hence the name carob (Batlle & Tous, 1997). Other names commonly used for carob pods are “St John’s bread” and “locust beans” (Kumazawa *et al*., 2002).

Agronomy and ecology – Carob is an evergreen tree with pinnately compound leaves. The carob is a drought resistant, perennial and long-producing tree (100 – 150 years) and often grows to between 6 and 12 m. In some instances, trees may grow to more than 20 m, especially when environmental conditions are favourable (Kumazawa *et al.*, 2002; Owen *et al.*, 2003). The tree in its wild form, can take up to 6 – 8 years before bearing pods, but improved, cultivated varieties only take 3 – 4 years after budding (Marakis, 1996). The length of the non-bearing period is also determined by environmental as well as horticultural factors, i.e. the better the conditions the shorter the non-bearing period (Zografakis & Dasenakis, 2000). Peak production is mostly reached by the age of 20 – 25 years (Batlle & Tous, 1997). Reportedly, first-time bearing can yield between 4 – 5 kg per tree, whereas a yield of up to 800 kg per tree may be attained at mature age (Marakis, 1996). These traits may vary, depending on horticultural conditions as well as farming practices such as the budding and grafting techniques applied, and differences between varieties (Zografakis & Dasenakis, 2000; Owen *et al.,* 2003).

The carob tree is said to grow very well under tropical warm temperature ranges (30° – 45°C) but is it also tolerant to the hot and humid coastal areas with hot summer winds (Zografakis & Dasenakis, 2000). They have also reported that the carob tree is able to withstand low temperatures of up to minus 6°C. Even though carob’s optimum growth temperature requirements are similar to that of many tropical fruit trees such as orange trees, carob can survive in poor soils and its water needs are much lower (Zografakis & Dasenakis, 2000). Since the tree has a deep tap root system (up to 20 m), carob production is possible even in areas with just 250 mm rainfall per year (Curtis & Race, 1998). Carob orchards hardly require any irrigation, fertilisers or annual pruning unlike many tropical fruit trees, but irrigation, fertiliser addition and annual pruning will improve the yield (Battle & Tous, 1996).

This species (*Ceratonia siliqua*) is trioecious, meaning that male trees, female trees and trees that bear both male and female flowers (hermaphrodites) exist within the species (Marakis, 1996). Economically speaking, growing more male (rather than hermaphrodites) carob trees as pollinators is of greater advantage since their hermaphrodite counter parts tend to have lower yields (Batlle & Tous, 1997). In addition, even though hermaphrodite

trees have the advantage of a longer flowering period than male trees, they are also more susceptible to disease, particularly a fungal disease well known as “oidium” (Curtis & Race, 1998).

The flowers of the carob tree are green tinted-red. Carob trees are the only Mediterranean trees with a main flowering season similar to that of tropical plants (September to November). Moreover, the exact time and length of flowering differs between geographic locations as flowering is strongly dependant on the local climatic conditions (Zografakis & Dasenakis, 2000). In orchards, the main pollinating agents are wind and insects (Curtis & Race, 1998). Curtis & Race (1998) also mentioned that inadequate pollination is mostly to blame for the low yields experienced in many carob orchards. Strategic horticultural practices should, therefore, be employed in order to facilitate and promote pollination, if good yields are to be achieved.

The carob pod is dark brown in colour and can have an elongated, compact, straight, curved, or twisted shape depending on the specific variety (Zografakis & Dasenakis, 2000). The length (up to 25 cm), weight (5 – 30 g), thickness (up to 1.3 cm) and width (up to 4 cm) varies somewhat between varieties, growth conditions and agricultural techniques applied during cultivation (grafting and budding) (Marakis, 1992).

The deseeded pod is made up of an inner softer layer known as the “mesocarp” and an outer leathery layer called the “pericarp”. The seeds in the pod are found embedded in the mesocarp region and arranged in transverse positions (Zografakis & Dasenakis, 2000). The seed contribution to the pod mass is about 5 to 40% (Calixto & Cańellas, 1982). For pod processing purposes, good quality pods are preferably ones with a low seed percentage contribution to total pod mass (Blenford, 1988; Marakis, 1992; Petit & Pinilla, 1995; Markis & Kefalas, 2004). External measurements of the pods give an indirect indication of the quality (i.e. the thicker the pod, the higher the pod to seed ratio and hence the better the pod quality) (Yousif & Alghzawi, 2000). A longitudinally opened carob pod with an exposed interior, showing the arrangement of the seeds, is illustrated in Fig. 1.

Economic importance

Evergreen beauty – The carob tree is an important component of the Mediterranean vegetation and its adaptability to both mild and dry areas makes it even more important both environmentally and economically (Zografakis & Dasenakis, 2000). Because of their shade-giving evergreen beauty, carob trees are useful in orchards, parks or in home back

Figure 1 Carob pods showing the arrangement of seeds in the pod.

yards (Zografakis & Dasenakis, 2000). In Spain, carob trees are normally grown close to villages for the “green beauty” as well as to provide a barrier to wind and field fires (Curtis & Race, 1998).

Other uses – Carob additionally has a range of industrial uses in pharmaceuticals, cosmetics, textiles, mining, explosives, chemicals, stationeries and carpentry building materials (Calixto & Cańellas, 1982; Albanell *et al.,* 1993; Battle & Tous, 1997; Zografakis & Dasenakis, 2000).

C. USE OF CAROB AS A FOOD SOURCE

General characteristics

Carob can be considered as a cheap food source (Makris & Kefalas, 2004). It has been reported that carob pods have a high sugar content (over 50%), making them a naturally sweet food (Biner *et al*., 2007). The protein content ranges between 1.0 and 7.6%, with a dietary fibre content of up to 40% (Marakis, 1996; USDA, 2006). Carob also contains a host of vitamins, minerals, and substantial amounts of up to 20% polyphenolic compounds (Binder *et al.*, 1958; Batlle & Tous, 1997; Makris & Kefalas, 2004; USDA, 2006). The nutritional value of carob pods is comparable to that of common cereal grains such as wheat and barley (Batlle & Tous, 1997).

Even though its use in food is less known in most parts of the world, carob is by no means a newly discovered food. As indicated by Brandt (2002), the use of carob in food dates back to ancient times even as far as 4 000 B.C. Marakis (1996) reported that many low-income groups in the world (for example in Greece) have consumed baked carob beans and an aqueous carob extract as part of their diets for hundreds of years. As stated by Owen *et al.* (2003), the carob pod has because of its high sugar content historically been collected and consumed as a food product. This was especially true in ancient times when it was used as a candy for children as well as in national emergencies such as war and famine (Berna *et al.*, 1997). Another of the earliest references to the use of carob as food in ancient times is the biblical story of John the Baptist, who is said to have survived in the desert on the carob pods for 40 days, and hence carob is often referred to as St. John’s bread (Batlle & Tous, 1997; Brandt, 2002; Haber, 2002). In the 1880s, the cavalries of British General Wellington in Spain and General Allenby’s soldiers in Palestine during World War I are reported to have fed on carob pods (Haber, 2002).

In modern communities carob pods are used in various processed food products such as confectioneries, as a flavourant, substitute or extender for cocoa in a variety of processed food products (Marakis, 1996; Biner *et al.*, 2007). Yousif & Alghzawi (2000) and Biner *et al.* (2007) clearly pointed out that this preference for carob over cocoa can mainly be attributed to the fact that carob powder contains no caffeine, thiobromine (stimulant) or oxalic acid, and has a very low fat content (maximum 2.3%). Oxalic acid, when consumed in large amounts can be toxic to humans. When the oxalic acid comes into contact with human tissue, it reacts with calcium to form calcium oxalate, which forms part of a kidney stone. This would eventually obstruct kidney tubules (urinary passage) and thus, causing kidney damage (Maroni *et al.,* 2005; Guo & McMartin, 2007). Carob pods have higher levels of dietary fibre when compared to cocoa (Yousif & Alghzawi, 2000). It is thus assumed that carob is much healthier than cocoa (Blenford, 1988). For individuals who are sensitive to caffeine, thiobromine or oxalic acid, or those who are simply health conscious and prefer to rather consume foods with a low fat content but still want to enjoy a nutty chocolate-like flavour, carob is an excellent alternative. A variety of food products with added carob ranging from confectioneries and sweets bars to ice creams and beverages are known today (Collins, 1978; Yousif & Alghzawi, 2000; Biner *et al.*, 2007).

Furthermore, because of its high sugar content (up to 89%) of which up to 70% is sucrose, carob as compared to cocoa (20-fold lower in sugar), reduces the need for sweeteners in some food products (Yousif & Alghzawi, 2000; Kumazawa *et al.*, 2002; Owen *et al.*, 2003; USDA, 2006). In fact, some authors describe carob as a sweetener with a flavour and appearance similar to that of chocolate (Yousif & Alghzawi, 2000). Zografakis & Dasenakis (2000) stated that carob syrup, obtained by extracting carob pods with water is one of the popular drinks in countries like Egypt. Curtis & Race (1998) speculated that carob syrup might have greater applications in the food processing industry than carob powder. This was mainly because the syrup can be incorporated into processed products more effectively than powders due to their low solubilities.

Primarily because of health-linked perceptions about carob, most of the carob-containing foods are found in health shops and are mainly targeted to the middle and higher income groups (Blenford, 1988). Carob is a cheap and generally available food source and, therefore, it can be considered as potentially a nutritious food source than cheaper, more common food products. Carob can also be targeted for use by the low-income groups (Muller, 2005).

Good examples of potential carob products are flavoured milks similar to chocolate-

flavoured milk, which has shown a fast growing market trend. Other examples are carob-based beverages, including instant powder mixes (Collins, 1978; Lang, 1982; Blenford, 1988; Yousif & Alghzawi, 2000; Biner *et al.*, 2007; USDA, 2006). Carob flavoured milk-based beverages such as "Good One" and "Moove" which were launched in Australia during the 1980's, as well as "Naturally Caroby^{TM} " launched by a company called Clover Leaf Creamery in Minneapolis showed good market performance, especially among the youth (Anon., 1979; Lang, 1982). Further examples are high fibre products mainly because of its high fibre content, for example in bakery and confectionery products (Wang *et al*., 2002; Gruendel *et al*., 2006; USDA, 2006). The possibility of using carob as a food source in a "South African context" has not been researched (Muller, 2005). Thus, exploration of new and modified food products with carob added as an ingredient, could facilitate opportunities to local food industries and communities at large, especially with regard to the use of carob in food.

One of the major attributes to the economic value of the carob is the fact that carob can be considered a high value cash crop, mainly due to the high industrial demands for its seeds from where gum is extracted (Albanell *et al.,* 1993; Biner *et al.*, 2007). This gum is commonly known as "carob bean gum" or "locust bean gum" (Calixto & Cańellas, 1982; Marakis, 1992; Battle & Tous, 1997; Bouzouita *et al*., 2006). The carob gum is obtained by grinding up the endosperm of carob seeds (Lazaridou *et al.,* 2001; Rizzo, 2004; Goncalves & Romano, 2005). This gum product exhibits good water-binding properties and thus it is used as a stabiliser, thickener and emulsifier in a variety of food products (Marakis, 1996).

Use in animal nutrition

For centuries (since 4 000 B.C.), mainly because of the high sugar content, carob pods have been used as animal fodder (Würsch *et al.*, 1984; Batlle & Tous, 1997). When fed to animals in feeding trials, carob pods have been shown to give results similar to those reported for barley in terms of the increase in animal body mass per amount of feed consumed per day (Marakis, 1996). This can be taken as an indication that carob pods have comparable nutritional values to some of the traditional animal feed sources like barley. Furthermore, being adaptable to dry and semi-arid conditions the carob tree is therefore, highly recommended for use as feed supplement for animal farming in drought stricken regions (Batlle & Tous, 1997). Cattle, horses, goats and sheep have also been reported to feed on the lower leaves and branches of the carob tree (Marakis, 1996). In

addition, carob gum is commonly used as a flavouring and emulsifier in a range of pet foods (Curtis & Race, 1998).

D. NUTRITIONAL VALUE OF CAROB

The chemical composition of the carob pod has been well studied (Binder, 1958; Calixto & Cańellas, 1982; Bravo *et al.*, 1994; Avallone *et al*., 1997; Yousif & Alghzawi, 2000). The chemical composition is known to vary mainly as a result of cultivation and environmental conditions, cultivar influences (genetic make-up), the origin and harvesting time. These variations in results caused data from researchers to notably differ (Zografakis & Dasenakis, 2000). The major chemical constituents of the carob pod are moisture, ash, carbohydrates, individual sugar constituents, proteins and amino acids, fat and fatty acids, minerals, vitamins, polyphenols and dietary fibre (soluble and insoluble). The data in Table 2 show the ranges of the major chemical constituents of carob pods (Calixto & Cańellas, 1982; Marakis, 1996; Avallone *et al*., 1997; Yousif & Alghzawi, 2000; USDA, 2006).

***Moisture* –** The moisture content of any material refers to the amount of water per unit mass. Water is an important substance necessary for all forms of life (Ihekoronye & Ngoddy, 1985). This includes foods of both plant and animal origin. However, water is an inexpensive filler, and therefore its removal from dry food materials increases convenience during packaging and transportation (Nielsen, 2003). The moisture content of raw food materials has a remarkable effect on the operational properties and thus on the choice of determination technique and equipment to be used during processing, as well as on the keeping quality of both raw materials and final products (Charley, 1982; Nielsen, 2003). This is because the rate of both biochemical reactions and microbiological degradations responsible for food spoilage are more rapid when the moisture content of the substrate is high (Duckworth, 1974). A high moisture content would also increase the chance of infestation by pests (Curtis & Race, 1998).

The major challenge in moisture removal from food products is the possibility for destruction of other important food components. For example, at elevated temperatures, the amino acid and sugar contents may be reduced (probably because of caramelisation and the Millard reaction during roasting), proteins may be denatured, and vitamins and volatile components might be lost (Nielsen, 2003). Another challenge is the uniformity of environmental factors such as climatic conditions at harvest, the moisture content of carob

Table 2 Proximate composition of carob pods (Calixto & Cańellas, 1982; Marakis, 1996; Avallone *et al*., 1997; Batlle & Tous, 1997; Yousif & Alghzawi, 2000; USDA, 2006)

Chemical constituent	**Concentration (g.100 g^{-1})**
Moisture	3.6 – 18.0
Ash	1.0 – 6.0
Fat	0.2 – 2.3
Protein	1.0 – 7.6
Carbohydrates	48.0 – 88.9
Total sugars	32.0 – 60.0
Dietary fibre	2.6 – 39.8
Polyphenols	0.5 – 20.0

pods is also dependent on factors such as the ripening status and relative atmospheric moisture removal from the materials. Care must, therefore, be taken when selecting a method for drying or roasting of a foodstuff. Beside species differences and other environmental factors such as climatic conditions at harvest, the moisture content of carob pods is also dependent on factors such as the ripening status and relative atmospheric humidity (Binder *et al*., 1958; Batlle & Tous, 1997). When still in a fresh state, the pods generally have a moisture content between 10 and 20% (Batlle & Tous, 1997). Batlle & Tous (1997) and Curtis & Race (1998) suggested that drying to a moisture content below 10% would be necessary to avoid rotting of the pods during storage prior to processing.

Determination of the nutritional value of foods requires that the moisture content must be known (Nielsen, 2003). According to the literature, the moisture content in carob pods ranges between 3.6 – 18.0 g.100 g^{-1} (Würsch *et al*., 1984; Battle & Tous, 1997; Marakis, 1996).

Ash and minerals – Ash refers to the inorganic residue remaining after either the ignition or complete oxidation of the organic matter in a foodstuff and gives an indication of the total mineral content in foods (Nielsen, 2003). Some minerals (for example calcium and phosphorus) form part of the human skeleton and if the body is not provided with adequate amount of such minerals, some physical malfunctions or deformations can be observed (Taylor & Pye, 1974). Other minerals have functions in the regulation of metabolic and circulatory systems, among other biological functions in the body. More than 100 µg of the macro minerals (Ca, P, Na, K, Mg, Cl and S) are required daily, while less than 10 µg quantities of the trace mineral nutrients (Fe, I, Zn, Cu, Cr, Mn, Fr, Se and Si) are required in the human diet daily (Nielsen, 1994).

The carob ash content normally ranges between 1 and 6 g.100 g^{-1} (Calixto & Cańellas, 1982; Bravo *et al.,* 1994; Avallone *et al*., 1997; Yousif & Alghzawi, 2000). In carob pods researchers have detected K, Na, P, B, Co, Mn, Fe, Cu, Zn, S, N, Cl, Mg, Ca and P. However, the content of each element are known to vary (Binder *et al*., 1958; Calixto & Cańellas, 1982; Petit & Pinilla, 1995; Shawakfeh & Erefej, 2005). Typical mineral components of carob are listed in Table 3 (Binder *et al*., 1958; Calixto & Cańellas, 1982; Bravo *et al.,* 1994; Yousif & Alghwiza, 2000; Batlle & Tous, 1997).

Ash determination, just like any other nutritional compositional analysis, requires critical care if accurate but reliable results are to be achieved. There are two types of ashing which may be used depending on what is intended to be determined namely: dry ashing and wet ashing (Nielsen, 2003). Dry ashing is normally used to determine

Table 3 Mineral constituents in carob pods (Binder *et al*., 1958; Calixto & Cańellas, 1982; Petit & Pinilla, 1995; Batlle & Tous, 1997; Shawakfeh & Erefej, 2005)

Mineral constituent	**Concentration (g.100 g^{-1})**
Potassium	0.60 – 0.86
Calcium	0.09 – 0.35
Magnesium	0.01 – 0.05
Sodium	0.04 – 0.08
Copper	0.01 – 0.03
Iron	0.03 – 0.29
Manganese	7.60 – 15.20
Zinc	0.05 – 0.14
Phosphorus	6.91 – 7.90
Sulphur	0.08 – 0.24
Cobalt	0.00 – 0.01
Nitrogen	4.60 – 7.40
Boron	0.01 – 0.02

proximate composition whereas wet ashing may be useful when analysis for specific minerals is required. Wet ashing methods are usually followed by other element detection techniques such mass spectrometric procedures, if the mineral profile of the food sample must be determined.

***Carbohydrates* –** Carbohydrates are the major constituents of many foods and are the major source of energy in the human diet. With one exception (lactose from milk), all carbohydrates are of plant origin (Nielsen, 2003). Clinical recommendations suggest that carbohydrates should account for more than 70% of the energy source of the human diet (Nielsen, 2003). In most carbohydrate-rich foods such as cereal grains as opposed to carob, carbohydrates are available in the form of polysaccharides, most of which (except for starch) are not digestible in the human gastro-intestinal tract due to the absence of the necessary digestive enzymes (Taylor & Pye, 1974).

The carob pod can serve as a rich source of carbohydrates. The carbohydrate content can be as high as 89 g.100 g^{-1}, depending on variety, climate and other horticultural conditions (Biner *et al.*, 2007; USDA, 2006). According to Biner *et al.* (2007) and Kumazawa *et al.* (2002), sugars contained in the pods are almost entirely sucrose, fructose and glucose. Of these three, sucrose accounts for up to 70%, with glucose and fructose sharing the remaining percentage in equal proportions (Zografakis & Dasenakis, 2000). Values of up to 95% sucrose (on total sugar basis) have also been reported (Bravo *et al.*, 1994). This identifies carob pods, as a good sucrose source, even as an alternative to sugar beet and sugar cane. It is worth noting that the use of carob pulp in food, more specifically in confectioneries and other sweet-tasting products, is mainly due to the high sugar (sucrose) content (Bravo *et al.*, 1994; Biner *et al.*, 2007). The ratios of individual sugars to the total sugar content are generally similar between cultivated and wild carob types (Bravo *et al.*, 1994).

It is generally agreed by nutritional chemists that the determination of carbohydrates should be carried out by way of the calculation (difference) method, i.e. by subtracting the mass of crude protein, total fat, moisture and ash from the mass of the test sample (Biner *et al.*, 2007). Most of the methods for carbohydrate determination are based on chromatographic techniques whilst commercial test kits for determining individual sugars are also available (Boehringer, 1992; Avallone *et al.,* 1997; Mecozzi, 1999; Biner *et al.*, 2007). Many researchers have undertaken studies regarding carob's individual sugar composition and profile determination (Shawakefh, 2005; Biner *et al.*, 2007).

Proteins and amino acids – Proteins are the building blocks of all, living cells and almost all except for storage proteins, are important for the normal biological functioning of the cell (Nielsen, 1994). Protein can also serve as an energy source in the human diet. Dietary requirements of protein are determined by factors such as body size, age, gender and physical activities. On average, a human adult daily requires 0.6 g protein per kilogram of ideal body weight (Carpenter & Calloway, 1981). Amino acids are building units of proteins and can be placed in two major groups namely essential and non-essential amino acids (Ihekoronye & Ngoddy, 1985; Erasmus, 2001). The human body has a capability to synthesise the non-essential amino acids but not others and those must, therefore, be obtained from the diet (Erasmus, 2001).

Carob's protein content ranges between 1.0 and 7.6 g.100 g^{-1} depending on cultivar differences, origin and farming practices (Calixto & Cańellas, 1982; Owen *et al.*, 2003). Batlle & Tous (1997) reported that seven amino acids were found in carob pods namely; alanine, glycine, leucine, proline, valine, tyrosine and phenylalanine. These included three of the essential amino acids - leucine, glycine and valine (Cooper *et al.,* 2000).

Several methods for the determination of the protein content of foods are known. Nielsen (2003) reported that the principles of all the techniques are based on measuring the nitrogen content, the amount of other protein components (such as peptides and amino acids), ultraviolet absorptivity, dye-binding capacity or light scattering properties (Nielsen, 2003). However, the choice of the test method to be used depends on factors such as method sensitivity, accuracy, precision, speed, the cost involved as well as the type of material being studied (Nielsen, 2003). For carob, methods based on the determination of the nitrogen content are most often applied. In this method, the percentage nitrogen concentration is then multiplied by a standard factor (6.25 for carob and other fruits) to correlate it to the corresponding protein content (Calixto & Canellas, 1982).

Fat – The terms "fats, oils and lipids" are sometimes used interchangeably. Nielsen (2003) defined lipids as a group of substances which are soluble in ether, chloroform or other organic solvents but which do not dissolve in water (Nielsen, 1994; Enujuigha & Ayodele-Oni, 2003). The term "fat" refers to a group of triacyglycerols esters, which are solid at room temperature, whereas "oils" are liquid triacylglycerols at room temperature (Penfield & Campbell, 1990). To be more specific, fat molecules are esters of fatty acids and glycerol (Nielsen, 1994).

Together with carbohydrates and proteins, lipids are the main components of foods (Nielsen, 2003). Lipids are an important source of energy and fat-soluble vitamins (A, D, E and K) in the diet (Nielsen, 2003). Similarly to some amino acids, some fatty acids (basic constituents of lipids) such as linoleic acid, are essential (the human body is not able to synthesise them) in the human diet (Carpenter & Calloway, 1981). The amount of fat in food products also affect the nutritional status, the keeping quality as well as other functional properties of various foods (Charley, 1982). In addition, fats (more especially the unsaturated fats) are generally associated with many health concerns such as cardiovascular diseases and obesity (Taylor & Pye, 1974). Fat determination in food products and raw materials is, therefore, important. In food product development, fat determination becomes vital, mainly for nutritional labelling purposes and for monitoring possible nutrient losses during certain processing steps.

Nutritional studies on carob have reported varying fat contents ranging between 0.2 – 2.3 g.100 g^{-1} (Binder, 1958; Calixto & Cańellas, 1982; Bravo *et al.*, 1994; Marakis, 1996; Avallone *et al.*, 1997; Yousif & Alghzawi, 2000; Biner *et al.*, 2007).

A number of methods for measuring the fat content of food have been developed (Hyvönen, 1996). The methods are mainly based on two principles, namely organic solvent extraction and non-solvent wet extraction (Nielsen, 2003). Some lipids present in foods are found bound to other components such as proteins (lipoproteins) and carbohydrates (liposaccharides) (Nielsen, 2003). For accuracy of the extraction process, the bonds between lipids and other components must, therefore, be broken. The solvent used in the extraction must (in terms of polarity) also be compatible with the type of lipids available in the food. This is necessary in order for the fat to be well solubilised in such a solvent (Nielsen, 1994). Generally, the application of gas chromatographically based techniques following acid hydrolysis, has been suggested for the determination of fatty acids in various food materials (Nielsen, 1994).

Vitamins **–** Vitamins are low-mass compounds essential for normal physiological functioning and have many nutritional body functions (Taylor & Pye, 1974). The lack of one or more vitamins in the body may result in physiological malfunctioning or a deficiency disease, for example stunting, scurvy and rickets resulting from a shortage of vitamin A, C and D, respectively (Mertz, 1974; Taylor & Pye, 1974). Unfortunately, the human body does not have the ability to synthesise many of the vitamins and, therefore, these vitamin requirements must be taken in with the diet (Nielsen, 2003). Dietary requirements have been determined for each vitamin. Additionally, determination of the vitamin content of

foods is, therefore, important for nutritional labelling. Labelling of food products with accurate nutritional information provides guidance to consumers during the selection of various food materials to include in the diet in order to meet daily requirements.

Fruits in general have shown to be an excellent source of vitamins (Aurand *et al.*, 1987). Agricultural Research Service of the United State Department of Agriculture (USDA, 2006) has listed the vitamins found in carob as A, B_6, C, E, folate, thiamine, riboflavin, niacin and pantothenic acid.

Nielsen (2003) gave a thorough description of the methods used for extracting most of the vitamins. The sensitivity of most of the vitamins to environmental factors such as heat, light and oxygen limits accurate vitamin determination in many food products, and hence the need for sophisticated techniques if a valid vitamin analysis needs to be carried out (Nielsen, 2003). Consequently, vitamin analyses are generally very expensive.

Polyphenols – Phenolics are compounds with an aromatic ring bearing one or more hydroxyl groups. The term polyphenols, therefore, refers to substances consisting of more than one aromatic ring. Polyphenols occur ubiquitously in foods of plant origin and because of their antioxidative properties and ability to modulate several proteins, polyphenols generally have beneficial effects on human health once consumed (Vinson, 2001; Sakakibara *et al.,* 2003). These benefits include the prevention of coronary heart diseases, promotion of anti-allergy effects, cancer prevention and vaso-relaxation (Sakakibara *et al.,* 2003). However, data on carob's antioxidant properties and the core functionality, with relation to its polyphenolic components, is still limited. Moreover, the profile as well as the nature of polyphenolic components of carob pods are still not fully understood and, therefore, need to be investigated.

Makris & Kefalas (2004) reported that carob polyphenolic extracts show better antioxidant potency due to better antiradical activity than that of well-aged red wines. The reducing power of carob extracts can also be more than four-fold that of many well known potent antioxidant agents such as gallic acid, caffeic acid and catechin in their pure forms (Makris & Kefalas, 2004). Carob is also reported to be a more efficient antioxidant source than some of the more popular sources such as red wines (Makris & Kefalas, 2004). However, part of the phenolic components of carob are available as condensed tannins, which may exhibit negative nutritional properties (e.g. reduced protein digestibility) once consumed (Bravo *et al*., 1994). For this reason, some researchers have indicated that carob pods might not be a very suitable feed material for either human or animals without first undergoing processing (Würsch *et al.*, 1984).

Several methods for extracting polyphenols from carob have been suggested by different authors, but the use of Folin-Ciocalteu's method is most commonly applied in the final analysis of the extract (Vernon, 1994; Kumazawa *et al.,* 2002; Owen *et al.*, 2003; Markis, & Kefalas, 2004; George *et al.*, 2005). Makris & Kefalas (2004) also studied the efficiency of different solvents in extracting polyphenols from carob powder. In their study, Makris & Kefalas (2004) found that non-polar solvents such as ethyl acetate are unsuitable for the extraction of polyphenols. However, their findings showed that aqueous 80% acetone and aqueous 80% acetonitrile (polar solvents) are very efficient solvents (Papagiannopoulos *et al.,* 2004). Avallone *et al*. (1997) also found that aqueous 70% acetone worked well. The polyphenolic compounds in carob range between 0.5 – 20.0 g.100 g^{-1} (Makris & Kefalas, 2004).

Dietary fibre – Dietary fibre can be defined as lignin plus plant polysaccharides that cannot be digested by human enzymes. These include pectin, hemicelluloses, hydrocolloids and resistant starch (Nielsen, 1994; Butler & Patel, 2000). Fibre is vital in the human diet as it aids in the digestion of foods in the gastrointestinal track (GIT) and, therefore, may protect against GIT cancer (Nielsen, 1994; Perez-Olleros *et al.,* 1999; Owen *et al*, 2003; Zunft *et al.*, 2003). Nielsen (2003) also reported that fibre helps in the normalisation of blood lipids and thereby reduces the chance of cardiovascular disease. It also helps in the prevention of biventricular disease.

As stressed by Englyst & Cummings (1988), a major problem facing researchers on dietary fibre has been the development of suitable analytical methods that yield reliable results. The Englyst & Cummings (1998) method has enjoyed popularity as a widely accepted method for the determination of dietary fibre in food in the past. In recent years the reproducibility of the Englyst & Cummings method has been questioned. This criticism is mainly based on the fact that the Englyst & Cummings method could be underestimating the dietary fibre content, as it only measures dietary fibre as the non-starch polysaccharides (NSP) (Butler & Patel, 2000). The AOAC 991.43 method (AOAC, 2005) on the other hand, also includes lignin and resistant starch and thus, it measures total dietary fibre as a sum of non-starch polysaccharides, lignin and resistant starch.

The dietary fibre content of carob pods has been reported in the range of 2.6 and 39.8 g.100 g^{-1} (Binder, *et al.*, 1958; USDA, 2006). The large variation reported by different researchers, might be attributed to the possibility that methods based on different principles have been followed by different investigators (Marakis, 1996). Some researchers followed methods based on the Englyst and Cummings' (1988) school of

thought, whereas others have followed the principles as given in the AOAC 991.43 method.

E. CAROB PROCESSING AND EFFECT ON COMPOSITION

Carob pods like many other fruits, coffee and cocoa beans, can to be processed into powder (roasted and non-roasted) (Yousif & Alghzawi, 2000). The resultant powder may then be used directly as an ingredient in other processed foods. Alternatively, carob powder may be processed further to extract other specific ingredients, for example sucrose and carob fibre (Marakis, 1992; Wang *et al*., 2002). In Fig. 2, a schematic flow diagram highlighting the various steps involved in the processing of carob pods and the products obtained with each step until carob powder is obtained (Batlle & Tous, 1997; Berna *et al.,* 1997), is presented.

Preparatory steps

Processing of carob pods begins with sorting where healthy looking, physically undamaged pods are selected. The pods are washed with water to remove any dirt, soil or dust. Drying, either sun or mechanical drying, may then follow (Batlle & Tous, 1997).

Kibbling

Kibbling refers to the coarse crushing of the carob pod to allow for an easy separation between the two major components, the seeds and pulp (Batlle & Tous, 1997; Zografakis & Dasenakis, 2000). In commercial processing, mechanical kibblers are mostly used for this purpose (Batlle & Tous, 1997).

Roasting

The kibbles (deseeded pod pieces) may be roasted if so opted. Roasting imparts certain sensory characteristics such as colour and flavour to the final product (Cantalejo, 1997; Yousif & Alghzawi, 2000). Different time/temperature combinations may be employed to give a preferred final product (Berna *et al.,* 1997). According to Yousif & Alghzawi (2000), the time/temperature combination of 150ºC for 60 min produces carob powder with the best sensory characteristics. Roasting temperatures below 80ºC require more than 24 hr before any changes in colour and flavour are found. The same study revealed that

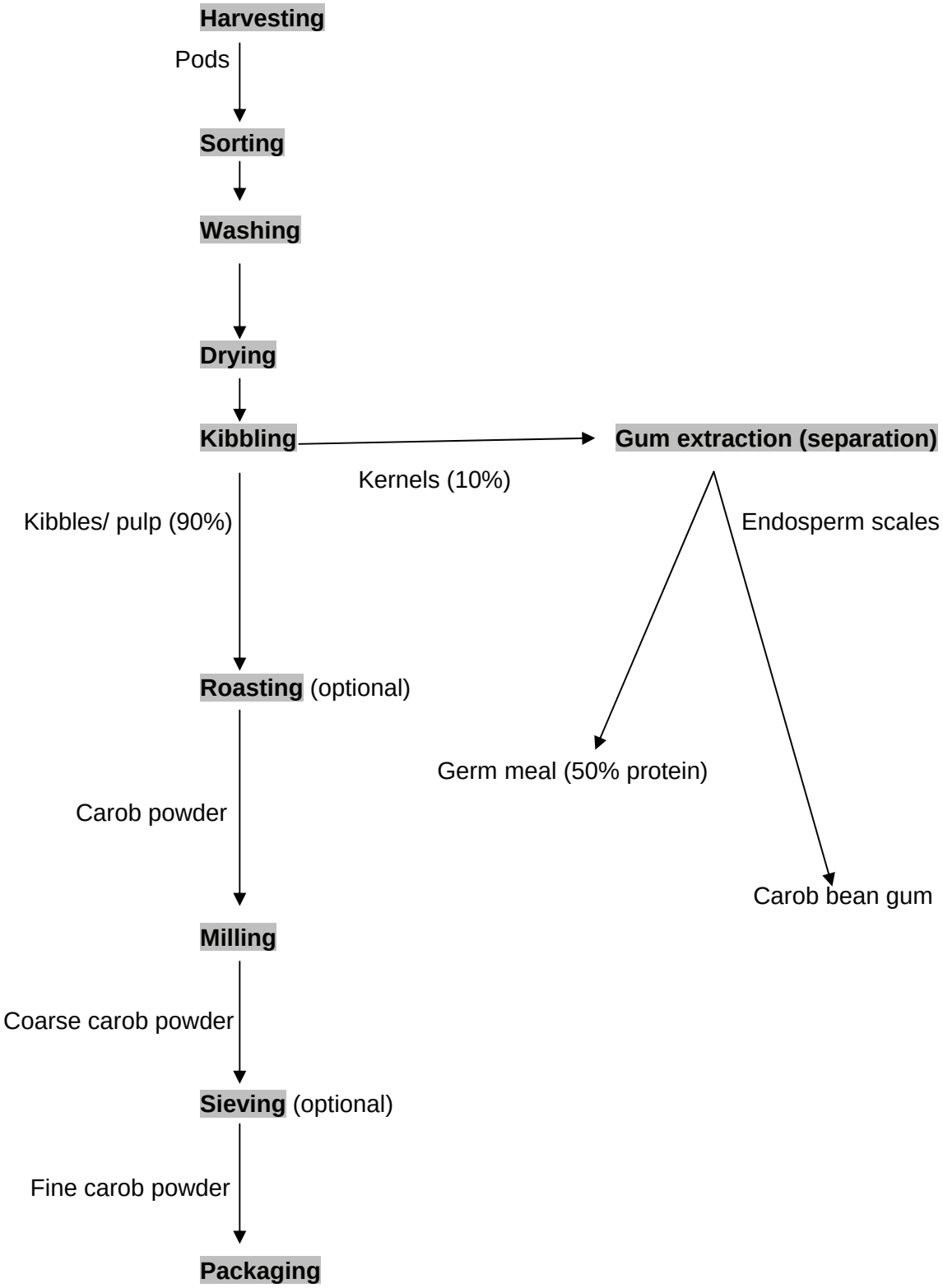

Figure 2 A schematic illustration of the processing steps of the carob pod (adopted from Batlle & Tous, 1997; Berna *et al.,* 1997).

temperatures between 150º and 400ºC do not exhibit good sensory characteristics. It was also found that temperatures above 400ºC are often difficult to control due to rapid changes they impart on colour and flavour (Yousif & Alghzawi, 2000). Similarly, roasting times of longer than 60 min showed a negative effect on the sensory quality of the powder. As suggested by Yousif & Alghzawi (2000), sieving of the kibbles prior to roasting might aid in removing fines and thus reducing the occurrence of a burnt flavour and very dark colour.

Milling

To obtain carob powder, milling of either roasted or non-roasted kibbles may be carried out by way of heavy milling equipment such as power-driven hammer mills (commercial processing) or by using small milling equipment (mortar and pestle) at kitchen level (Batlle & Tous, 1997). Hammer mills are commonly used in the grinding of food materials provided the moisture and lipid contents are reasonably low (Pomeranz & Meloan, 1978). A high moisture and fat content will contribute to difficulties experienced during milling (Yousif & Alghzawi, 2000).

The granule size of the resultant powder is mainly determined by the type and size of the milling equipment used, the piece (kibble) sizes and the composition, especially the moisture content of the raw materials before milling, as well as the period for which milling is carried out (Biner *et al.*, 2007).

Packaging

Food packaging refers to covering or wrapping of food material with other material(s) with the objective of providing an inert barrier to external conditions (Rooney, 1995). Other objectives may be based on the convenience in handling and during transportation and storage. As in the case of many powdery products, moisture tight materials (such as polythene) have proven to be most appropriate for the packaging of carob powder (Yousif & Alghzawi, 2000).

Effect of processing on the nutritional composition

As it applies to any raw food material, depletion or loss of nutrients during processing of carob cannot be under-estimated. In most cases, the loss is more pronounced for sensitive nutrients such as vitamins, fatty acids, sugars and proteins, but almost all

nutrients can be affected in one way or another. In many instances, processing reduces the moisture content of carob largely.

Carob pods are sometimes roasted at variable temperature-time combinations to enhance certain colours and flavours as may be preferred (Batlle & Tous, 1997). Both the roasting time and temperature greatly affect the nutritional content of the carob pods. For example, the roasting step may cause a reduction in sugar and protein content (Yousif & Alghzawi, 2000; Doxastakis *et al.,* 2007). Such reduction might be as a result of the Maillard reaction and caramelisation during the roasting process (Blenford, 1979; Calixto & Canellas, 1982; Yousif & Alghzawi, 2000; Sikorski, 2002; Jones, 2005).

Upon exposure to elevated temperature in the presence of oxygen (for example during carob roasting), lipids are oxidised, which leads to their decomposition into secondary products such as alcohols, aldehydes, ketones, carboxylic acids and hydrocarbons (Sikorski, 2002). This may as a result reduce the lipid fraction of the material in question (Yousif & Alghzawi, 2000). The content of some volatile components such as short chain volatile fatty acids may also be affected. For example, the isobutyric content of pods may be reduced from 9.3 to 6.9 $g.kg^{-1}$ after roasting at 160°C for 30 min (Berna *et al.*, 1997). Even though the loss of nutritional components is undesirable in most cases, loss of some of the components such as iso-butyric acid may be beneficial (Berna *et al.*, 1997) as it imparts an unpleasant but characteristic aroma to the final carob product.

F. NEW FOOD PRODUCT DEVELOPMENT

Definition of a new product

A new product according to Fuller (2004), is one that has not previously been manufactured by a company and introduced by such a company into the market or the presentation by a company of an established product in a new form or into a new market not previously explored by that particular company. A new product is thus seen as one that is new and has never been presented in the local market. However, this is rare. Most products advertised as “new” usually have an analogue, a similar product, produced locally by a competitor or which has been imported (Fuller, 2004; Backley, 2007). Thus, this definition should not to be applied too rigidly as new products can be grouped as: line extensions; repositioned existing product; new form or size of existing product; reformulation of existing product; repackaging of existing product; innovative products; and even creative variants (CCFRAG, 1996; Baker *et al*., 2004).

Added value

Added value is a characteristic many new products are pre-assumed to possess. Added value describes the degree of innovation that makes a product more desirable to customers and consumers. Such novelty might be an improvement in one or more of the following: appearance, stability, functionality, texture, taste, flavour or convenience (Fuller, 2004; Backley, 2007). Value addition simply refers to the act that would effect a physical, chemical or sensory change in a food product such that it becomes more desirable to the consumer.

Customers and consumers

Although commonly used interchangeably, there is a clear distinction between the terms “customer” and “consumer”. The term “customer” specifically refers to the person who purchases in a given market. A consumer on the other hand is the person who directly partakes in the use (consumption) of the product. The consumer could, therefore, also be the customer. The consumer’s hedonic demand of “I want” should therefore not be confused with the customer’s practical barrier of “I need” or “I can afford” (Fuller, 2004). The consumer is the key to the success of any new food product. The consumer’s needs and/or desires regarding such a product, therefore, should be carefully considered prior to the development of a new food product (Backley, 2007).

Markets

The terms “market” and “marketplace” are also commonly used synonymously but the two have different meanings. A market refers to a perceived need (and hence a potential sell) in customers and consumers, of a certain product. The market is thus, conceptual (Fuller, 2004; Backley, 2007). A marketplace is the real physical entity where customers access the products. Even electronic food marketplaces which operate via Web sites are real, not conceptual.

Product life cycles

Every product has a defined lifecycle. Such a cycle consists of five distinctive phases namely: an introductory period; a strong growth period; the beginning of a decline in the growth of the sales volume; a no-growth period; and the beginning of a decline in sales

volume (Fuller, 2004). The length of a lifecycle should also be anticipated prior to the development of new food product.

Phases of new product development

The development of a new product is, according to Baker *et al.* (1988), comprised of a number of phases. These include: the idea stage; development stage; taste-panelling stage; consumer sampling stage; shelf-life stage; packaging stage; production stage; market testing stage; and commercialisation stage. In a commercial environment, the generation of new food product ideas involves identification of new products with excellent market potentials that are in line with the company's objectives.

The development stage which involves the practical creation of the new product should work concurrently with the taste-panelling stage. This is important since organoleptic effects of any change made during the development stage must be carefully monitored. Although not compulsory, consumer sampling may lead to useful information regarding the consumers' perception of the new product (Baker *et al.*, 1988). Once the product has been developed, shelf-life evaluation normally follows, in order to obtain information as to how long the product will keep under specified environmental conditions. In the presence of such information and the right technology and personnel the shelf-life of a product may be improved if required. The type of the packaging used is also crucial and it is affected by a number of factors. These include, but are not limited to the type of product in question, the consumer's convenience, attractiveness, production costs and transportation involved.

In most instances, it is important to perform a practical evaluation of the full-scale production (using a small-scale pilot production line). At the production stage, factors for consideration must include the cost and maintenance of equipment, cost of energy, yield, safety, labour requirements, sanitation and government regulations (Baker *et al.*, 1988; CCFRAG, 1996). All these must be considered with a main objective of producing the best product possible at the lowest cost.

Furthermore, the market-testing stage is mostly aimed at obtaining information on the product's sales potentials. This is not a common practice in many companies. Most companies bypass this phase and proceed straight to the commercialisation stage, which is the last phase of determining the success or failure of a new product (Baker *et al.*, 1988). Commercialisation involves the practical presentation of such a product into the market which might require advertising and other marketing aids.

G. DISCUSSION

When studying the literature, it is clear that a large part of the Southern African population has nutritional problems as most households do not have access to adequate foods and thus many people are malnourished. It was also found that carob is widely distributed in South Africa especially in the Western Cape Province but most of this highly nutritious product goes to waste every year. The reason for this is probably because local communities are not aware of the nutritional potential of carob. The availability and adaptability to local environmental conditions of carob could present nutritional opportunities for the whole Southern Africa, as carob pods are known to be highly nutritious. However, carob's nutritional composition is known to vary among cultivars as well as between geographical areas. There is, therefore, a need for more research and exploration to determine the nutritional potentials of South African grown carob cultivars and to evaluate the potential of local cultivars to be used in the development of more new nutritious food products. Efforts should also be made to educate the relevant communities about the possible use of carob as a source of food.

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CHAPTER 3

CAROB PROXIMATE COMPOSITIONAL ANALYSIS

Summary

Five South African cultivated carob cultivars (Tylliria, SFax, Aaronsohn, Santa Fe and one "Unknown" cultivar) were analysed for proximate (moisture, ash, total sugars, protein, fat and dietary fibre) and polyphenol composition as well as for mineral, amino acid and fatty acid content. The average proximate composition range of raw carob pods was: 8.17 – 9.56% moisture; 89.57 – 91.12% carbohydrates; 40.69 – 54.74% sugars; 29.88 – 36.07% dietary fibre; 3.07 – 4.42% protein; 2.58 – 3.08% polyphenols; 0.45 – 0.86% fat; and 2.13 – 2.69% ash. In all carob cultivars, sucrose (33.70 – 45.09%) was the major sugar while glucose and fructose contributed relatively less (1.79 – 4.92% and 1.80 – 5.19%). A total of 17 amino acids were detected in all five carob cultivars studied, including the seven essential amino acids. All cultivars analysed were found to be of good nutritional quality in terms of long-chain fatty acid proportions, i.e. PUFA: SFA and *n*-6: *n*-3 ratios. Small amounts of acetic, iso-butyric, butyric and iso- valeric acids were detected in all five carob cultivars whereas valeric acid was only detected in the Santa Fe cultivar. All cultivars contained all nine minerals analysed for, in this study. Slight compositional variations (although statistically significant) were found among cultivars and thus selection of cultivars for use in food processing should be based on consumer sensory evaluation rather than on compositional characteristics. The results obtained in this study suggest that the locally cultivated carob cultivars are highly nutritious and thus carob can be considered as an alternative food source for South Africa.

The impact of various roasting times (45, 60 and 75 min) at 150ºC, on the temperature sensitive components such as sugars, protein and fat, was also examined. An increase in roasting time had a significant ($P<0.05$) reducing effect on the sugars and protein contents but no significant ($P>0.05$) effect was observed in the fat content. This implies a necessity to optimise roasting conditions such that the sugars and protein contents are less impacted, whilst producing organoleptically acceptable carob powders for use in food.

Introduction

Carob (*Ceratonia siliqua* L.) is an evergreen sclerophyllous tree of Mediterranean origin

(Rizzo *et al*., 2004; Shawakfeh & Ereifej, 2005). The species belongs to the *Caesalpinaceae* subfamily of the *Leguminoceae* family (Yousif & Alghzawi, 2000; Biner *et al.*, 2007). The tree is perennial in nature and has pinnately compound leaves (Marakis, 1996). On average, the carob tree has a pre-fruiting period of 6 – 8 years but, improved varieties can bear fruits (pods) within 3 – 4 years. Once peak production has been attained (20 – 25 years of age), up to 800 kg pods may be produced per tree per harvest (Marakis, 1996).

The fruit is a dark brown pod, with a straight, curved or twisted shape and it might be elongated or compressed in structure (Zografakis & Dasenakis, 2000). The pod mass ranges between 5 and 30 g (Marakis, 1996). The pods can be up to 25 cm long, up to 1.3 cm thick and up to 4 cm wide (Marakis, 1996). Seeds contribute between 5 and 40% of the total pod mass (Calixto & Cańellas, 1982). Up to 70% of the carob pulp is mainly sucrose (up to 95% of the carbohydrate content), with fructose and glucose contributing the remainder in relatively equal proportions (Bravo *et al.,* 1994). Carob contains substantial amounts of protein (up to 7.6%) and because of its low fat content (0.2 – 2.3%), carob may be regarded as a healthy food source (Marakis, 1992; Bravo *et al.,* 1994; Petit & Pinilla, 1995, Marakis & Kefalas, 2004). Dietary fibre and polyphenols have been reported to exhibit nutritional benefits in the human diet (Aurand *et al*., 1987; Li *et al.,* 2007). Carob contains up to 39.8% dietary fibre and 20% polyphenols (Würsch *et al*., 1984; Makris & Kefalas, 2004; USDA, 2006).

The chemical composition of carob pods has been studied (Binder *et al.*, 1958; Calixto & Cańellas, 1982; Blenford, 1988; Bravo *et al.*, 1994; Petit & Pinilla, 1995; Marakis, 1996; Avallone *et al*., 1997; Marakis, 1992; Makris & Kefalas, 2004) but the findings from the researchers from different locations, differ. Such variations can be attributed to the fact that carob composition is strongly influenced by differences between cultivars and horticultural conditions (Zograakis & Dasenakis, 2000).

Roasting (normally at 150°C) was shown to improve the sensory acceptability of carob since it has been proven to impart desired sensory characteristics such as excellent colour and flavour to the final product (Berna *et al.,* 1997; Cantalejo, 1997; Yousif & Alghzawi, 2000). However, roasting might also have an effect on the carob's chemical composition (Yousif & Alghzawi, 2000) but this has not been well studied.

Since there are several reports of variations in the chemical composition of carob from different parts of the world, and since the composition of South African grown cultivars is not known, the aim of this study was to determine the proximate composition (moisture, ash, fat as well as short and long chain fatty acids, protein and the individual

amino acids, carbohydrates (dietary fibre and individuals sugars) and polyphenols) of five South African grown carob cultivars. The impact of different roasting conditions on the composition will also be determined.

Materials and methods

Carob pods

Carob pods of four known cultivars were obtained with the kind assistance of Mr Freddy Rust (a commercial farmer) from trees at the Eenboom farm, in the Western Cape Province (Malmesbury district), South Africa. The known cultivars included: “Santa Fe” of Santa Fe Springs, California, USA origin; “SFax” originating from Menzel bon Zelfa or SFax, Tunisia; “Tylliria” from Cyprus and; “Aaronsohn” of Israel (Batlle & Tous, 1997). Pod samples belonging to the different cultivars were collected and kept in separate, well aerated, nylon bags to prevent mixing of the cultivars. Additionally, pods from a “Unknown cultivar” (i.e. trees obtained from a commercial nursery and planted for ornamental purposes) were also obtained with the kind assistance of Dr Carel Muller (researcher) from the Elsenburg Research Station of the Department of Agriculture, in the Western Cape Province, South Africa.

Kibbling

Carob pods were cut open length wise using a manually hinged cutting blade. This allowed for an easy separation of the seeds from the pod. All off-cuts from the kibbling, including the powder were collected, to ensure that a completely representative husk was obtained. The kibbles and the off-cuts were vacuum-packed in polyethene bags and stored at 4°C until needed for further analysis.

Milling

Carob kibbles were milled by passing through a hammer mill (Scientec, Cape Town) with a 0.5 mm size sieve. The resultant powder was immediately vacuum-packed in polyethene bags and stored at 4°C until needed.

Analytical methods

The following components were determined using standard methods (AOAC, 2005): moisture (925.09), dietary fibre (991.43), protein (960.52), fat (920.85) and ash (923.03). A conversion factor (6.25) was used to determine the actual protein content of each sample (Avellone *et al*., 1997).

Total Carbohydrate determination

The total carbohydrate content was determined by subtracting the percentage protein, lipid, ash and polyphenol from the percentage dry matter i.e. % carbohydrates = % dry matter - (% protein + % lipid + % ash + % polyphenol) (Biner *et al.*, 2007).

Determination of individual sugars

A combined analysis for determining sucrose, D-glucose and D-fructose was carried out using a commercially available enzymatic test kit ("Sucrose/D-Glucose/D-Fructose", Test kit, Boeringer Mannheim, cat. no. 1071626035).

Amino acids determination

The individual amino acids composition was determined by using a modified HPLC method described by Mostert & Hoffman (2007).

Polyphenol determination

The method of Makris & Kefalas (2004) was used for the extraction of polyphenols. Acetone (80%) (Merck, analytical grade) was used as an extraction solvent. This provided samples for total polyphenol content determination via the Folin-Ciocalteu method (Singleton & Rossi, 1965; Singleton *et al*., 1999). This method measures polyphenols as gallic acid equivalent (g.100 g^{-1}).

Short chain volatile fatty acids determination

An extraction medium consisting of 1 part 35% formic acid and 3 parts distilled water was prepared. Carob powder (1 g) was weighed into a McCartney bottle and 10 mL extraction medium was added and the suspension was vortexed for 1 min. The suspension was allowed to stand for 5 min before it was filtered (Whatman no.1) using a Büchner extraction funnel. Part (4 mL) of the clear filtrate was measured into another McCartney bottle and then stored at -18°C. Immediately, before analysis, the sample extract was brought to room temperature and 2 µL internal standard (hexanol) was added.

A Varian 3700 gas chromatograph (GC) equipped with a flame ionisation detector and a 30 m bonded phase Nukol (Supelco, Inc., Belafonte, PA) fused silica capillary column (0.53 mm diameter and 0.50 µm film thickness) was used to determine the short chain volatile fatty acid content in the extracts. Initially, the column temperature was held at 105ºC for 2 min, increased at the rate of 10ºC.min^{-1} to 190ºC and held for 10 min for the remainder of the run. The injector temperature was set at 150ºC, while the detector temperature was set at 300ºC. The flow rate of the carrier gas (nitrogen) was set at 6.1

mL.min^{-1}. Borwin Version 1.2 integration software (JMBS Developments, Le Fontail, France), operating in the internal standard mode was used to quantify for short chain fatty acids. GC values were converted to the original sample concentration as discussed by Sigge *et al*. (2005).

Long-chain fatty acids determination

The long-chain fatty acid composition was determined by using a method described by Tichelaar *et al*. (1998) and Mostert & Hoffman (2007).

Mineral determination

The AgriLASA (Agricultural Laboratory Association of South Africa) method (6.1.1), as described by Mostert & Hoffman (2007) was used to determine the mineral content (Ca, P, K, Mg, Na, Mn, Fe, Cu and Zn).

Roasting

One (the Tylliria) of the five cultivars evaluated in the overall study was selected for use in the roasting study. Kibbles of relatively similar shapes and sizes were roasted at 150°C for 45, 60 and 75 min in a computer-controlled oven (Defy, SS395). The roasted kibbles were then allowed to cool down to room temperature, after which they were vacuum-packed in polyethene bags and stored at 4°C until needed for further analysis (for moisture, sugars, protein, and fat contents).

Statistical analysis

Each component (except for amino acids which were only determined once due to high costs) was determined in triplicate for each treatment (cultivar or roasting time). Composition data were statistically analysed using Statistica software, release 8 (StatSoft, USA). Variations between the treatment mean values were determined using a one way analysis of variance (ANOVA) with significance defined at $P<0.05$.

Results and discussion

Moisture

The moisture contents of the five carob cultivars analysed in this study are given in Table 1. The data showed that the Tylliria, SFax and Aaronsohn cultivars showed no significant difference ($P>0.05$) in terms of the moisture content. The Santa Fe and the Unknown cultivar differed from one another and from the Tylliria, SFax and Aaronsohn cultivars. This could be due to the fact that the moisture content in carob pods is also

Table 1 Proximate and polyphenol composition of raw South African grown carob pods (g.100 g^{-1} dry mass)

Component *	Carob cultivar				
	Tylliria	**SFax**	**Aaronsohn**	**Santa Fe**	**Unknown**
Moisture	9.27 ± 0.40^{a}	9.56 ± 0.19^{a}	9.29 ± 0.02^{a}	8.91 ± 0.00^{b}	8.17 ± 0.01^{c}
Carbohydrates	90.69 ± 0.25^{ab}	89.57 ± 0.33^{a}	90.79 ± 0.51^{b}	91.12 ± 0.27^{b}	90.31 ± 0.42^{ab}
Total sugars	54.74 ± 1.35^{a}	40.69 ± 0.77^{c}	50.55 ± 0.64^{b}	45.61 ± 1.09^{d}	51.46 ± 1.98^{ab}
Sucrose	45.09 ± 1.49^{a}	33.70 ± 0.72^{b}	40.41 ± 0.55^{a}	42.02 ± 1.05^{a}	44.84 ± 3.13^{a}
Glucose	4.92 ± 0.15^{a}	3.54 ± 0.15^{c}	4.95 ± 0.26^{a}	1.79 ± 0.08^{b}	2.22 ± 0.51^{b}
Fructose	4.73 ± 0.43^{ab}	3.45 ± 0.24^{a}	5.19 ± 0.16^{b}	1.80 ± 0.16^{c}	4.40 ± 0.83^{ab}
Dietary fibre	31.47 ± 1.04^{ab}	36.07 ± 2.71^{c}	33.35 ±1.56^{abc}	35.85 ± 2.10^{ac}	29.88 ±1.30^{b}
Protein	3.57 ± 0.11^{a}	4.42 ± 0.01^{b}	3.07 ± 0.01^{a}	3.26 ± 0.02^{a}	3.42 ± 0.00^{a}
Polyphenol$^{\#}$	2.65 ± 0.26^{a}	2.87 ± 0.27^{a}	3.08 ± 0.51^{a}	2.58 ± 0.33^{a}	2.94 ± 0.36^{a}
Fat	0.71± 0.06^{b}	0.45± 0.04^{a}	0.74± 0.02^{b}	0.86± 0.05^{c}	0.85± 0.04^{c}
Ash	2.37 ± 0.02^{a}	2.69 ± 0.09^{d}	2.31 ± 0.07^{ab}	2.17 ± 0.02^{bc}	2.13 ± 0.06^{c}

$^{a, b, c, d}$ Values in a row without a common superscript are significantly different (P<0.05).
$^{\#}$ Expressed as gallic acid equivalent.
* The values are given as means of a triplicate determinations ± standard deviation.

influenced by factors such the ripening stage and moisture absorbency of the skin (Batlle & Tous, 1997). They reported that while in a fresh state, pods generally have a moisture content ranging between 10 and 20% (m/m). Both Binder *et al*. (1958) and Batlle & Tous (1997) recommended that the moisture content of carob pods be reduced to 10% (m/m) before storage. This is essential to prevent spoilage during storage, and to prolong shelf-life (Charley, 1982; Curtis & Race, 1998; Nielsen, 2003). All carob powders analysed in this study had moisture contents below 10% (m/m) (Table 1). In addition, a low moisture content has other operational advantages such as facilitating easier milling and final packaging (Batlle & Tous, 1997).

Total carbohydrate content

The carbohydrate content of the carob cultivars analysed in this study is given in Table 1. The data showed that the values varied from 89.57 – 91.12 g.100 g^{-1} dry mass. This is slightly above the range (48.00 – 88.88 g.100 g^{-1}) reported in the literature (Zografakis & Dasenakis, 2000; USDA, 2006). These results (the higher carbohydrate content) indicate that carob could be a good energy source once included in the diet (Nielsen, 2003).

Neither the Tylliria nor the Unknown cultivar differed significantly (P>0.05) from any other cultivar. Aaronsohn and Santa Fe did not significantly differ from one another but both these cultivars differed significantly (P<0.05) from SFax. The differences in carbohydrate content between the various cultivars were found to be very small. However, this does not necessarily imply similarities in types of carbohydrates present. Variations could exist among cultivars in terms of carbohydrate types such as dietary fibre and sugars as discussed below.

Total sugar content

The total sugar content of the five carob cultivars analysed is given in Table 1. The total sugar content in the cultivars involved, ranged between 40.69 and 54.74 g.100 g^{-1}. The highest value (54.74 g.100 g^{-1}) found in this study is higher than that reported by other researchers (49.08 g.100 g^{-1}) (USDA, 2006). The data from the study agrees with the findings of other investigators in that sucrose is the major sugar whilst glucose and fructose contributed smaller values but are relatively similarly (Biner *et al.*, 2007). The total sugar content is reported as the sum of the three major soluble sugars i.e. sum of sucrose, glucose and fructose (Shawakefh & Erefej, 2005; Biner *et al.*, 2007). This study revealed significant differences (P<0.05) between the Tylliria, SFax, Aaronsohn, and Santa Fe cultivars. The Unknown cultivar differed significantly from both SFax and Santa Fe but not from either the Tylliria or the Aaronsohn.

Generally, the sugar content and composition of plants are known to be influenced by their habitats (Li *et al.*, 2007). This factor could not have greatly influenced the values observed in this particular study because the pod samples involved were collected from the same orchard, and therefore, it was concluded that the differences found could only have been as a result of inter-cultivar variations.

Sucrose content – The sucrose content of each of the five carob cultivars is given in Table 1. The sugar analysis showed that sucrose contents of the cultivars included in study ranged between 33.70 and 45.09 g.100 g^{-1}. The SFax was the only cultivar which significantly (P<0.05) differed (lowest content) from the other four cultivars included in the study. The high sucrose content as found in the present study explains carob's sweet taste in general, and it is suggested that food products containing high amounts of carob would be naturally sweet, thus reducing the need for additional sweeteners (Biner *et al.,* 2007).

Glucose content – The glucose contents of the carob cultivars studied are given in Table 1 and were found to vary from 1.79 for the Santa Fe cultivar and 4.95 g.100 g^{-1} for the Aaronsohn cultivar. Tylliria and Aaronsohn were similar in glucose content but differed significantly (P<0.05) from the other three cultivars. Similarly, the glucose content in Santa Fe and the Unknown cultivar were not significantly different but the two were significantly lower (glucose content) than that of the SFax cultivar.

Fructose content – The fructose contents of the carob cultivars are given in Table 1 with variations in the range of 1.80 to 5.19 g.100 g^{-1} dry mass. Santa Fe was significantly (P<0.05) lower in fructose content than the other cultivars. Tylliria and the Unknown cultivar did not differ significantly from either SFax or Aaronsohn. The differences were significant (P<0.05) for SFax, Aaronsohn and Santa Fe.

Dietary fibre content

The "Englyst and Cummings" method (Englyst & Cummings, 1988) has, in the past, enjoyed popularity as a widely accepted method for the determination of dietary fibre in food. In recent years, the "Englyst and Cummings" method has been criticised, based on recent scientific developments. The problem is the fact that the "Englyst and Cummings" method underestimates the dietary fibre content, since it only measures dietary fibre as the non-starch polysaccharides (NSP) (Butler & Patel, 2000). The AOAC 991.43 method (AOAC, 2005) on the other hand, additionally considers lignin and resistant starch as part of the dietary fibre content. Thus, the AOAC 991.43 method was used in this study to determine the dietary fibre content.

Some investigators have reported the dietary fibre content in carob pods to be as low as 3.7 g.100 g^{-1}, whereas others have reported content as high as 39.88 g.100 g^{-1} (Binder *et al.*, 1958; USDA, 2006). Besides inter-cultivar differences, such large variations might be attributed to the possibility that methods based on different principles have been applied (Marakis, 1996; Butler & Patel, 2000). Some researchers (especially those from older literature sources such as Calixto & Cańellas (1982)) might have followed methods based on the Englyst and Cummings (1988) school of thought, whereas recent ones such as the USDA (2006) followed the principles of the AOAC 991.43 method.

The dietary fibre contents of the five carob cultivars analysed in this study are given in Table 1. In this study, high dietary fibre contents were found using the AOAC (991.43) method. The dietary fibre contents in the carob pods of the various cultivars investigated were found to range between 29.88 and 36.07 g.100 g^{-1} (Table 1). These values are also in line with recent findings (39.8 g.100 g^{-1}) by other researchers (USDA, 2006). In this study it was found that no significant (P>0.05) difference was observed between Tylliria, SFax and Santa Fe. SFax differed significantly (P<0.05) from Tylliria and the Unknown cultivar (Table 1).

The high dietary fibre content of carob can be seen as nutritionally beneficial since carob fibre has been reported to exhibit valuable health-promoting attributes such as blood cholesterol lowering, anti-oxidative properties and the reduced risk of gastro-intestinal cancer (Brandt, 2002; Haber, 2002).

Protein content

The protein content of the five carob cultivars analysed in this study is given in Table 1. Overall, the cultivars contained appreciable amounts of protein (3.07 – 4.42 g.100 g^{-1}). These values were similar to findings of other investigators who reported ranges between 1.0 and 7.6 g.100 g^{-1} (Calixto & Cańellas, 1982; Owen *et al.*, 2003). In all five cultivars studied, the protein content was slightly lower than the minimum level (5 g.100 g^{-1}) required for any food to be labelled as a source of protein, as set by the labelling regulations (Anon., 2002). No significant (P>0.05) differences were observed between the protein content of Tylliria, Aaronsohn, Santa Fe, and the Unknown cultivar. SFax had a significantly (P<0.05) higher protein content than all the others cultivars.

Amino acid content

The amino acid content of the five carob cultivars investigated is given in Table 2. A total of seventeen amino acids were detected in the carob cultivars. Except for the Unknown cultivar, which did not contain cysteine and methionine, all the carob cultivars

Table 2 Amino acid composition of five locally grown carob pod cultivars (mg.100 g^{-1} dry mass)

Component	Carob cultivar				
	Tylliria	**SFax**	**Aaronsohn**	**Santa Fe**	**Unknown**
Aspartic acid	280	770	270	220	400
Glutamic acid	330	420	250	240	460
Serine	520	530	450	440	150
Glycine	20	20	40	40	110
Histidine[ce]	60	80	40	70	120
Arginine[ce]	70	190	40	90	160
Threonine[e]	620	550	540	520	140
Alanine	120	220	130	110	310
Tyrosine	140	140	100	100	70
Valine[e]	390	330	200	180	390
Proline	450	370	220	410	570
Methionine[e]	10	40	20	20	0
Isoleucine[e]	200	180	110	100	160
Leucine[e]	210	290	50	40	320
Cysteine	10	10	10	10	0
Phenylalanine[e]	40	70	30	30	50
Lysine[e]	260	270	190	220	280

[e] Essential amino acid
[ce] Conditionally essential amino acid i.e. arginine is essential in children with severe childhood undernutrition (Jahoor *et al.*, 2007) whereas histidine is essential in clinical conditions such as uraemia (Kopple & Swendseid, 1974)

contained the same types of amino acids. Although the protein content was low in all cultivars studied, the carob protein was found to be of good quality, as it contained all seven essential amino acids.

Polyphenol contents

The data from this study showed that there were no significant differences (P>0.05) among the five cultivars as far as polyphenolic content is concerned (Table 1). The polyphenolic content ranged between 2.58 and 3.08 g.100 g^{-1}. These values are in agreement with findings of other researchers (1.3 – 20 g.100 g^{-1}) (Makris & Kefalas, 2004).

Polyphenolic compounds have been reported to exhibit health benefits, especially with regard to cardiovascular diseases, due to their ability to scavenge free radicals, superoxide and hydroxyl radicals (Li *et al.*, 2007). Sakakibara *et al.* (2003) also reported that phenolic compounds have anti-allergic, cancer preventative and vasorelaxing effects. In fact, carob is known to be a more efficient antioxidant source than some of the popular sources such as red wines (Makris & Kefalas, 2004). It should be noted that the analytical method used (Folin-Ciocalteau) in this study does not measure absolute contents of specific phenolic materials since the values are expressed as gallic acid equivalents (g.100 g^{-1}) (Li *et al.*, 2007).

Although success has been achieved in setting recommended dietary allowances (RDA) for various specific classes of polyphenols (for example 2 g.kg^{-1} body weight, for proanthocyanidin), setting an overall RDA which would fit all classes (collectively) still has not been achieved (Kroon & Williamson, 2005).

Fat content

The fat content of each of the five carob cultivars (pods) investigated is given in Table 1. SFax had a significantly (P<0.05) lower fat content than all the other cultivars. Tylliria and Aaronsohn did not differ (P>0.05), but both differed from SFax and from Santa Fe and the Unknown cultivar. Santa Fe and the Unknown cultivar did not differ, but both significantly differed (P<0.05) from all the other cultivars. Overall, the fat content in all five cultivars was very low (below 1.0 g.100g^{-1}). This is in agreement with previous reports by other authors giving values of 0.2 – 2.3 g.100g^{-1} (Marakis, 1996).

Foods with high fat content are often highly perishable as a result of lipid hydrolysis and oxidation leading to rancidity. A low fat content can, therefore, be assumed to have an positive effect on the shelf-life (2 – 3 years) of carob pods (Curtis & Race, 1998). The consumption of high fat containing foods is also associated with increased risk of coronary

circulatory disease, obesity and some types of cancers (Sikorski, 2003). Because of its low fat, carob may therefore be regarded as a healthier food source (Biner *et al.*, 2007).

Short-chain fatty acid (SCFA) content

The short-chain fatty acid content of the five carob cultivars (pods) investigated is given in Table 3. Acetic, iso-butyric, butyric and iso-valeric acids were detected in all the carob cultivars analysed in this study. Valeric acid was only detected in the Santa Fe cultivar. As shown Table 3, the contents vary between the carob cultivars especially in terms of specific SCFA. The SCFA content was very low in all the carob cultivars analysed (34.73 – 106.84 mg.100g^{-1}). Santa Fe had a significantly (P<0.05) higher total SCFA content than all other cultivars. The Unknown cultivar was significantly lower than Aaronsohn but significantly higher than Tylliria and SFax. Tylliria and SFax did, however, not differ significantly (P>0.05).

Short-chain fatty acids are reported to be a good source of energy in the diet since they can easily be absorbed due to their high solubility (Sikorski, 2003). Also worth noting is the presence of iso-butyric acid, as it is said to contribute to the unpleasant aroma found in carob (Berna *et al.,* 1997). Santa Fe was significantly (P<0.05) higher in iso-butyric acid than all other cultivars. Tylliria and SFax did not differ significantly from one another. The Unknown cultivar did not significantly (P>0.05) differ from either Tylliria and SFax or Aaronsohn. Aaronsohn differed significantly from Tylliria and SFax.

Long-chain fatty acid content

Although the total fat content of carob found in this study and that reported by other researchers (0.45 – 0.86 and 0.2 – 2.3 g.100g^{-1}, respectively) is low, it is still important to comment on the fatty acid composition. The long chain fatty acid content (%) of the carob cultivars analysed in this study is shown in Table 4. The results show that the fatty acid content varied between the carob cultivars. No significant differences (P>0.05) were observed amongst Tylliria, Aaronsohn, Santa Fe and the Unknown cultivar in terms of polyunsaturated fatty acid (PUFA) to saturated fatty acid (SFA) ratio. SFax did also not significantly differ from Aaronsohn but differed (SFax) significantly (P<0.05) from all the other cultivars.

Consumption of foods which have a low unsaturated fatty acid (UFA) to saturated fatty acid (SFA) ratio are associated with a high risk of cardiovascular diseases (Sikorski, 2003). Nutritional health organisations around the world have, therefore, set recommendations regarding the polyunsaturated fatty acid (PUFA) to saturated fatty acid (SFA) ratio. For example, the British Department of Health has recommended a PUFA:

Table 3 Short-chain fatty acid composition of raw South African grown carob pods (mg.100g^{-1} dry matter)

Component*	Carob cultivar				
	Tylliria	**SFax**	**Aaronsohn**	**Santa Fe**	**Unknown**
Acetic acid	9.71 ± 1.57^{a}	7.39± 0.31^{a}	39.39 ± 2.56^{c}	35.45 ± 2.3^{c}	19.96 ± 1.96^{b}
Iso-butyric acid	23.78 ± 1.89^{a}	24.57 ± 0.31^{a}	30.41 ± 1.32^{b}	53.07 ± 2.03^{c}	27.99 ± 1.76^{ab}
Butyric acid	2.26 ± 0.24^{a}	2.18 ± 0.03^{a}	6.46 ± 0.28^{b}	9.88 ± 0.80^{c}	2.58 ± 0.25^{a}
Iso-valeric acid	0.41 ± 0.09^{a}	0.59 ± 0.03^{a}	3.94 ± 0.54^{b}	4.91 ± 0.37^{c}	3.98 ± 0.18^{b}
Valeric acid	ND^{a}	ND^{a}	ND^{a}	3.54 ± 0.54^{b}	ND^{a}
Total SCFAs	36.16 ± 3.52^{a}	34.73 ± 0.61^{a}	80.20 ± 4.35^{c}	106.84 ±4.90^{d}	54.51 ± 3.82^{b}

$^{a, b, c, d}$ Values in a row without a common superscript are significantly different (P<0.05).
* The values are given as means of a triplicate determination ± standard deviation.
ND = Not Detected

Table 4 Long-chain fatty acid content (%) of raw South African grown carob pods

LCFAs*	Cultivar				
	Tylliria	**SFax**	**Aaronsohn**	**Santa Fe**	**Unknown**
C6:00	7.51	3.75	1.93	1.29	0.50
C8:00	n.d.	0.73	0.01	0.03	0.30
C10:00	n.d.	0.57	0.18	n.d.	0.44
C11:00	4.09	1.43	0.12	0.06	1.17
C12:00	1.81	1.27	3.10	1.19	0.73
C13:00	n.d.	1.36	3.79	0.48	1.87
C14:00	3.34	3.20	2.77	6.24	1.12
C15:00	3.96	3.18	2.40	4.36	2.19
C16:00	19.49	8.57	11.37	20.01	21.45
C18:00	7.42	4.20	6.41	7.20	11.05
C20:00	2.60	3.45	0.96	2.29	0.67
C21:00	1.66	6.07	0.28	1.34	1.23
C22:00	2.25	2.50	1.02	2.46	2.09
C24:00	0.83	0.66	0.16	1.59	1.73
Total *SFA*	***50.26*^{a}**	***40.95*^{bc}**	***34.50*^{c}**	***48.54*^{a}**	***46.53*^{a}**
C14:01	0.53	0.45	0.63	0.18	0.39
C15:01	n.d.	0.34	23.92	0.04	0.57
C16:1n7	1.21	0.70	1.42	4.41	0.54
C18:1n9t	7.42	4.20	4.47	7.68	17.73
C18:1n9c	3.19	2.34	0.90	4.06	0.86
C20:1n9	5.02	1.77	0.34	0.45	0.66
C22:1n9	3.43	0.41	0.40	0.56	2.92
C24:1n9	6.09	3.45	0.29	0.27	0.24
Total *MUFA*	***16.66*^{c}**	***13.73*^{c}**	***32.34*^{a}**	***17.64*^{bc}**	***23.92*^{b}**
C18:2n6t	9.92	26.05	7.20	5.58	5.88
C18:2n6c	1.09	0.61	0.44	2.17	0.80
C18:3n3	1.30	2.50	5.82	0.34	0.74
C18:3n6	4.51	1.39	0.88	4.61	0.90
C20:02	3.94	1.48	0.18	0.53	4.25
C20:3n6	1.23	0.23	0.13	0.06	0.44
C20:3n3	3.09	2.00	0.99	7.02	2.02
C20:4n6	1.19	3.02	0.46	0.37	3.33
C20:5n3	0.13	1.02	12.62	0.66	0.09
C22:02	1.13	0.30	n.d.	0.65	0.69
C22:5n3	3.47	4.14	3.77	0.44	6.05
C22:6n3	2.04	2.59	0.66	11.43	4.34
Total PUFA	***33.08*^{b}**	***45.32*^{a}**	***33.16*^{b}**	***33.81*^{b}**	***29.55*^{b}**
***n*-3**	**10.04^{c}**	**12.25^{c}**	**23.87^{a}**	**19.88^{b}**	**13.24^{c}**
***n*-6**	**20.66^{b}**	**32.55^{a}**	**9.16^{d}**	**13.26^{c}**	**15.17^{c}**
PUFA: SFA	**0.66^{b}**	**1.11^{a}**	**0.96^{ab}**	**0.70^{b}**	**0.64^{b}**
n-6: n-3	**2.06^{ab}**	**2.66^{a}**	**0.38^{d}**	**0.67^{c}**	**1.15^{b}**

*Values are presented as mean percentages of the total fatty acid methyesters (FAME) extracted.
$^{a-d}$Values in a row without a common superscript are significantly different (P<0.05).

SFA ratio above 0.4 (Mostert & Hoffman, 2007). The PUFA: SFA ratios in all carob cultivars analysed in the present study (Table 4) ranged between 0.64 and 1.11. This is well in agreement with the above mentioned recommendation.

Also worth considering is the ratio of essential *n*-6 (linoleic acid) to essential *n*-3 (linolenic acid) (popularly classified as Omega 6 and Omega 3). These fatty acids are termed “essential fatty acids” as the human body does not have the ability to synthesize them in sufficient amounts (Williams, 1993) and they must therefore be included in the diet. The human body requires docosahexanoic acid (DHA) (Omega 3) for its normal functioning. The body is also able to synthesize its own DHA provided it has sufficient supply of linolenic acid (Omega 3) (Williams, 1993). However, this process could be very slow especially when the diet is high in linoleic acid (Omega 6) and arachidonic acid (Omega 6) as these two groups of essential fatty acids (Omega 3 and Omega 6) compete for metabolic enzyme systems (Simopoulos, 1999). These enzyme systems prefer the *n*-6 over the *n*-3. Many people tend, therefore, to be deficient in *n*-3 as a result of imbalanced proportional (*n*-6: *n*-3) intake.

In terms of the *n*-6: *n*-3 ratio, SFax, Aaronsohn, Santa Fe and the Unknown cultivar differed significantly ($P<0.05$) from each other. Tylliria only differed significantly from Aaronsohn and Santa Fe. Although some differences were observed among cultivars in terms of *n*-6: *n*-3 ratio, all cultivars analysed had good quality long-chain fatty acid compositions in terms of the *n*-6: *n*-3 ratio, when applying the British recommendations. This is evident since all cultivars were below (0.38 – 2.66) the recommended maximum level of 4. Although South African legislation does not specify a specific *n*-6: *n*-3 ratio, the British Department of Health recommends a maximum ratio of four (4) (Mostert & Hoffman, 2007).

Ash

The ash contents of the five carob cultivars are given in Table 1. Overall, the ash content in all cultivars in this study ranged between 2.13 and 2.69 g.100 g^{-1}. Slight variations in the ash content were present for the five carob cultivars. The ash content of Tylliria differed significantly ($P<0.05$) from that of Sfax, Santa Fe and the Unknown cultivar. SFax differed significantly from all the other cultivars, while Aaronsohn only differed significantly from SFax and the Unknown cultivar.

The ash content gives a general indication of the mineral content present in the cultivars. The values found fall well within the range found by other researchers (1.00 –

4.1 g.100 g^{-1}) (Calixto & Cańellas, 1982; Bravo *et al*., 1994; Avallone *et al*., 1997; Yousif & Alghzawi, 2000; USDA, 2006).

Mineral content

The mineral content of the five carob cultivars (pods) investigated is given in Table 5. The average mineral composition ranges of raw carob pods were (in mg.100 g^{-1} dry matter): 135.67 – 302.67 calcium; 44.00 – 92.33 phosphorus; 852.33 – 1 091.33 potassium; 55.00 – 99.00 magnesium; 4.41 – 14.45 sodium; 0.59 – 1.23 manganese; 0.47 – 0.98 iron; 0.07 – 0.23 copper; and 0.11 – 0.69 for zinc. Overall, significant (P<0.05) differences in individual mineral compositions could also be observed among the cultivars. Moreover, all nine minerals (calcium, phosphorus, potassium, magnesium, sodium, manganese, iron, copper and zinc) analysed for in this study were detected in all five carob cultivars.

The current (since 2002) and the proposed (June 2007) recommended dietary allowances (RDA) for some mineral nutrients are given in Table 6 (Anon., 2002; Anon., 2007). According to guidelines provided by the current South African food labelling regulation (Anon., 2002), a food substance can be labelled as a source of any mineral nutrient as long as it contains 15% or more of that specific nutrient's RDA.

Similarly, a food can be labelled as a high source if the content for that particular mineral is 30% or more of the RDA. Based on these guidelines, SFax, Aaronsohn and Santa Fe may be considered as sources of calcium. The current regulation does not specify RDAs for copper and manganese but based on the proposed regulation, all cultivars, except the Unknown cultivar, would be sources of copper whilst for manganese; all cultivars would qualify as sources. None of the cultivars analysed could be considered as a source of iron, phosphorus or zinc. All cultivars may be considered as sources of magnesium. Neither the current nor the proposed regulation has specified RDA for potassium but some amounts were detected in all cultivars analysed in this study.

Another fact worth noting is the very low sodium content. This implies that if used as a food ingredient, carob powder would have a low contribution to the sodium content of final product. In South Africa, a sodium content of 40 mg.100 g^{-1} or lower, is required, for any food to be labelled "very low in sodium", as per regulation set by the South African Department of Health (Anon., 2002).

Based on observations from this study the cultivars may be considered as of relatively good nutritional quality, especially in terms of mineral composition.

Effect of roasting time on carob composition

Tylliria was the cultivar selected for use in the roasting study. The decision to use Tylliria

Table 5 Mineral composition of raw South African grown carob cultivars (mg.100g^{-1} dry matter)

Component*	Carob cultivar				
	Tylliria	**SFax**	**Aaronsohn**	**Santa Fe**	**Unknown**
Calcium	135.67 ± 0.00^{a}	302.67 ± 0.00^{b}	301.67 ± 0.00^{b}	297.33 ± 0.00^{b}	145.33 ± 0.00^{a}
Phosphorus	77.00 ± 0.00^{a}	92.33 ± 0.00^{b}	62.33 ± 0.00^{c}	77.00 ± 0.00^{a}	44.00 ± 0.00^{d}
Potassium	1 065.33 ± 0.00^{ab}	1 091.33 ± 0.06^{a}	995.56 ± 0.00^{c}	852.33 ± 0.00^{d}	1 024.00 ± 0.00^{b}
Magnesium	66.00 ± 0.00^{a}	92.00 ± 0.00^{b}	99.00 ± 0.00^{b}	91.67 ± 0.00^{b}	55.00 ± 0.00^{a}
Sodium	4.41 ± 0.19^{b}	14.45 ± 1.32^{a}	7.24 ± 0.17^{c}	9.73 ± 0.17^{d}	14.08 ± 0.06^{a}
Manganese	0.59 ± 0.04^{b}	0.99 ± 0.04^{a}	1.23± 0.01^{c}	1.09 ± 0.03^{d}	0.93 ± 0.03^{a}
Iron	0.73 ± 0.23^{ab}	0.75 ± 0.04^{ab}	0.47 ± 0.02^{a}	0.98 ± 0.16^{b}	0.67 ± 0.22^{ab}
Copper	0.20 ± 0.05^{a}	0.15 ± 0.07^{ab}	0.16 ± 0.04^{ab}	0.23 ± 0.04^{a}	0.07 ± 0.01^{b}
Zinc	0.67 ± 0.05^{a}	0.69 ± 0.02^{a}	0.67 ± 0.06^{a}	0.11 ± 0.12^{b}	0.54 ± 0.05^{a}

a, b, c, d Values in a row without a common superscript are significantly different (P<0.05).
* The values are given as means of triplicate determinations ± standard deviation.

Table 6 Minimum recommended dietary allowances (RDA) of mineral nutrients for persons 4 years and older (Anon., 2002; Anon., 2007)

Mineral nutrient	**RDA (mg) as per current guidelines**	**RDA (mg) as per proposed guidelines**
Calcium	1 100.0	1 300.0
Copper	N.S.	0.9
Iron	14.0	18.0
Magnesium	350.0	420.0
Manganese	N.S.	2.3
Phosphorus	880.0	1 250.0
Zinc	15.0	11.0
Potassium	N.S.	N.S.

N.S. = Not specified

was based on the results of the proximate compositional analysis of the raw pods as given in Table 1. These results showed that there were relatively minor (although statistically significant) differences among the five carob cultivars in terms of protein and fat contents. In terms of the sugar content, Tylliria was much higher than all the other cultivars analysed. It was thus assumed that a high initial sugar content, as found in the raw Tylliria pods, could be used to illustrate the effect of roasting time, more clearly. It was further assumed that the effect of roasting time in the other cultivars would follow a pattern similar to that would be found when using Tylliria for the roasting study.

The results of the roasting study are presented in Table 7. Data clearly indicates that roasting at 150°C for 45 min resulted in a significant (P<0.05) reduction in the moisture content with further reductions after roasting for longer periods (60 and 75 min). This clearly illustrates how roasting can be used advantageously to reduce the moisture content in order to extend the shelf-life of carob as well as to allow for easier milling.

A significant reduction (equivalent to 22%) was also observed in the total sugar content after roasting for 45 min. It is interesting to note that roasting for a further 15 min (total of 60 min) gave no significant (P<0.05) reduction in the total sugar content. There was a significant (P<0.05) reduction after roasting for a total of 75 min (in comparison to the 45 min roasting time). Roasting for 75 min did not make a further significant (P>0.05) reduction on the total sugar content, when compared to the 60 min roasting time. The impact of roasting time on the sucrose content which is the major sugar, followed a similar trend to that on the total sugar content.

After roasting for 45 min it was found that the glucose content was reduced significantly (P<0.05) but, roasting for further 15 min (total of 60 min) showed no further significant reduction. Although there was a significant reduction between 45 min and 75 min roasting times, no significant reduction was observed between the 60 min and the 75 min roasting times. Similarly, the fructose content was significantly reduced after 60 min of exposure. Although there was a significant reduction between 45 min and 75 roasting times, no significant reduction was observed either between the 45 min and 60 min roasting times or between the 60 min and 75 min roasting times.

The impact of roasting time on the protein content followed a similar trend to that of the glucose content. A reduction in the protein (and sugar) content observed after roasting could be ascribed to the Maillard reaction and caramelisation taking place during the roasting process (Blenford, 1988; Sikorski, 2003; Jones, 2005). The products of these two chemical reactions (Maillard reaction and caramelisation) could be responsible for carob's desirable nutty chocolate-like flavour and colour.

Table 7 The impact of roasting time (at 150°C) on the moisture, protein, sugar and fat content of Tylliria carob pods (g.100 g^{-1} dry matter)

Component*	Roasting time			
	0 min (Unroasted)	**45 min**	**60 min**	**75 min**
Moisture	9.27 ± 0.40[a]	3.54 ± 0.15[b]	2.53 ± 0.08[c]	1.33 ± 0.00[d]
Total sugars	54.74 ±1.35[c]	40.48 ± 2.26[b]	35.91 ± 1.16[ab]	32.53 ± 0.84[a]
Sucrose	45.09 ±1.49[c]	32.74 ± 2.24[b]	28.90 ± 1.59[ab]	26.58 ± 0.52[a]
Glucose	4.92 ± 0.15[b]	3.61 ± 0.31[a]	3.31 ± 0.19[a]	1.79 ± 0.45[a]
Fructose	4.73 ± 0.43[a]	4.14 ± 0.36[ab]	3.70 ± 0.09[bc]	3.26 ± 0.69[c]
Protein	3.59 ± 0.11[b]	3.30 ± 0.11[a]	3.29 ± 0.11[a]	3.18 ± 0.01[a]
Fat	0.71 ± 0.06[a]	0.70 ± 0.04[a]	0.66 ± 0.04[a]	0.63± 0.04[a]

[a, b, c, d] Values in a row without a common superscript are significantly different (P<0.05).
* The values are given as means of a triplicate determinations ± standard deviation.

In principle, a reduction in the lipid content would be expected after exposure to high temperature such as during roasting (Yousif & Alghzawi, 2000). When exposed to high temperatures in the presence of oxygen, lipids would normally be oxidised, which leads to their decomposition (Sikorski, 2003). In this study, roasting showed no significant (P>0.05) effect on the fat content, even after the longest exposure (75 min). The low initial fat content (and thus, low surface area to volume ratio) of raw carob powder is perhaps a reason for this.

The findings of the roasting study are in agreement with those of other investigators (Yousif & Alghzawi, 2000). Roasting significantly reduced protein and sugar content. Roasting is, however, reported to improve the sensory acceptability of carob (Yousif & Alghzawi, 2000). The fact that an increase in roasting time significantly reduced the sugar and protein content indicates the necessity for the optimisation of the roasting conditions, such that the protein and sugar content in the final product (roasted powder) would not be severely affected.

Conclusion

Overall, this study has revealed that there are statistically significant (at 95% confidence level) differences in composition among the five carob (*Ceratonia siliqua*) cultivars studied, in terms of all components analysed in this study, except for the polyphenolic content which did not differ (P>0.05) among cultivars. This study also confirmed that South African grown carob has a good nutritional value. This is made evident by its carbohydrate, individual sugars, dietary fibre, polyphenol, calcium, magnesium and essential amino acids (nutritionally important components) content. Although the protein content was slightly low for all five cultivars studied, carob's protein is still worth highlighting because of its good quality (seven essential amino acids detected). Carob can be regarded as a healthy food source because of its very low fat content. Although it had a very low total fat content (as confirmed in this study), carob's fatty acid composition was of good quality, in terms of *n*-6: *n*-3 and PUFA: SFA ratio. Furthermore, the low fat content might also contribute to carob's long shelf-life.

Although roasting significantly reduced the sugar and protein contents, the levels in both raw and roasted carob (Table 7) still represent a potentially nutritious food source. Based nutritional compositional information revealed in this study, it can, therefore, be concluded that South African grown carob cultivars are a rich source of various nutrients. It can thus be concluded that when incorporated in "new product" formulations, carob

would add to the nutritional value thereof. The results from the study on the effect of roasting time on the chemical composition could be useful when further processing is done, as it may allow for an informed choice of roasting time whereby the temperature sensitivity of nutritionally important components can be taken into consideration.

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Chapter 4

DEVELOPMENT OF NUTRITIONAL FOOD PRODUCTS FOR A RANGE OF MARKET SECTORS USING CAROB (*Ceratonia siliqua*) AS AN INGREDIENT

Summary

Semi-arid climatic conditions, large human populations, high unemployment rates, hunger and malnutrition, are typical features of Southern African countries. Nutritional, alternative food sources are one means of alleviating hunger and malnutrition. The carob (*Ceratonia siliqua*) tree is well adapted to arid conditions, and its pods can be ground into a nutritious powder which can be used as an ingredient for a variety of nutritional products. This species is also found in South Africa, especially in the Western Cape Province where the climate is similar to that of its origin (Mediterranean climate). In this work, the feasibility of using carob as an alternative food source was explored by developing new food products with or by incorporating carob , and thus five food products (bread, porridge, breakfast cereal flakes, a mousse and a milk-based beverage) were successfully developed. All developed products were microbiologically safe (after production) and organoleptically acceptable. Overall, the study has proven that carob can be used or incorporated in food products as an alternative or to enhance the nutritional content of the products.

Introduction

Like in many other third-world countries, the South African population is faced with nutritional problems such as energy and micro-nutrient deficiencies (Bayley, 1995; Walker *et al*., 1996). One way to improve the nutritional status is by making good use of what is available locally. Carob (*Ceratonia siliqua*), an evergreen tree species from the Mediterranean region, has been widely propagated in South Africa, especially in the Western Cape Province (Muller, 2005). Although carob has mainly been used as an animal feed source, few people in South Africa are aware of its potential in human nutrition.

Carob is reported to have been used as a food source for many centuries. In ancient societies (more than 4 000 years B.C.), carob pods were consumed by children as candies, mainly due to the high sugar content (as high as 54.74 g.100 g^{-1}) (Chapter 3). Carob is sometimes also known as “St. John’s bread”. This is based on the assumption that John the Baptist survived on carob pods for 40 days in the desert (Kumazawa *et al*.,

2002). Generally, the use of carob as food is reported to have been very common during difficult times, for instance during wars or famine periods (Avallone *et al*., 1997; Berna *et al.*, 1997; Brandt, 2002; Haber, 2002).

Carob imparts a nutty flavour similar to that of cocoa and chocolate but unlike cocoa, carob does not contain any caffeine or thiobromine (Marakis, 1996; Yousif & Alghzawi, 2000; Biner *et al.*, 2007). Furthermore, carob has a very low (0.45 – 0.86 g.100 g^{-1}) fat content and is high in dietary fibre (up to 36.07 g.100 g^{-1}) (Chapter 3). Carob is regarded as a healthier source of food than cocoa and has thus been commonly used as an alternative (replacer or extender) to cocoa (Blenford, 1988). The deseeded pod (roasted or non-roasted) can be milled into a powder which may then be used as an ingredient in a variety of processed food products (Yousif & Alghzawi, 2000).

Cereal products generally form a major part of the diet. Some of the common, but basic cereal products found on the South African market today include bread, porridge and breakfast cereals. The development of recipes which include carob as an ingredient present an opportunity for the food industry. In many parts of the world, bread is generally regarded as a cheap and convenient source of energy in the diet (Wang *et al*., 2002). A bread formulation with carob (6%) as one of the ingredients would not only introduce a completely new exotic flavour to the consumer market but would also improve the dietary profile, especially since the fibre content of carob makes it a good source (up to 36%) of dietary fibre (USDA, 2006). Similarly, the incorporation of carob (49%) into breakfast cereals (Ackermann *et al*., 2006) would not only result in an increase of dietary fibre, but also an increase in polyphenol content, as well as giving a product with a low fat content (Marakis, 1996; USDA, 2006).

Another opportunity for using carob as a food ingredient is in desserts (mousse) (Lötter *et al*., 2006) and dairy-based beverages. Although such products come in a variety of flavours (for example caramel, vanilla, chocolate and fruity flavours), chocolate as a flavour, dominates especially among young to middle-aged consumers (Lang, 1982; T Flandorp, 2007, personal communication). Due to similarities between carob and cocoa flavour, a carob flavoured mousse (Lötter *et al*., 2006) and a carob milk-based beverage (classified as a shake) could definitely compete with similar products currently available on the South African market.

The aim of this study was to develop food products with improved nutrient profiles for a range of market sectors, using carob as an ingredient. To attain this objective, the following products were developed: a bread and a porridge type product targeted to the low-income market sector (LSM 1-4); breakfast flakes for the middle-income sector (LSM

4-6); and a mousse and a milk-based drink for the high income consumer sector (LSM 7-10). Another objective was to evaluate all products developed for nutritional composition, microbiological safety and for consumer acceptability in terms of appearance and flavour.

Materials and methods

Carob pods

Five South African grown cultivars were used in this study. These included four known cultivars (Tylliria, SFax, Aaronsohn and Santa Fe) collected from the Eenboom Farm, in the Western Cape Province (Malmesbury district), South Africa and one "Unknown" cultivar collected from the Elsenburg Agricultural Research Station of the Western Cape Department of Agriculture, South Africa. The cultivars were identified by Mr Freddy Rust (F Rust, 2007, personal communication).

Cleaning and storage

The pods were cleaned with a brush and water to remove any soil, mould or dirt from the pod surface. The pods were then air dried at 37˚C for 48 h and stored at room temperature in well aerated nylon bags until further processing.

Kibbling

The seeds were separated from the husk by cutting open the pods with a hinged cutting blade. The kibbles and all the off-cuts were collectively vacuum-packed in polyethene bags and stored at 4°C until needed for further processing.

Roasting

Kibbles were roasted at 150°C for 60 min (Berna *et al.*, 1997; Yousif & Alghzawi, 2000; Viljoen *et al*., 2002) in an oven (Defy 831) attached to a computerised temperature control system. After cooling to room temperature, the roasted kibbles were then vacuum-packed in polyethene bags and stored at 4°C until needed for further processing.

Milling

Carob kibbles (roasted or non-roasted) were milled using a small-scale hammer-mill (Scientec, Cape Town) with a 0.5 mm pore size sieve. The resultant powder was vacuum-packed in polyethene bags and stored at 4°C until needed.

Sieving

All carob powders used in the product development process were sieved through a laboratory test sieve (300 µm size) (Endecotts, BS 410, London) by using a laboratory-scale shaker (Endecotts, EFL 2000/1, London). The resultant powders were then vacuum-packed in polyethene bags and stored at 4°C until needed for further processing.

Microbiological analysis

The carob powders and all final products were analysed for total aerobic mesophilic microorganisms, coliforms and *Escherichia coli*, yeasts and moulds, aerobic endospore-forming bacteria *and Staphylococcus aureus*. The powders were also analysed for anaerobic endospore-forming bacteria.

Plate count agar (PCA) (Biolab), violet red bile agar (VRBA) (Biolab) and potato dextrose agar (PDA) (Biolab) were used for enumerating the total aerobic mesophilic microorganisms, coliforms and *E.coli* and the yeasts and moulds, respectively. Tryptic Soy Agar (TSA) (Biolab) was used for the detection of both the aerobic and anaerobic endospore-forming bacteria whereas for *S. aureus,* Baird Parker's agar (Biolab) supplemented with 50% egg yolk emulsion (Biolab) (5 mL per 90 mL agar) and 1% potassium tellurite (Biolab) (1 mL per 90 mL agar), was used. All microbiological media were prepared according to the manufacturers' instructions.

The sample (10 g) and 90 g sterile physiological saline solution (PSS) (0.75% m/v) were aseptically transferred to a stomacher bag. The bag was placed in a stomacher (Interscience, W (window door)) and the content (10^{-1} dilution) was homogenised for 4 min. A serial dilution (up to 10^{-8}) of the sample was prepared for each sample type. Microbiological tests were then carried out according to standard, using the South African National Standards (SANS) (as adopted from International Standards Organisation (ISO)), namely: SANS 4833 (2007) for total aerobic mesophilic microorganisms; SANS 4832 (2007) for coliforms and *Escherichia coli*; SANS 7954 (1987) for yeast and moulds; SANS 7932 (2005) for *Bacillus cereus* and other aerobic endospore-forming bacteria; SANS 7937 (2007) for *Clostridium perfringens* and other anaerobic endospore-forming bacteria; and SANS 6888-1 (1999) for *Staphylococcus aureus*.

In-house panel of judges

The in-house panel of judges consisted of six semi-trained panellists from the Department of Food Science, University of Stellenbosch. This panel, at the various stages of the product development process, reviewed (including sensory evaluation) and discussed the outcomes. Such discussions were necessary as vital qualitative information derived

provided guidance for further product development until a final formulation was eventually reached, for every product developed (CCFRAG, 1996).

As the compositional variations between the five cultivars were too small (Chapter 3), selection of the two cultivars to proceed with during further product development had to be based on sensory characteristics. The in-house panel was involved in making this choice. Porridges (Table 2) made with specific cultivars were used for this purpose.

Formulation and production of bread

The straight dough method was followed for bread making (Mariotti *et al*., 2006). For all final products, ingredients (Table 1) were weighed by using a top loading balance (Mettler, PJ6000). Bread flour (250 g) and all other ingredients (excluding margarine) were kneaded for 10 min using an electric mixer (Kenwood, KM 220), after which a small amount of bread flour (10 g) was added in order to prevent the dough from sticking onto the mixer's side wall and the dough was mixed once again for 1 min. A small amount of margarine (5 g) was melted and used to cover the dough so as to prevent the dough from drying out during subsequent dough proving steps. The dough was placed into a porcelain bowl, which was then covered using a plastic wrap. The dough was left to raise (initial proofing) for 20 min at 35˚C. The dough was then punched down and uniformly formed by hand, placed into a baking pan and once again left to prove (final proving) for 20 min at 35˚C. Bread was then baked for 25 min at 200˚C. The semi-trained in-house panel of judges evaluated three formulations (1A, 1B and 1C) to determine which formulation produced the most optimal sensory profile (Table 1). This involved variations in the amounts of carob powder used in the formulation. Similarly, three baking times (20, 25 and 30 min) were also compared to determine the optimal baking time.

Formulation and production of porridge

All the ingredients (Table 2), except milk powder and boiling H_2O, for the production of the carob porridge were weighed into a glass bowl. After mixing, boiling H_2O was added and the whole mixture was again well stirred. The mixture was then cooked in a microwave oven (Sharp, Model 4184) which was set at the highest power (600 watts), for 9 min. During the heating phase, the porridge was stirred four times (after 1, 2, 6 and 8 min) in order to prevent the formation of lumps. A small amount of salt (Table 2) was added for taste. Lastly, milk powder was stirred into the mixture. The respective formulations (Table 2) were evaluated by the semi-trained in-house panel of judges in terms of sensory characteristics such as texture, colour and flavour, in order to select the most optimal formulation for the final analysis (consumer analysis).

Table 1 Formulation used in determining the optimal ingredient combination for bread

Ingredient	Formulation (g)		
	1A	**1B**	**1C**
Whole wheat bread flour	260.0	260.0	260.0
Carob powder	30.0	35.0	40.0
Sugar	30.0	30.0	30.0
Yeast (instant)	5.0	5.0	5.0
NaCl	5.0	5.0	5.0
Margarine	5.0	5.0	5.0
H_2O (37˚C)	220.0	220.0	220.0
Total mass	**555.0**	**560.0**	**565.0**

Table 2 Formulation used to determine the optimal ingredient combination for the porridge

Ingredient	Formulation (g)				
	2A	**2B**	**2C**	**2D**	**2E**
Carob powder	88.0	40.0	30.0	75.5	69.4
Maize meal	-	50.0	55.0	-	-
Corn flour	-	-	-	13.7	12.6
Milk powder (28% fat)	-	7.5	12.5	8.8	16.1
NaCl	2.0	2.5	2.5	2.0	1.9
H_2O	360.0	360.0	360.0	360.0	360.0
Total mass	**450.0**	**460.0**	**460.0**	**460.0**	**460.0**

Formulation and production of breakfast cereal flakes

Carob breakfast cereal flakes were processed according to a method originally developed by Ackermann *et al.* (2006). The optimal formulation for the flakes is given in Table 3. All ingredients were weighed by using a top loading balance (Mettler, PJ6000). Carob and water were mixed for 2 min using a manual whisk in a ceramic bowl. All other ingredients were added and the whole mixture was blended for another 2 min. The mixture was covered with a plastic shrink-wrap and placed in an oven (Defy, 427) at 60°C for 10 min, to form the dough. The dough was then rolled into flat sheets using a manual pasta machine (Atlas, 150 mm-DELUXE) initially adjusted to aperture setting 1, then setting 3 and finally at setting 5, to produce dough sheets of uniform thickness. The dough sheets were hand-broken into flakes. The flakes were then placed on an oven tray and toasted at 80°C for 3.5 h.

The inclusion of oat bran and flour was based on the sensory characteristics such as colour and perceived health-related attributes such as a high dietary fibre content (Mariotti *et al.,* 2006). Oats would also provide binding properties. Sodium chloride was added for taste. The addition of cocoa malt powder was to incorporate a flavour dimension commonly associated with breakfast cereals, so that the product would not be completely foreign (malt is commonly present in breakfast cereals). Sodium carbonate (Na_2CO_3) was added as a drying agent to prevent moisture absorption once the product was dried.

Formulation and production of mousse

The carob mousse was made according to a method originally developed by Lötter *et al.* (2006). The formulation used for making the mousse is shown in Table 4. A top loading balance (Mettler, PJ6000) was used for weighing all ingredients. Ingredients were initially weighed into three separate bowls, i.e. bowl A consisting of gelatine, sugar and boiling H_2O; bowl B consisting of a non-dairy vegetable and palm oil-based créme (Orley Whip) and yoghurt; and bowl C containing beaten egg white and carob powder. The contents of each bowl were mixed separately using an electric whisk (Kenwood, HM220). The content of bowl C was combined with that of bowl B and mixed well. This combined mixture (bowl C and B) was added to bowl A and the latter was mixed using a manual whisk. The mousse products made with roasted and non-roasted carob powder were compared and evaluated by the semi-trained in-house panel of judges to determine which is the most suitable for use in mousse.

Table 3 Formula used for the production of carob breakfast cereal flakes

Ingredient	**Amount (g)**
Carob powder	48.8
Oat flour	7.6
Oat bran	7.4
Malt powder*	2.7
NaCl	0.3
Na_2CO_3	0.1
H_2O	33.1
Total mass	**100.0**

*Milo: A powdered malted cocoa beverage mix product of Nestlé, South Africa (Pty) Ltd.

Table 4 Ingredient combination used for making the carob mousse

Ingredient	**Amount (g)**
Plain yoghurt	33.3
Orley Whip*	29.2
H_2O	17.6
Carob powder	7.3
Sucrose	6.2
Egg white	4.2
Gelatine	2.2
Total mass	**100.0**

*A non-dairy vegetable and palm oil-based créme produced by Orley Foods (Pty) Ltd., South Africa

Formulation and production of the milk-based drink

In order to determine the optimal formulation for a carob milk-based drink, a number of developmental trials (Table 5) were carried out, which mainly involved adjustments in the amount of some of the ingredients such as water, sucrose, guar gum (Chemimpo) and vanilla essence (a product of Pioneer Foods (Pty) Ltd., South Africa). The in-house panel of judges was used to determine which formulation resulted in the most optimum combination of sensory characteristics such as texture, flavour and colour. All the dry ingredients (except sucrose) were weighed in a ceramic bowl. Weighing was done using an analytical top loading balance (Precisa, 310 M). Boiling H_2O was added and the mixture was mixed for 2 min using an electric beater (Kenwood, HM220). Sucrose was added and the mixture was mixed for 1 min. The mixture was then covered and allowed to stand for at least 1 h at refrigeration temperature (approximately 4˚C). A standing time was necessary for the foam (developed during blending) to set.

A small amount of sugar (sucrose) and vanilla essence was added to enhance the flavour. The addition of a stabiliser (guar gum) was aimed at improving consistency and the overall texture of the product. Adjustments to the amount of stabiliser were aimed at obtaining an optimum consistency and texture (as determined by the in-house panel of judges). The panel was further used to compare the milk-based drink made from roasted carob with the one made with non-roasted carob, so as to determine the carob powder type that is most suitable for the production of the milk-based drink.

Nutritional content

The nutritional content of the products developed was estimated using Food Finder III software (MRC, 2006) and the chemical composition of carob (as reported in Chapter 3 of this thesis). Food Finder III was thus used to approximate the nutritional content of the non-carob ingredients. Nutritional contents were calculated for 100 g of product.

Consumer sensory analysis

For each of the five product types developed, the overall degree of liking was determined using the target consumer. For each product type, two variants (made with two different carob cultivars) were included, i.e. the Tylliria and the Unknown cultivar. The nine-point hedonic scale, which is the most common and internationally accepted method for assessing preference and acceptability of food and beverages, was used (Lawless & Heymann, 1998). This scale uses the following measurement categories: 9 = Like extremely; 8 = Like very much; 7 = Like moderately; 6 = Like slightly; 5 = Neither like nor dislike; 4 = Dislike slightly; 3 = Dislike moderately; 2 = Dislike very much; 1 = Dislike

Table 5 Formulations used for determining optimal formulation for the carob milk-based drink

Ingredients	**Formulations (g)**				
	5A	**5B**	**5C**	**5D**	**5E**
H_2O	81.55	81.15	81.00	81.10	81.20
Carob powder	3.00	3.00	3.00	3.00	3.00
Full cream milk powder	15.00	15.00	15.00	15.00	15.00
Sucrose	-	0.40	0.40	0.40	0.40
Guar gum	0.15	0.15	0.30	0.20	0.20
Vanilla essence	0.30	0.30	0.30	0.30	0.20
Total mass	**100.00**	**100.00**	**100.00**	**100.0**	**100.00**

extremely.

For bread, eighty (N = 80) consumers between the ages of 18 and 60 were sourced to analyse the product. For the carob milk-based drink and carob mousse, sixty-three (N = 63) consumers between the ages of 18 and 60 were sourced while 90 consumers (N = 90) between the ages of 18 and 60 were sourced for both carob porridge and carob flakes. The samples were served in a complete randomised order (Lawless & Heymann, 1998). The consumers were asked to complete a standard questionnaire (an example is attached as Addendem A) in comparing the samples in front of them and to give an indication of the acceptability of the colour and flavour of the samples. Each variant was served in a suitable odour-free container coded with a three digit random code.

The sample size for the bread enriched with carob was a 1 cm thick half-slice of bread, uniformly spread with 0.5 g margarine. For the porridge, the sample size was 10 g for each variant. The carob breakfast cereal flake samples (sample size 4 g) were served in containers along with 20 g of full cream ultra heat treated (UHT) milk in a separate container. Consumers were instructed to prepare the samples by pouring the milk over the samples. For the milk-based drink each consumer was presented with approximately 10 mL of each sample variant. To eliminate the influence of temperature differences among samples, all samples were evaluated within 10 min of removal from the refrigerator (4°C). Before serving, the product was briefly swirled with an electric whisk (Kenwood, HM220) in order to ensure that no carob particulates settled at the bottom of the container, and thus to ensure consistency in samples among consumer panellists. For the mousse each consumer was presented with approximately 5 g of each sample variant. All samples were evaluated within 10 min of removal from the refrigerator (4°C).

The experimental design was a complete randomised block design with each consumer analysing all possible treatments. Based on the guidelines in the literature, statistical analysis of the consumer sensory data obtained was done via the analysis of variance (ANOVA), whilst the t-test was used to ascertain whether there were significant differences between samples within the same product type. Differences with a significance level of 5% ($P \leq 0.05$) were considered as significant (Ott, 1998; SAS, 2002).

Results and discussion

Carob pods

Selection of cultivars for product development

The in-house panel of judges recommended to use of the Tylliria and the Unknown cultivar

during further product development. According to the in-house panel, these two cultivars had the best sensory quality (colour and flavour) when compared to the other three.

Sieving

Sieving through a 300 µm sieve showed that during milling, the inner flesh was ground into smaller particles than the pod skins. This was evident since the finer sections of the powder were lighter (typical of the non-skin part) in colour than the coarser skin sections. It was also found that the resultant powder (finer fraction) was still slightly bitter. The in-house sensory panel found that there was a clear reduction in the bitter flavour. This reduction in bitterness after sieving (<300 µm) suggests that the condensed tannins which are responsible for bitterness in carob pods (Silanikove *et al*., 2006) were more concentrated in the pod skins (pericarp) than the inner flesh. The use of other technologies such as abrasive peeling to remove the pericarp part (Radhakrishnaiah *et al*., 1993) before milling might contribute in resolving the problem of bitterness in carob.

Microbiological analysis

The results of microbiological tests preformed on the carob powders as well as on the final products are given in Tables 6 and 7. The data is presented as number of colony forming units per gram sample ($cfu.g^{-1}$).

Carob powders – For the non-roasted powders, the presence of microorganisms was detected (Table 6) on PCA, TSA and PDA plates, indicating the presence of aerobic mesophilic microorganisms (PCA), aerobic spore-forming bacteria (TSA), and yeast and moulds (PDA). However, no coliforms, *E. coli, S. aureus* or anaerobic spore-forming bacteria were detected in any of the samples evaluated.

Based on the data obtained, it is recommended that powders that have not been exposed to a heat treatment only be used in products which would subsequently undergo a heat treatment (for example, baking, cooking or toasting). The results from this study indicated the necessity for monitoring for these microorganisms during carob processing to ensure a good microbial quality and thus minimise spoilage of the final product.

The data (Table 6) for the roasted powders showed the presence of much lower counts in terms of aerobic mesophilic microorganisms. No counts were found on any other growth media. This clearly shows that roasting had a positive effect on the microbiological quality of the carob powders.

Although no South African legislation (Anon., 2004) specifies microbiological requirements for carob, the powders would comply when compared to legal specifications

Table 6 Microbial examination of the different carob powders used in the product development study

Sample type	Microorganisms (cfu.g^{-1})				
	Aerobic mesophilic microorganisms	**Coliforms, *E.coli* and *S. aureus***	**Aerobic spore-formers**	**Anaerobic spore-formers**	**Yeast and moulds**
Non-roasted Tylliria	2 400	ND	230	ND	2 200
Non-roasted Unknown	5 800	ND	3 300	ND	260
Roasted Tylliria	80	ND	ND	ND	ND
Roasted Unknown	1 900	ND	ND	ND	ND

cfu = Colony forming units
ND = None detected

Table 7 Microbiological contamination found in the developed products

Sample type	Microorganisms (cfu.g^{-1})				
	Aerobic mesophilic microorganisms	**Coliforms, *E.coli* and *S. aureus***	**Aerobic spore-formers**	**Anaerobic spore-formers**	**Yeast and moulds**
Tylliria Bread	200	ND	ND	ND	ND
Unknown Bread	50	ND	ND	ND	ND
Tylliria Porridge	10	ND	ND	ND	ND
Unknown Porridge	ND	ND	ND	ND	ND
Tylliria Flakes	190	ND	ND	ND	ND
Unknown Flakes	80	ND	ND	ND	ND
Tylliria Mousse	40	ND	ND	ND	ND
Unknown Mousse	30	ND	ND	ND	ND
Tylliria Drink	50	ND	ND	ND	ND
Unknown Drink	20	ND	ND	ND	ND

cfu = Colony forming units
ND = None detected

set for some other food products. For example, the aerobic mesophilic counts of all powders conformed well to specifications for rooibos tea and honeybush tea of "not more than 75 000 cfu.g^{-1}" (Anon., 2004). The absence of coliforms and *E. coli* suggests that the powders contained no microbial contamination of a faecal nature. Anaerobic spore-forming bacteria were also not detected in any of the carob powders.

Developed products – Relatively low (≤200 cfu.g^{-1}) aerobic mesophilic microbial counts were found in the products developed (Table 7). The only organisms detected were aerobic mesophiles, and the counts complied to legislation when compared to standards specified for other products. Dairy products for example, may contain up to 50 000 cfu.g^{-1}, whereas up to 20 000 cfu.g^{-1} is permitted in eggs and egg products (Anon., 2004). None of the products developed in this study contained more than 200 cfu.g^{-1}, aerobic mesophilic microorganisms (Anon., 2004). Coliforms, *E. coli, S. aureus*, aerobic and anaerobic spore-forming bacteria, yeast and moulds, were not detected in any of the products developed (Table 7). This provides assurance of microbial safety for such products after product. Nevertheless, the importance of hygienic processing and handling must never be underestimated.

Bread

Formulation and bread production process

Although Formulations 1A, 1B and 1C (Table 1) were all acceptable in terms of the sensory characteristics, Formulation 1B had a more acceptable carob flavour than Formulation 1A and 1C, as judged by the in-house panel. Formulation 1B was thus selected as the optimal formulation for the carob bread, and was used in further trials. Formulation 1B was subjected to further baking-time trials to determine the optimum baking time at 200°C. Three baking times (20, 25 and 30 min) were tested. According to the in-house panel, the 25 min baking time produced the bread with the best overall bread quality in terms of appearance and flavour. Baking for 20 min produced an under-baked product, whilst baking for 30 min produced over-baked bread, characterised by an undesirable dark-coloured and hard crust. Bread made using Formulation 1B and the 25 min baking time was, therefore, selected for further analysis by a consumer panel.

Nutritional content

The nutritional information of the final bread is given in Table 8. According to the current (Anon., 2004) and the proposed (Anon., 2007) guidelines provided by the South African

Table 8 Estimated nutritional contents of the products developed (amounts per 100 g)

Component	Product				
	Bread	**Porridge**	**Flakes**	**Mousse**	**Drink**
Energy (kJ)	904.1	265.5	1362.0	470.5	319.9
Carbohydrates(g)	47.9	18.9	85.9	15.0	12.6
Total sugar (g)	8.6	10.4	39.6	2.5	11.2
Sucrose (g)	8.2	6.7	34.4	2.0	2.0
Glucose (g)	0.1	0.3	1.6	0.2	0.2
Fructose (g)	0.3	0.7	3.4	0.3	0.1
Lactose (g)	0.0	0.0	0.0	0.0	0.0
Dietary fibre (g)	5.3	4.6	25.5	2.5	1.1
Protein (g)	6.4	1.5	6.1	4.1	2.7
Total fat (g)	1.6	0.7	3.2	8.4	2.0
SFAs (g)	0.3	0.6	0.9	6.5	1.8
MUFAs (g)	0.3	0.1	1.0	1.2	0.2
PUFAs (g)	0.7	0.1	1.1	0.1	0.0
Cholesterol (mg)	0.0	0.0	1.6	3.0	1.0
Sodium (mg)	353.3	200.9	238.0	47.9	0.5

SFAs = Saturated fatty acids
MUFAs = Monounsaturated fatty acids
PUFAs = Polyunsaturated fatty acids

Department of Health regarding labelling and advertising of foodstuffs, the developed bread can be a source of energy (904.1 kJ.100 g^{-1}), protein (6.4 g.100 g^{-1}) and dietary fibre (5.3 g.100 g^{-1}). The bread was also high in total carbohydrates (47.9 g.100 g^{-1}) and it had a low fat content (1.6 g.100 g^{-1}). Based on the nutritional information, the bread may therefore be considered as a nutritious (due to its energy, protein and carbohydrates contents) and healthy (because of its dietary fibre and fat contents) product. When compared to other bread products currently available on the South African market, this product had a similar nutritional profile, although it was slightly richer in terms of energy, protein and dietary fibre contents.

Consumer acceptability

The degree of liking for two carob bread samples based on colour is illustrated in Table 9. The total group of consumers preferred the colour of the two bread samples equally (P>0.05), with no significant difference in their preference. The males and females illustrated a similar pattern (P>0.05), preferring the two samples equally. The overall mean hedonic value for colour for the total group was higher than 7 for both Tylliria and the Unknown cultivar, illustrating that the addition of 10.3% carob (either Tylliria or the Unknown cultivar) had a positive effect on the appearance of the bread. As clearly illustrated in Fig. 1, 80% of the consumers (N = 80) described the colour of both carob bread samples as highly acceptable, by choosing the following positive classes on the nine-point hedonic scale: *Like moderately, Like very much* and *Like extremely*. This illustrates that the fortification of bread with carob has an extremely positive effect on the colour of bread.

According to the data in Table 10, the total group of consumers preferred the overall flavour of the two bread samples equally. There was thus no significant difference in the degree of liking for flavour (P>0.05). The male and female consumers illustrated a similar pattern (P>0.05). The overall mean value for flavour for the total group is higher than 7 for both Tylliria and the Unknown cultivar. This indicates that the addition of carob powder (at 10.3%) had a positive effect on the flavour of the product. Based on flavour, more than 80% of the respondents rated the product as *Like extremely* to *Like moderately* (Fig. 2) on the nine-point hedonic scale. This indicates that the flavour of the enriched product is highly acceptable. It is assumed that the natural sweet taste of both carob cultivars (Batlle & Tous, 1997; Zografakis & Dasenakis, 2000) has played a significant role. This result further illustrates that the fortification of bread with carob was highly successful.

Table 9 Degree of liking for bread samples based on colour

Treatment	Mean hedonic scale values		
	Total group (N = 80)	Female consumers (N = 57)	Male consumers (N = 23)
Tylliria	7.23[a]	7.21[a]	7.26[a]
Unknown	7.30[a]	7.30[a]	7.30[a]
LSD (*P* = 0.05)	0.26	0.26	0.65

LSD - Least Significant Difference at *P* = 0.05.
[a]Means within the same column with different superscripts differ significantly (*P*≤0.05).

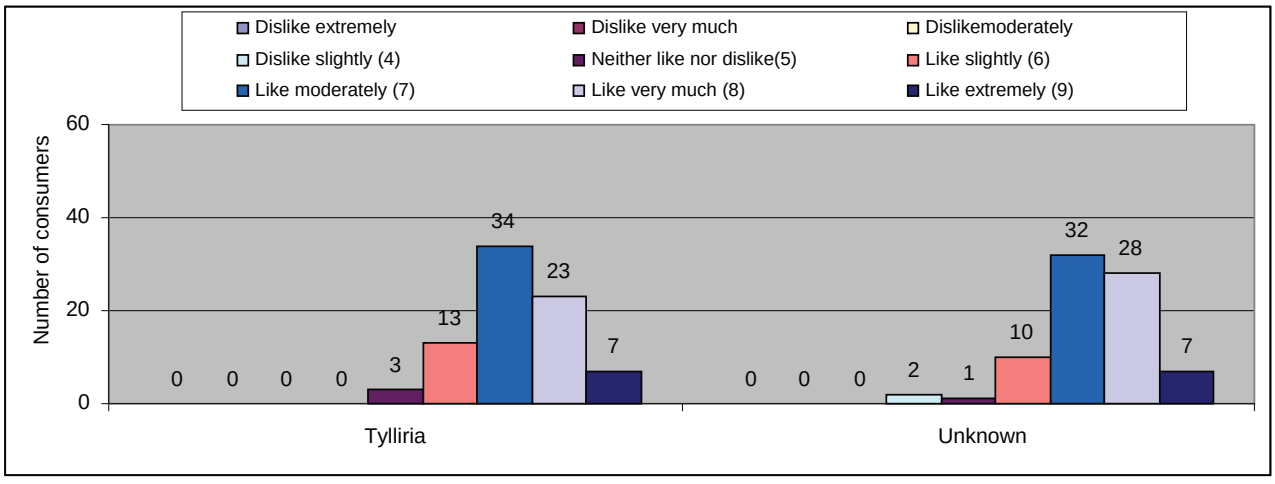

Figure 1 Acceptability of the colour of bread samples fortified with carob for the total consumer group (N = 80).

Table 10 Degree of liking for bread samples based on flavour

Treatment	Mean hedonic scale values		
	Total group (N = 80)	Female consumers (N = 57)	Male consumers (N = 23)
Tylliria	7.43[a]	7.43[a]	7.44[a]
Unknown	7.56[a]	7.54[a]	7.61[a]
LSD (*P* = 0.05)	0.27	0.32	0.56

LSD - Least Significant Difference (*P* = 0.05).
[a]Means within the same column with different superscripts differ significantly (*P*≤0.05).

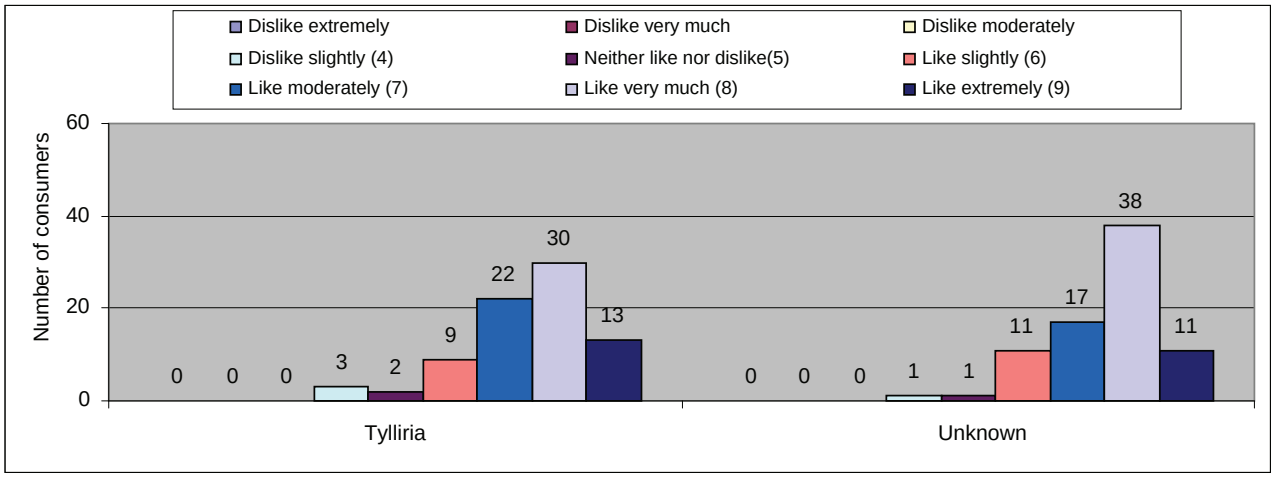

Figure 2 Acceptability of the flavour of bread samples for the total group of consumers (N = 80).

Porridge

Formulation and production process

Different formulations for the carob porridge (Table 2) produced products of varying sensory characteristics. Since carob has poor gelatinisation properties, the exclusion of cereal raw materials (maize meal and corn flour) as in Formulation 2A, did not result in a product of acceptable porridge-like consistency. Combinations of carob powder and maize meal (Formulation 2B and 2C) resulted in products with an astringent mouthfeel and bitter after-taste. The resultant porridges also had an undesirable grainy mouthfeel. The inclusion of a low percentage of corn flour in the formulation resulted in an acceptable texture and appearance, whilst the addition of milk powder introduced a slight creamy mouthfeel (Formulation 2D). Increasing the amount of milk powder (Formulation 2E) further enhanced the creaminess as intended. Formulation 2E was, therefore, selected as the optimal formulation for "carob porridge" by the in-house panel of judges to be analysed further by the consumer panel. This porridge (2E) had a characteristic sweet flavour in the absence of any added sugar. The natural sweetness (due to the high sucrose content = 45 g.100 g^{-1}) is typical of carob (Chapter 3 of this thesis).

Nutritional content

The nutritional content of the final formulation for the porridge developed is presented in Table 8. The porridge developed can be considered as source of energy (265.5 g.100 g^{-1}) (Anon., 2004; Anon., 2007), mainly due to a high carbohydrate content (18.9 g.100 g^{-1}). A large contribution of sugar (over 50%) to the total carbohydrate content was as expected since a high sugar content is typical of carob (Biner *at al.*, 2007). The product's appreciable dietary fibre content (4.6 g.100 g^{-1}) and its low fat content (0.7 g.100 g^{-1}) (Anon., 2004; Anon., 2007) would also imply that it could have health benefits (Taylor & Pye, 1974; Perez-Olleros *et al.,* 1999). The protein content was found to be low (1.5 g.100 g^{-1}). The nutritional profile was close to that of similar products currently on the market, except for the energy and fat contents which were considerably lower for the porridge developed in this study. For this reason (lower fat content) the "carob porridge" can be viewed as a healthier alternative to similar products currently on the South African market.

Consumer acceptability

The total group of consumers as well as female consumers preferred the overall colour of the two porridge samples equally (P>0.05). Male consumers preferred the colour of porridge made from the Unknown cultivar significantly (P≤0.05) more than that made with

Tylliria (Table 11). The overall mean value for colour for the total group is higher than 6 for both Tylliria and the Unknown cultivar. This illustrates that the use of up to 70% carob powder in a porridge formula will result in a product of an acceptable colour. More than 60% of the total group (N = 90) of consumers described both products as *Like extremely, Like very much, Like moderately* and *Like slightly (*Fig. 3*)*. This indicates, therefore, that the colour of both samples was acceptable.

The total group as well as female consumers indicated a significantly ($P \leq 0.05$) higher preference for the flavour of the Unknown cultivar (Table 12). Male consumers liked the flavour of both samples equally ($P > 0.05$). The overall mean values for flavour were between 5 and 6 for both samples, which illustrate that this group of consumers liked the flavour of the products moderately. More than 50% of the total group of the consumers described both products as *Like extremely, Like very much, Like moderately* and *Like slightly (*Fig. 4*)*. This illustrates that the flavour of both porridge samples was reasonably acceptable.

Breakfast cereal flakes

Formulation and production process

The breakfast cereal flakes produced had a good appearance, in terms of colour and texture, according to the in-house panel. Once soaked in cold or hot milk, the flakes could easily wet, and did not turn soggy even after a 10 min of soaking. The in-house panel liked the product for its overall flavour and appearance, although a formal consumer analysis was still necessary, in order to scientifically determine the acceptability of the product.

Nutritional content

The nutritional information of the final breakfast cereal product is given in Table 8. Based on the current (since 1997) and the proposed (June 2007) guidelines of the South African Department of Health regarding the labelling and advertising of foodstuffs (Anon., 2004; Anon., 2007), the flakes are a high source of energy (1362 g.100 g^{-1}) and carbohydrates (85.9 g.100 g^{-1}). The product was also high in sugar (39.6 g.100 g^{-1}); and a source of protein (6.1 g.100 g^{-1}). The flakes may be regarded as healthy due to a low fat content of 3.2 g.100 g^{-1} (Anon., 2004; Anon., 2007). The product was similar to other breakfast cereals found on the market, in terms of the nutritional content. Clearly notable was the dietary fibre content (25.5 g.100 g^{-1}), which was even higher than that of whole grain breakfast cereal products found on the market. This indicates that the use of carob as an

Table 11 Degree of liking for carob porridge samples based on colour

Treatment	Mean hedonic scale values		
	Total group (N = 90)	Female consumers (N = 69)	Male consumers (N = 21)
Tylliria	6.40[a]	6.49[a]	6.10[b]
Unknown	6.65[a]	6.57[a]	6.90[a]
LSD (*P* = 0.05)	0.28	0.32	0.54

LSD - Least Significant Difference (*P* = 0.05).
[a-b]Means within the same column with different superscripts differ significantly (*P*≤0.05).

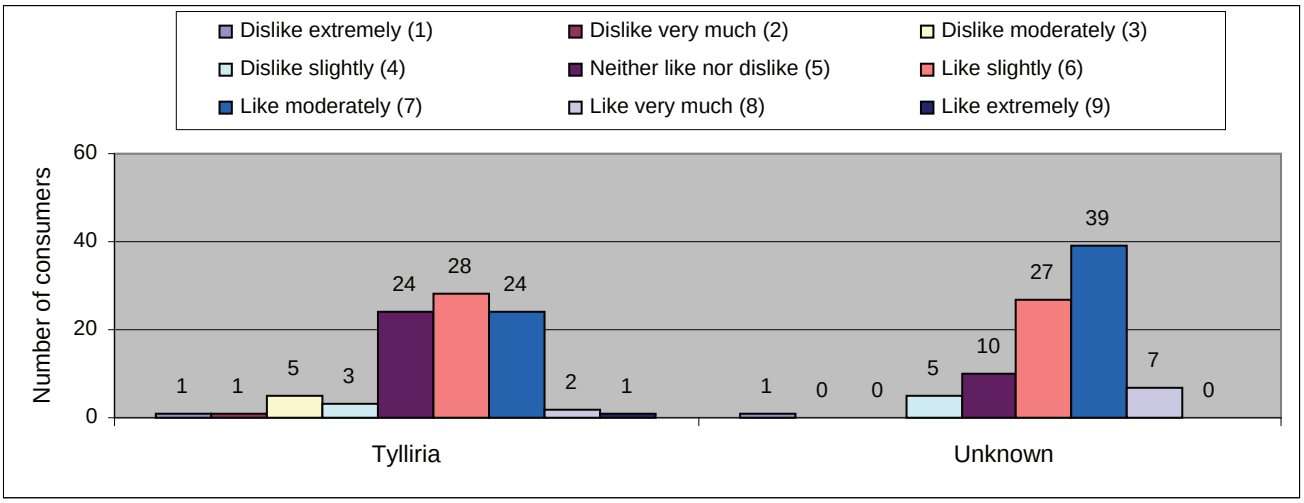

Figure 3 Acceptability of the colour of carob porridge samples for the total group (N = 90).

Table 12 Degree of liking for carob porridge samples based on flavour

Treatment	Mean hedonic scale values		
	Total group (N = 90)	Female consumers (N = 57)	Male consumers (N = 23)
Tylliria	5.06[b]	4.87[b]	5.70[a]
Unknown	5.98[a]	5.96[a]	6.05[a]
LSD (*P* = 0.05)	0.60	0.45	0.73

LSD - Least Significant Difference (*P* = 0.05).
[a-b]Means within the same column with different superscripts differ significantly (*P*≤0.05).

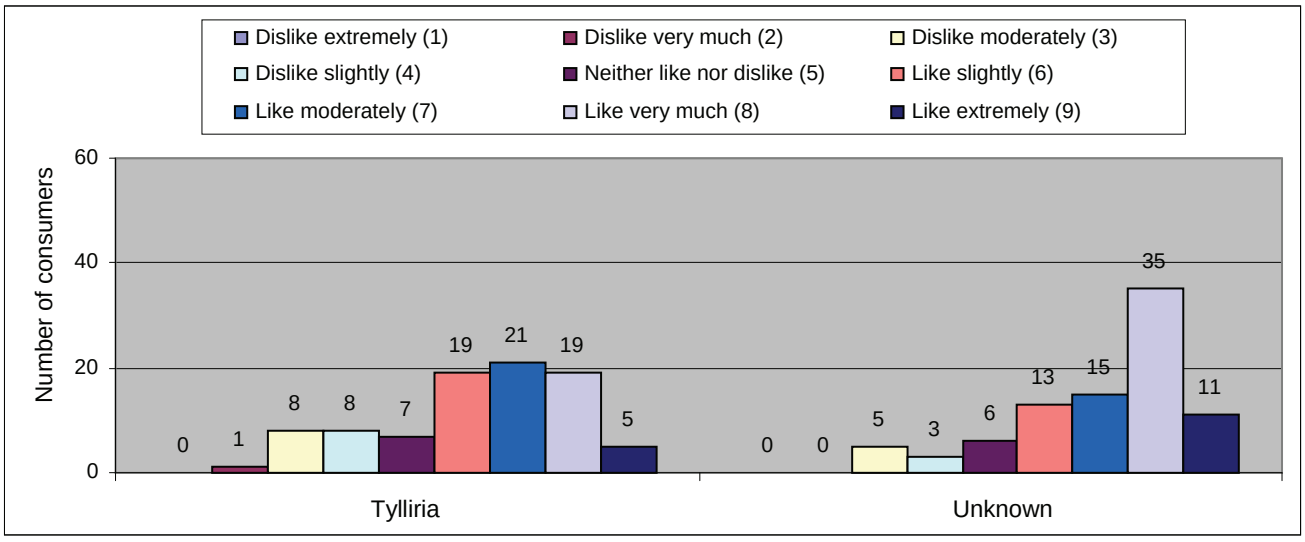

Figure 4 Acceptability of the flavour of carob porridge samples for the total group (N = 90).

alternative raw material could be more effective than other sources of fibre in improving the dietary fibre (and hence add to health benefits) content in this range of products.

Consumer acceptability

The degree of liking for the two carob flake samples based on colour is illustrated in Table 13. The total group of consumers preferred the colour of the Unknown cultivar significantly ($P \leq 0.05$) to that of Tylliria. A similar pattern was illustrated by both male and female consumers. The overall mean value for colour for the total group is higher than 6 for Tylliria and higher than 7 for the Unknown cultivar. This illustrated that the use of carob (up to 73%) has a positive effect on the degree of liking of the porridge colour. More than 60% of the total group of the consumers described both products as *Like extremely, Like very much, Like moderately* and *Like slightly* (Fig 5). This indicates therefore that the colour of both products (carob flakes) is acceptable.

The degree of liking for the two breakfast cereal flakes based on flavour is presented in Table 14, whilst data regarding acceptability is summarised in Fig. 6. The total group of consumers (N = 90) as well as the female consumers preferred the overall flavour of the flakes made with the Unknown cultivar to those made with Tylliria ($P \leq 0.05$). Male consumers on the other hand, preferred the flavour of both samples equally. Again, the mean values for flavour ranged between 6 and 7. Both samples were highly acceptable, as more than 70% of the total group of the consumers rated both products positively (Fig 6). This indicates that the use of carob powder as the main ingredient (73%) in making breakfast cereal flakes was highly successful. Carob's natural sweetness (Batlle & Tous, 1997) and the product's unique crunchiness and nutty aroma might possibly played a role.

Mousse

Formulation and production process

The processing method of Lötter *et al.* (2006) as used in this study produced a mousse of acceptable sensory characteristics (flavour and colour) according to the in-house panel of judges. The in-house panel preferred the mousse with roasted rather than with non-roasted carob powder. The use of roasted carob powder resulted in mousse with a distinctive chocolate-like carob flavour and colour. This could be an added advantage from a marketing point of view, considering the fact that chocolate flavour is popular on the market (Lang, 1982; T Flandorp, 2007, personal communication). Although the use of non-roasted carob also gave a good creamy colour, it had a slightly undesirable sour taste.

Table 13 Degree of liking for carob breakfast cereal flake samples based on colour

Treatment	Mean hedonic scale values		
	Total group (N = 90)	Female consumers (N = 69)	Male consumers (N = 21)
Tylliria	6.69[b]	6.74[b]	6.55[b]
Unknown	7.30[a]	7.33[a]	7.20[a]
LSD (*P* = 0.05)	0.25	0.28	0.53

LSD - Least Significant Difference (*P* = 0.05).
[a-b]Means within the same column with different superscripts differ significantly (*P*≤0.05).

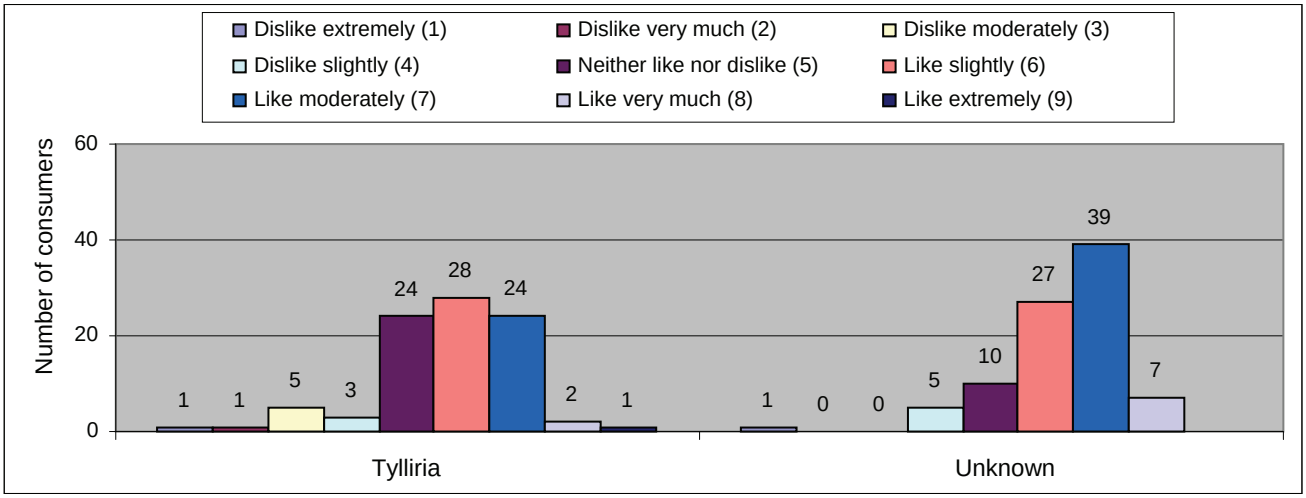

Figure 5 Acceptability of the colour of carob breakfast cereal flake samples for the total group (N = 90).

Table 14 Degree of liking for carob breakfast cereal flake samples based on flavour

Treatment	Mean hedonic scale values		
	Total group (N = 90)	Female consumers (N = 57)	Male consumers (N = 23)
Tylliria	6.26[b]	6.15[b]	6.65[a]
Unknown	7.03[a]	7.02[a]	7.10[a]
LSD (*P* = 0.05)	0.34	0.41	0.56

LSD - Least Significant Difference (*P* = 0.05).
[a-b]Means within the same column with different superscripts differ significantly (*P*≤0.05).

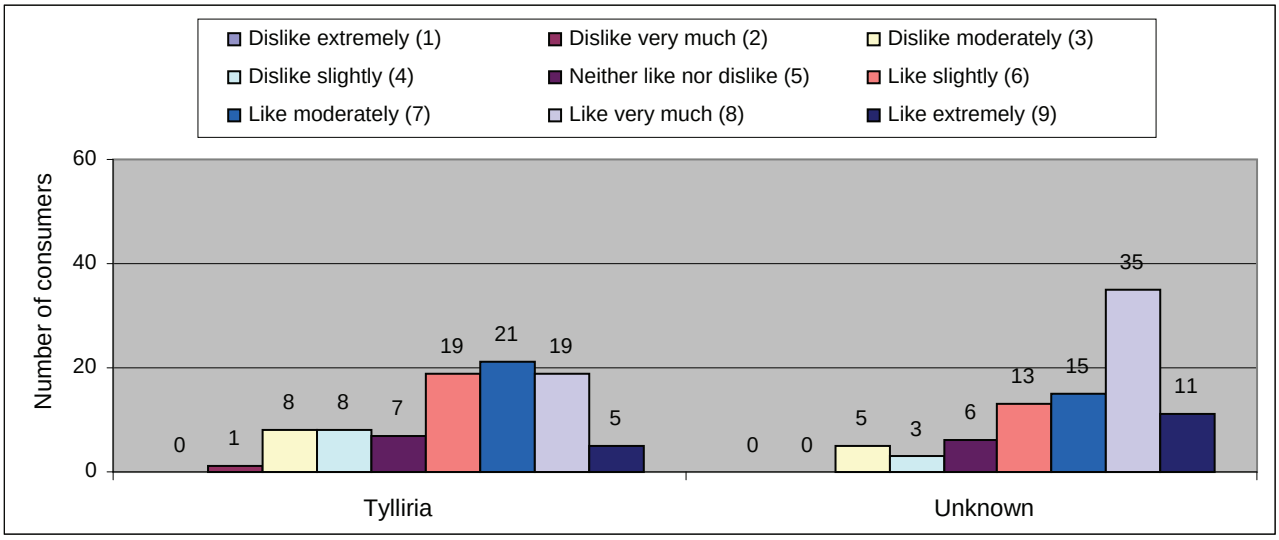

Figure 6 Acceptability of the flavour of carob breakfast cereal flake samples for the total group (N = 90).

Thus, the in-house panel preferred the mousse made with roasted carob over the one made with non-roasted carob, on the basis of flavour and colour. The mousse made with roasted carob was, therefore, selected for consumer sensory analysis. The product could be suitable for consumption as a dessert.

Nutritional content

The information regarding the nutritional contents of the final mousse is given in Table 8 The mousse could be a source of energy (470.5 g.100 g^{-1}) (Anon., 2004; Anon., 2007). A high energy value was mainly due to high fat (8.4 g.100 g^{-1}) and carbohydrate contents (15.0 g.100 g^{-1}) (Anon., 2004; Anon., 2007). The protein content (4.1 g.100 g^{-1}) was lower than the minimum level (5 g.100 g^{-1}) required for any food to be labelled as a source of protein (Anon., 2004; Anon., 2007). Like many other dessert products containing a large number of ingredients from animal sources, the mousse was high in cholesterol (4.6 g.100 g^{-1}) (Anon., 2004; Anon., 2007). Care should, therefore, be taken regarding the amount of such products (desserts) consumed, because the consumption of large amounts of cholesterol and/or fat present a risk of cardiovascular related illnesses and cancers (Sikorski, 2003; Bosco *et al*., 2007). The nutritional content was similar to those of similar products on the market, except that the mousse developed in this study was lower in carbohydrates (and hence in the energy content).

Consumer acceptability

The mean hedonic scale values for the colour of the two carob mousse samples are given in Table 15. The total group of consumers (N = 63) as well as female consumers preferred the colour of the carob mousse made with the Unknown cultivar more than the one with Tylliria ($P \leq 0.05$). Male consumers liked both mousse samples equally ($P > 0.05$). The overall mean values were above 5 for Tylliria and above 6 for the Unknown cultivar. This shows that the incorporation of 7.3% roasted carob powder had a positive effect on the colour of mousse. As depicted in Fig. 7, more than 50% of the total group of the consumers rated the colour of both products positively. The colour of the product was, therefore, reasonably acceptable.

The mean hedonic scale values for the flavour of two carob mousse samples are given in Table 16. The total group of consumers (N = 63) preferred the overall flavour of the two mousse samples equally ($P > 0.05$). The males and females illustrated a similar pattern. Overall mean value for flavour for the total group is more than 5 for both Tylliria and the Unknown cultivar. This illustrated that 7.3% roasting carob had a reasonably positive effect on the acceptability of the flavour. As indicated in Fig. 8, approximately

Table 15 Degree of liking for carob mousse samples based on colour

Treatment	Mean hedonic scale values		
	Total group (N = 63)	Female consumers (N = 47)	Male consumers (N = 16)
Tylliria	5.81[b]	5.72[b]	6.47[a]
Unknown	6.15[a]	6.04[a]	6.06[a]
LSD (*P* = 0.05)	0.23	0.28	0.66

LSD - Least Significant Difference (*P* = 0.05).
[a]Means within the same column with different superscripts differ significantly (*P*≤0.05).

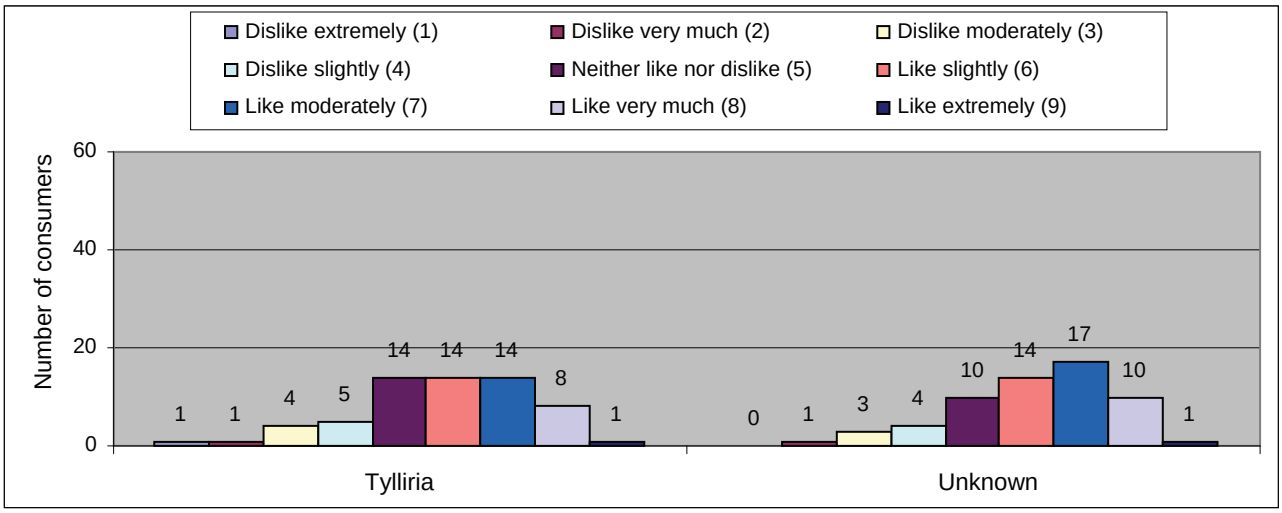

Figure 7 Acceptability of the colour of carob mousse samples for the total group (N = 63).

Table 16 Degree of liking for carob mousse samples based on flavour

Treatment	Mean hedonic values		
	Total group (N = 63)	Female consumers (N = 47)	Male consumers (N = 16)
Tylliria	5.29[a]	5.36[a]	5.25[a]
Unknown	5.71[a]	5.60[a]	6.19[a]
LSD (*P* = 0.05)	0.77	0.60	0.94

LSD - Least Significant Difference (*P* = 0.05).
[a]Means within the same column with different superscripts differ significantly (*P*≤0.05).

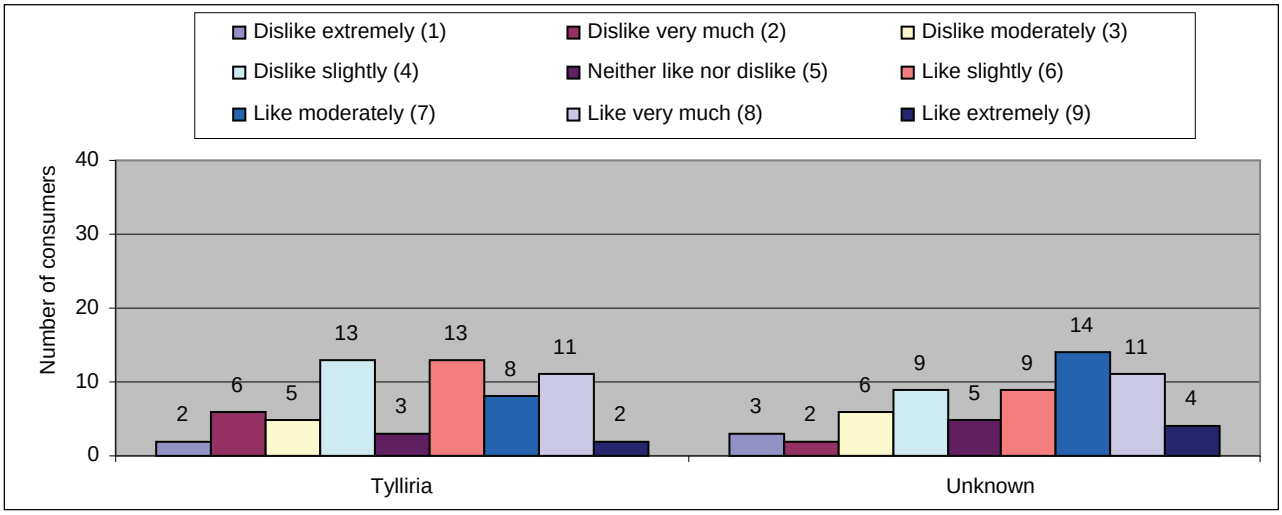

Figure 8 Acceptability of the flavour of carob mousse samples for the total group (N = 63).

60% of the total group of the consumers rated both products positively. The flavour of both samples was, therefore, acceptable. This illustrated that the fortification of mousse with roasted carob powder (7.3%) was successful. Commercially produced chocolate mousse is usually highly acceptable (T Flandorp, 2007, personal communication). It could be assumed that carob mousse may also have a good chance to be successful within the commercial arena.

Milk-based drink

Formulation and production process

Formulation 5A resulted in a product of good flavour and colour. The addition of a small amount of sucrose (Formulation 5B, 5C, 5D and 5E) enhanced the product sweet flavour. Formulation 5B resulted in a product with better sensory characteristics than Formulation 5B in terms of flavour. The texture of the product (Formulation 5B) was a concern as the carob powder did not dissolve completely or suspend uniformly in the product. The product thus had a slightly coarse mouthfeel. Some of the non-dissolved carob particulates also sank to the bottom of the product. An increase in the amount of stabiliser (as in 5C) was, therefore, aimed at resolving this problem.

The product of Formulation 5C was too thick according to the in-house judging. A slight reduction in the amount of stabiliser (as in 5D) was, therefore, aimed at slightly reducing the thickness. The in-house panel preferred Formulation 5D as it resulted in a product of a more acceptable thickness than Formulation 5A, 5B and 5C.

When compared against the use of non-roasted carob using Formulation 5D, the use of roasted carob resulted in more acceptable sensory characteristics such as flavour and colour, according to the in-house panel of judges. The use of non-roasted carob resulted in a product of which the vanilla flavour was too strong, to such an extent that it masked the carob flavour. This therefore necessitated a reduction in the amount of vanilla added (as in Formulation 5E). Formulation 5E gave a product of a more acceptable (in comparison to Formulation 5D) flavour, for a product made with non-roasted carob. For roasted carob on the other hand, Formulation 5D produced a better flavour according to the in-house panel of judges. Formulation 5D and 5E were thus selected as optimal formulations for the processing of milk-based drinks made with roasted and non-roasted carob powders, respectively. The in-house panel preferred the product made with roasted carob over the one of non-roasted carob as roasted carob gave a better chocolate-like carob flavour. Formulation 5D (involving roasted carob) was thus selected for the final

product analysed by the consumer panel.

Due to its thick texture, the developed carob milk-based drink could have a satiating (stomach-filling) effect, making it an ideal (and healthy) breakfast drink. The product could also be used as a convenient "on-the-go" snack.

Nutritional content

The nutritional information of the final milk-based drink is given in Table 8. Mainly due to its high carbohydrate content (12.6 g.100 g^{-1}), the milk-based drink is a good source of energy (319.9 g.100 g^{-1}) (Anon., 2004; Anon., 2007). Other nutrients such as protein, dietary fibre and fat were found to be low (2.7, 1.1 and 2.0 g.100 g^{-1}, respectively). A low dietary fibre content may be ascribed to the fact that carob is added in very small amounts. More research aimed at increasing the amount of carob added to the product could result in an improved dietary fibre content. The product virtually contained no sodium (Anon., 2004; Anon., 2007). Similar products on the South African market contain as high as 66 mg.100 g^{-1} of sodium. Consumption of elevated amounts of sodium results in a higher risk of hypertension (Karanja *et al*., 2007). The carbohydrate content (and hence energy content) was half the amount found in other mousses available on the market. Overall, the product's nutritional profile was closely similar to other mousse products especially in terms of the protein, fat and dietary fibre contents.

Consumer acceptability

The mean hedonic scale values for the colour of the two milk-based drinks are given in Table 17. The total group of consumers (N = 63) preferred the overall colour of the two samples equally (P>0.05). The males and females illustrated a similar pattern. The overall mean value for colour for the total group is more than 6 for both Tylliria and the Unknown cultivar illustrating that 3% roasted carob had a positive effect on the degree of liking of the colour. More than 80% of the total group of the consumers rated both samples positively (Fig. 9). This indicates, therefore, that the colour of the milk-based drink was highly acceptable.

The degree of liking and acceptability for flavour of the two milk-based samples are shown in Table 18 and Fig 10, respectively. The total group of consumers (N = 63) preferred the overall flavour of the two milk-based drink samples equally (P>0.05) (Table 17). The males and females illustrated a similar pattern. Again the overall mean value for flavour for the total group is substantially more than 6 for both Tylliria and the Unknown cultivar illustrating that 3% carob has a positive effect on the degree of liking of flavour. Approximately 80% of the total group of consumers rated both samples positively (Fig. 10).

Table 17 Degree of liking for milk-based carob drink samples based on colour

Treatment	Mean hedonic values		
	Total group (N = 63)	Female consumers (N = 47)	Male consumers (N = 23)
Tylliria	6.45[a]	6.53[a]	6.20[a]
Unknown	6.48[a]	6.51[a]	6.33[a]
LSD (*P* = 0.05)	0.25	0.88	0.55

LSD - Least Significant Difference (*P* = 0.05).
[a]Means within the same column with different superscripts differ significantly (*P*≤0.05).

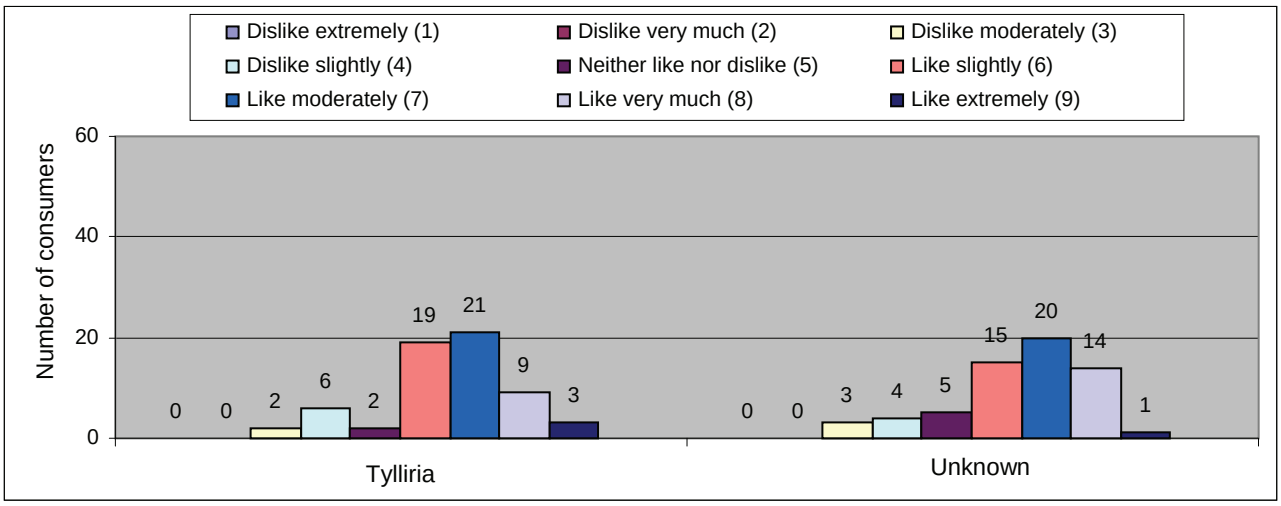

Figure 9 Acceptability of the colour of milk-based carob drink samples for the total group (N = 63).

Table 18 Degree of liking for milk-based carob drink samples based on flavour

Treatment	Mean hedonic values		
	Total group (N = 63)	Female consumers (N = 57)	Male consumers (N = 23)
Tylliria	6.84[a]	6.83[a]	6.88[a]
Unknown	6.71[a]	6.64[a]	6.94[a]
LSD (*P* = 0.05)	0.37	0.40	0.90

LSD - Least Significant Difference (*P* = 0.05).
[a]Means within the same column with different superscripts differ significantly (*P*≤0.05).

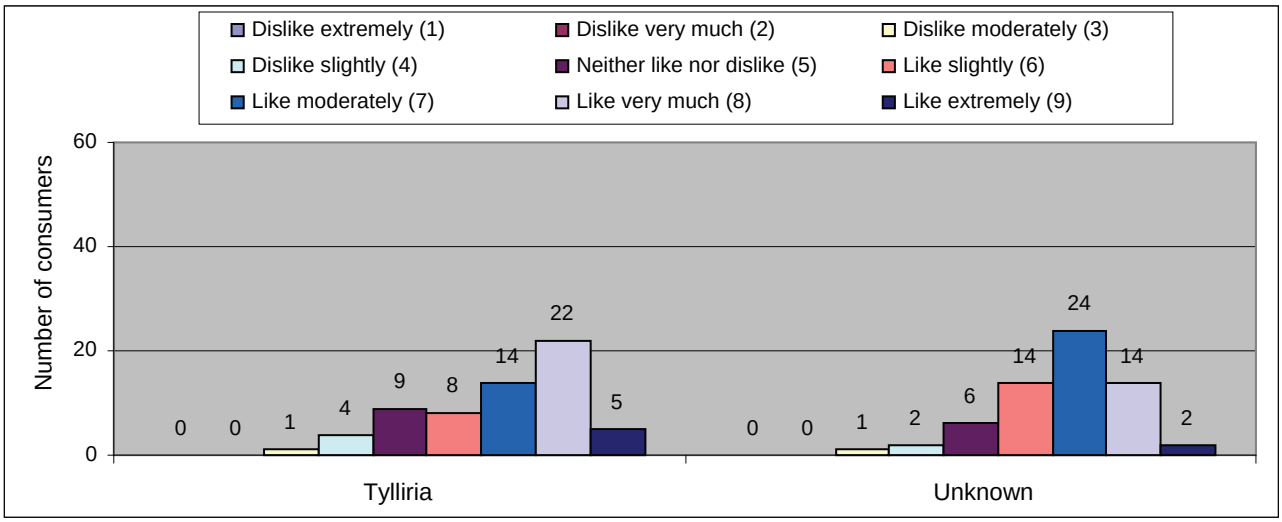

Figure 10 Acceptability of the flavour of the milk-based carob drink samples for the total group (N = 63).

The product had a characteristic nutty chocolate-like flavour and one can assume that the latter would have a positive effect on the overall acceptability of the flavour of the product.

Conclusion

New nutritional food products were developed using carob as a major ingredient. Formulations were developed where specific carob cultivars were successfully incorporated. Valuable information regarding the processing of carob and its use was obtained. These for example include the amount to be added, sensory variations between cultivars, roasted versus non-roasted and the importance of sieving. Such information may be useful to the food industry and anyone interested in carob's application in food. Microbiological analyses showed that all products were safe for human consumption. Consumer sensory analysis showed that all five products were acceptable. Overall, it was proven that carob can be used as an ingredient for the various processed foods, targeted at different South African market sectors. Based on the findings of this study, carob is specially be recommended for use as a cheap, but nutritionally rich food source so as to improve the nutritional status of low-income rural communities.

Addendum A

_____Code_____ _____code_____

QUESTIONNAIRE: ACCEPTABILITY OF CAROB BREAD

NAME OF JUDGE: _____ JUDGE NO:_____

PLEASE CIRCLE WHICHEVER IS APPLICABLE:

GENDER:	*AGE:*	*CONSUMPTION OF BREAD:*
Male/ Female	***18-20/ 21 – 25/ 26-30/ 31+***	***7X per week / 3x per week / 1x per week / 1x per month/ / NEVER***

INSTRUCTIONS

PLEASE TASTE THE 2 SAMPLES IN THE ORDER PRESENTED, I.E. FROM LEFT TO RIGHT. RINSE YOUR MOUTH WITH WATER BEFORE BEGINNING.

TAKE A GENEROUS BITE FROM EACH SAMPLE AND **RANK THE SAMPLES ON THE FOLLOWING SCALES.**

IN EACH CASE, CIRCLE THE NUMBER NEXT TO THE PREFERRED DEGREE OF LIKING

	CODE		**CODE**	
DEGREE OF LIKING FOR THE **OVERALL COLOUR** OF BREAD	9	Like extremely	9	Like extremely
	8	Like very much	8	Like very much
	7	Like moderately	7	Like moderately
	6	Like slightly	6	Like slightly
	5	Neither like nor dislike	5	Neither like nor dislike
	4	Dislike slightly	4	Dislike slightly
	3	Dislike moderately	3	Dislike moderately
	2	Dislike very much	2	Dislike very much
	1	Dislike extremely	1	Dislike extremely

	CODE		**CODE**	
DEGREE OF LIKING FOR THE **OVERALL TASTE** OF BREAD	9	Like extremely	9	Like extremely
	8	Like very much	8	Like very much
	7	Like moderately	7	Like moderately
	6	Like slightly	6	Like slightly
	5	Neither like nor dislike	5	Neither like nor dislike
	4	Dislike slightly	4	Dislike slightly
	3	Dislike moderately	3	Dislike moderately
	2	Dislike very much	2	Dislike very much
	1	Dislike extremely	1	Dislike extremely

THANK YOU VERY MUCH FOR YOUR INVALUABLE ASSISTANCE. PLEASE COLLECT A SMALL "GIFT" AS YOU LEAVE THE SENSORY AREA

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CHAPTER 5

GENERAL DISCUSSION AND CONCLUSIONS

Background

Carob's (*Ceratonia siliqua*) good nutritional value, its availability (especially in the Western Cape Province) and adaptability to harsh environmental conditions, could make it a suitable crop and an ideal alternative food source in South Africa. Carob pods have a long history (>4 000 years) of use as a food product. Its use as a food source may be associated with numerous nutritional benefits and a long shelf-life (2 – 3 years). These pods also have a nutty chocolate-like flavour but unlike cocoa, carob does not contain any caffeine, thiobromine (stimulants) or oxalic acid (can be toxic). In addition, carob is normally regarded as a healthy food because it has a low fat content (0.2 – 2.3%).

A literature review (Chapter 2) showed that cultivars from different regions differ in chemical composition. Currently, knowledge on the nutritional composition and hence the overall nutritional potential of locally (South African) grown carob cultivars, is limited (Muller, 2005). The general objective of this research was, therefore, to determine the chemical composition (Chapter 3) and the potential to incorporate five locally cultivated carob cultivars into food product formulations (Chapter 4).

Compositional analysis

Each of the five locally cultivated cultivars (Tylliria, Sfax, Aaronsohn, Santa Fe and a "Unknown" cultivar) was analysed for chemical composition with the aim to establish if there were chemical variations in composition. Additionally, the compositional information obtained would allow for comparisons between locally grown carob and those from elsewhere as published in the literature.

The data obtained in this study showed that carob is a good source of energy as all the cultivars analysed contained carbohydrates as the main component (over 90%). Sugars were found to constitute the largest portion of the carbohydrates (over 60%) while the rest was made up dietary fibre. Sucrose was found to be the main sugar (over 80% of the total sugar content) with glucose and fructose contributing the remainder. The high sucrose content explains carob's natural sweet taste and, thus, the use of carob as a natural sweetener, especially in food combinations where carob is present in large concentrations. All the cultivars studied were found to be a good source of dietary fibre

(29.88 – 36.07 g.100 g^{-1}). The dietary fibre values found in this study and in other recent studies (up to 39.8 g.100 g^{-1}) (USDA, 2006) were much higher when compared to those reported in older studies (as low as 3.7 g.100 g^{-1}) (Binder *et al.*, 1958). These variations can be attributed to differences in the analytical method used. The method used in this and the other more recent studies (AOAC 991.43) (AOAC, 2005) measures dietary fibre as the sum of non-starch polysaccharides (NSP), lignin and resistant starch (Butler & Patel, 2000). Older studies on the other hand, only considered the non-starch polysaccharides (NSP) portion. Thus, it is important to note that harmonisation of analytical methods would ensure uniform dietary fibre analysis.

The local cultivars studied were also found to contain protein (3.07 – 4.42 g.100 g^{-1}) which is also of nutritional importance. Although carob contains good quality proteins, levels present (<5 g.100 g^{-1}) do not allow any claims to be made that carob is a “good” source of protein (Anon., 2004; Anon., 2007). The amino acid profiles of all five cultivars can also be considered of good quality (seven essential amino acids were detected).

Due to its polyphenolic compounds (2.58 – 3.08 g.100 g^{-1}), carob also has anti-oxidative properties. The values reported in this study fall well within the ranges reported by other investigators (1.3 – 20.0 g.100 g^{-1}) (Makris & Kefalas, 2004). Anti-oxidants are generally reported to have health benefits such as anti-allergic, cancer preventative and vasorelaxing effects (Sakakibara *et al.*, 2003) and, therefore, the consumption of carob could contribute in this regard.

All the cultivars studied contained less than 1% fat. This low fat content reaffirms the fact that carob may be considered a healthy food as the consumption of fatty foods is associated with an increased risk of cancer, circulatory diseases and obesity among others. For all the cultivars analysed in this study, fatty acid ratio of the PUFA:SFA and *Omega*-6:*Omega*-3 were found to fall within recommendations of international health organisations. Even though only very low concentrations of short-chain fatty acids (SCFA) were detected, iso-butyric acid was found to be the SCFA present in the highest concentration. This fatty acid has been reported to contribute to the unpleasant aroma found in raw carob (Berna *et al.,* 1997). Heat treatments such as roasting, baking and cooking are, therefore, essential during carob processing as they will reduce the iso-butyric acid (and possibly other undesirable volatiles) levels.

Furthermore, the five cultivars analysed were found to contain calcium, phosphorus, potassium, magnesium, sodium, manganese, iron, copper, and zinc. These minerals are all known to have various physiological functions in the human body. All cultivars were low in sodium (4.41 – 14.45 mg.100 g^{-1}), which is another positive attribute of carob as the

consumption of sodium in large concentrations has been reported to increase the risk to health conditions such as increased blood pressure and hypertension (Guàrdia *et al*., 2006).

The roasting study (part of Chapter 3) showed that an increase in roasting time will reduce the sugar and protein contents. Roasting is, however, normally applied as one of the steps during carob processing and is aimed at enhancing sensory characteristics such as colour and flavour (Yousif & Alghzawi, 2000). For carob processing, it can thus be recommended that roasting conditions be optimised so that the sugar and protein reductions are minimised, whilst producing acceptable sensory quality. The ability of roasting to reduce the carob's moisture content presents other processing operational advantages.

Overall, the compositional analysis research has shown that all five cultivars analysed had a good nutritional value and, therefore, could be used as a food ingredient. Compositional variations among the cultivars were also found to be small.

Product development

In order to establish the practicality of incorporating locally grown carob into food products, a total of five different nutritional food product formulations (bread, porridge and breakfast cereal, mousse and milk-based drink) were developed (Chapter 4). Since the compositional variations among cultivars were too small, selection of the two cultivars to proceed with during further product development was based on sensory characteristics rather than on composition. The in-house panel of judges recommended the cultivars, Tylliria and the "Unknown" cultivar for further product development.

In certain of the products, such as the porridge and breakfast cereal, carob was used as the main ingredient while in the others (bread, mousse and the milk-based drink), carob only made up a small proportion of the ingredients. Consequently, carob's good nutritional profile was more positively reflected in the porridge and breakfast cereal whereas its nutritional contribution in the bread, mousse and milk-based drink were less dominant with the result that the carob can be seen more as a flavourant and colourant in those products.

The results of the consumer sensory analysis (N = 63 – 90) performed on the products developed gave a clear indication of the successful formulation development using carob as an ingredient. This was evident as all products were ranked positively by over 50% of consumer (sensory) panellists. Some of the products such as the bread and

the milk-based drink might have good market potential as over 80% and 90% of the panellists, respectively responded positively. The study proved that the two local carob cultivars (Tylliria and the Unknown cultivar) exhibited acceptable sensory characteristics. Variations between these two cultivars were small in terms of consumer sensory acceptability, which suggests that inter-cultivar variations might not necessarily affect consumer preferences.

From the results obtained in this study, it was concluded that heat treatment enhances carob's sensory characteristics such as colour and flavour. The use of roasted carob is, therefore, recommended in products where heat application is not part of the production step (e.g. in the milk-based drink and mousse).

Microbiological analysis of all the carob products also showed that all the products developed in this study were microbiologically safe for human consumption. This proved that carob can safely be incorporated into food products.

Concluding remarks and recommendations

This study showed that South African grown carob compares favourably with carob from around the world in terms of chemical content. The nutritional profile (Chapter 3) of carob and the high degree of acceptance of carob-containing products by the consumer panel (Chapter 4) further showed that carob has potential as an alternative nutritious food source. This is the first study of its kind on carob composition in South Africa and the findings can, therefore, be used as the scientific basis for the promotion of carob as an alternative food source in Southern Africa. The pods can directly serve as food to local inhabitants in places where carob is found (for example in the Western Cape Province). Furthermore, commercialisation of locally grown carob pods is recommended as it would (directly or indirectly) substantially contribute towards improving the nutritional status of local communities. The information gathered in this study could, therefore, be of value to local inhabitants, the food industry, community upliftment organisations and governments.

Carob has its own unique flavour, which many local communities are not familiar with. Success in promoting carob as a new flavour and as an alternative source of food would require a highly intensive marketing campaign. In the health-food market on the other hand, carob has already been well established. The processing of carob powder and the manufacture of carob products locally, would lead to a reduction in retail prices as currently most of these products or their raw material (carob) are being imported.

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