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# **Lentil (*Lens culinaris* Medikus): A Whole Food Rich in Prebiotic Carbohydrates to Combat Global Obesity**

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Additional information is available at the end of the chapter

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## **Abstract**

Lentil (*Lens culinaris* Medik.) is a cool season food legume that is high in protein (20–30%) and in a range of micronutrients (e.g., minerals, carotenoids, folates) but very low in phytic acid. Recent research indicates that lentil contains a wide array of low-molecular weight carbohydrates (LMWC) or prebiotic carbohydrates, such as mono- and disaccharides, raffinose-family oligosaccharides (RFO), fructooligosaccharides (FOS), and sugar alcohols, and high-molecular weight resistant starches. Lentil provides more than 13 g of prebiotic carbohydrates per 100 g serving, and this level increases almost two-fold upon cooking, cooling, and reheating. In addition, prebiotic carbohydrate levels vary with lentil genotype and growing location/country. Intestinal microbiome and prebiotic studies suggest a prebiotic-rich, low-calorie diet can reduce the prevalence of obesity and related non-communicable diseases. Lentil thus represents a whole food source of prebiotics that can play a role in efforts to reduce obesity and non-communicable diseases. This chapter provides an overview of the current obesity-related health issues, holistic approaches to reduce obesity, worldwide lentil production, and the promise of pulses, mainly lentil, to be a whole food solution to combat global obesity. In addition, lentil's superior LMWC profile and the genetic potential for further enrichment of prebiotic carbohydrates are briefly discussed.

**Keywords:** lentils, low-molecular weight carbohydrates, prebiotics, gut microbiome, obesity

## 1. Introduction

Obesity has become an epidemic. Chronic, non-communicable diseases associated with obesity result in 36 million deaths globally each year, more than all other causes combined [1]. Obesity has been a severely neglected global public health concern for decades [2] and, today, “globesity” — the global epidemic of overweight and obesity — is taking over many parts of the world despite continued economic development [2]. In 1995, 200 million adults were obese; by 2000, 300 million adults were obese; and today, more than 1.9 billion adults are overweight and 600 million are obese [2]. Both obesity and overweight increase the risk of health conditions including hypertension, adverse lipid concentrations, type 2 diabetes, and several cancers (endometrial, breast, prostate, and colon) [3]. This situation calls for immediate public health awareness to reduce obesity and the risk of related health disorders.

Obesity and overweight are preventable health conditions. Several prevention approaches are available, including dietary therapy (low caloric diets), changes in physical activity and/or social behavior, surgical procedures, and combinations thereof. However, because of the nature of these metabolic disorders, solutions will by necessity have a focus on diet. The intestinal microbiome and a prebiotic-rich, low-caloric diet are beginning to be recognized as being important for preventing obesity. Prebiotic-rich diets change microbial species in the human gut, which leads to increased satiety, regulation of intestinal motility, production of short-chain fatty acids, prevention of diarrhea and constipation, and reduction of pathogen colonization [4, 5]. Furthermore, consumption of a prebiotic-rich diet may stimulate the immune system [6], promote mineral absorption (especially iron and selenium), decrease the risk of colon cancer [7, 8], reduce cholesterol levels and excess circulation of glucose, and improve insulin sensitivity [9]. As such, products high in prebiotics are becoming more popular health-promoting foods around the world. Lentil (*Lens culinaris* Medik.), also known as *poor man's meat*, is one such “superfood” and has the potential to provide daily prebiotic requirements. Compared with cereal food products, prebiotics are found at high levels in lentils [10]. The objective of this chapter is to provide an overview of current obesity-related health issues, holistic food approaches to prevent obesity, global lentil production, and the promise of pulse crops, particularly lentil, as a whole food solution to combat global obesity.

## 2. Obesity

### 2.1. Global obesity prevalence

Since the 1960s, obesity and overweight prevalence has increased enormously, resulting in a global health burden far surpassing infectious diseases. The numbers tell a clear story. From 1980 to 2013, the number of overweight or obese individuals rose from 857 million to 2.1 billion [11–13]. Worldwide, 37% of men and 38% of women are overweight or obese [13]. In some regions, the rate of obesity in adults exceeds 50% [13]. The prevalence of overweight and obesity among children and adolescents is also rising [11], with these conditions affecting 23.8% of boys and 22.6% of girls in developed countries and 12.9% of boys and 13.4% of girls

in developing countries, respectively [13]. Unfortunately, awareness of the problem has not resulted in any decline in obesity and overweight rates, especially in developing countries [13].

Health effects and comorbidities associated with obesity are numerous and include cardiovascular disease, type 2 diabetes mellitus, chronic kidney disease, osteoarthritis, hypertension, stroke, dyslipidemia, gall bladder disease, and some cancers [14–16]. The severity of many of these comorbidities increases with the degree of obesity, i.e., increasing body mass index (BMI) [16]. In an analysis of risk factors contributing to global disease burden, high BMI alone is the sixth highest risk factor for chronic disease even before the consideration of associated comorbidities [14].

The costs of obesity are very high, both for afflicted individuals and for national healthcare systems [14, 17–19]. Obese individuals have impaired physical function and health-related quality of life [19], as well as socioeconomic and emotional consequences such as decreased work force, completion of fewer years of school, stigmatization, decreased self-esteem, and increased likelihood of experiencing poverty [18, 20]. Furthermore, obesity and overweight cause an estimated 3.4 million deaths worldwide, accounting for 4% of life years lost and 4% of disability-adjusted life years [14]. The financial burden of overweight and obesity is equally high, consuming between 0.7 and 9.1% of total healthcare expenditures among countries included in a meta-analysis from 1990 to 2009 [17]. Had associated comorbidities been included, these expenditure estimates would be significantly higher. The etiology of obesity is complex, but three contributing factors have been identified: diet, metabolic dynamics, and physical inactivity [21]. The complexity of obesity arises because of interactions of these factors with genetics and environmental stimuli (e.g., stress). To illustrate, over 150 gene loci relate to obesity and diabetes through effects on body processes, including insulin and insulin receptors, fat deposition and distribution, lipolysis, and hypothalamic function [22]. Obesity is associated with chronic low-grade inflammation, which subsequently leads to a host of downstream pathological conditions [23–26]. Proinflammatory markers, such as interleukin-6 and tumor necrosis factor (TNF)- $\alpha$  and - $\beta$ , are found in higher concentrations in the liver of obese individuals, resulting in local and systemic insulin resistance [27].

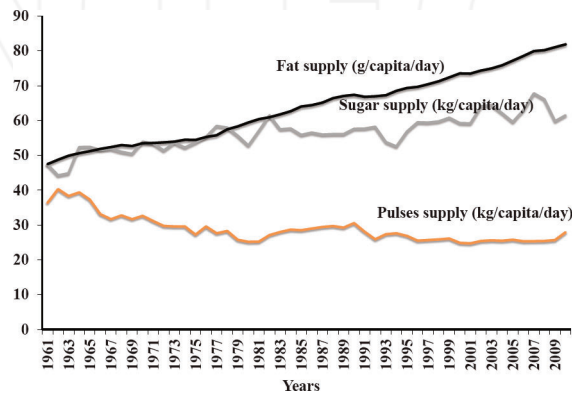
## 2.2. What causes obesity?

Popkin et al [12]. suggest an evolutionary rationale for the obesity pandemic, pointing out clashes between human biology and the technological and industrial revolutions (**Table 1**). Three problems the authors identify relate directly to diet: sweet preference, disconnect between thirst and hunger mechanisms, and fatty food preference. Food processing, added sweeteners, caloric beverages, and ease of vegetable oil production exploited these biological tendencies, resulting in dramatically increased consumption of sugars, oils, and milled grains. The per capita supply of fats increased from 47 to 82 g/capita/day and of sugars increased from 47 to 61 kg/capita/day between 1961 and 2010; however, the per capita supply of pulse crops (including lentil) dropped significantly over the same period (**Figure 1**) [28]. Furthermore, food availability per capita overall has significantly increased [11].

Biology	Technology
Sweet preferences	Cheap caloric sweeteners, food processing benefits
Thirst and hunger/satiety mechanisms not linked	Caloric beverage revolution
Fatty food preference	Edible oil revolution, high-yield oilseeds, cheap removal of oils
Desire to eliminate exertion	Technology in all phases of movement/exertion

Reproduced from ref [12].

**Table 1.** Technological clashes with human biology.



**Figure 1.** Global supply of sugar, fat, and pulses from 1961 to 2009 [28].

**2.3. Holistic approaches to preventing obesity**

Consumption of foods rich in sugars/fat and low physical activity are major contributors to obesity. Environment and human genetics also play a fair role, but societal changes are driving the obesity epidemic. In 2002, experts from United Nations, Food and Agricultural Organization (FAO), and World Health Organization (WHO) collaborating centers joined forces to identify an immediate action plan to prevent global obesity. They recommended (1) correcting social food intake and physical activity patterns, (2) intervention and commitment to the above actions (food intake and physical activity) at all levels (e.g., individual through to community, national, and international levels), and (3) developing new government policies for populations to improve individual lifestyle characteristics [29].

Economic growth, urbanization, and globalization of food markets have led to nutritional transitions around the world. Consequently, consumption of high-fat high-energy diets and/or reduced physical activity have become common practice. For thousands of years, world agriculture adopted simple rotations of profitable cash crops, i.e., wheat, maize, and rice, with nutritionally rich legume crops. However, modern calorie-centric agriculture is devoid of these traditional or more diverse cropping systems, leading to nutrition transitions that are linked

to increased rates of obesity in both developing and developed countries. To overcome these nutritional transitions, appropriate food systems should feature a host of activities related to the distribution, utilization, and consumption of nutritious foods including fruits, vegetables, cereals, and pulse crops [30]. Holistic systems approaches use healthy soil, water, seeds, fertilizer, human labor, and capital and have outputs beyond food—primarily the long-term health of both the people and the environment. Such approaches have proven successful with respect to the prevention of micronutrient malnutrition in developing countries. For example, introduction of biofortified staple food crops through HarvestPlus to severely malnourished populations has promoted distribution, utilization, and consumption of these food crops. Traditionally, agriculture, food science and technology, economics, nutrition, and sociology were separate disciplines. However, the newly designed system has combined all of these activities into one compressive larger unit termed a “food system”. As a result, the undernourished population around the world is declining, from approximately 1 billion in 1991 to 792 million today [28]. Is it possible to use the same approach to prevent obesity?

Indeed, experts are proposing a holistic approach to prevent obesity. Proposed approaches include (1) increasing the diversity of locally available foods through food hub, (e.g., developing home gardens to be managed by women as a source of highly nutritious food for their families), (2) diet diversification with whole foods (pulse crops, fruits, and vegetables) and reduction of the intake of foods rich in refined sugar and fat, (3) developing appropriate technologies to preserve and store foods for local communities, (4) population-based guidelines to control food intake, e.g., food-based dietary guidelines (eat more fruits, vegetables, and legumes) or a nutrient-based dietary approach (10% energy from protein, 15–30% energy from fat, and >50% energy from complex carbohydrates), along with limited salt and alcohol intake, and (5) population-based guidelines for physical activity and a greater focus on basic social and biological research to understand household decision-making, food habits, child care, and food purchasing power [29, 31]. Notably, social level solutions for obesity prevention require attention from national government, food supply, media, non-government organizations, healthcare services, workforce, education, neighborhoods, homes, and families [29, 31].

### 3. The potential role of pulse crops in combating obesity

Diet modulates local and systemic inflammatory markers, suggesting a mechanism of action on obesity, diabetes, and other related conditions. In a fairly detailed study, a healthy diet, characterized by fruits, vegetables, tomato, poultry, pulses, tea, fruit juices, and whole grains, was inversely associated with inflammatory markers C-reactive protein, TNF- $\alpha$ , soluble vascular cell adhesion molecules (sVCAM-1), and E-selectin; conversely, a diet rich in refined grains, red meat, butter, processed meat, high-fat dairy, sweets, pizza, potato, and soft drinks was positively associated with systemic inflammation [32]. In particular, prebiotic food ingredients can decrease systemic inflammation through associations with hindgut microflora [33]. Pulse crops, and lentil in particular, are key whole foods that provide significant nutritional benefits in terms of micronutrient and prebiotic carbohydrate content and could thus play a role in obesity prevention.

3.1. Lentil production

Global lentil production has increased six-fold since the 1960s, from 0.85 million tons (Mt) in 1961 to 4.98 Mt in 2013; this has been accompanied by a 150% increase in sown area and a more than doubling of average yields from 528 to 1150 kg ha<sup>-1</sup> (**Table 2**) [28]. Lentil is currently grown in as many as 51 countries, including Canada, India, Turkey, Australia, the United States, Nepal, China, Ethiopia, Syria, and Bangladesh. Although the area under lentil cultivation in Turkey has declined in the last decade, lentil growing areas in Australia, Canada, Ethiopia, India, Nepal, and the United States have considerably increased. Production in Asia is concentrated in a belt stretching from Turkey in the west to Bangladesh in the east, accounting for ~58% of the world sown area. China has recently started releasing lentil-related data, and Bangladesh has increased its productivity through release and cultivation of improved varieties. During the 1990s, Iran, Nepal, and Syria substantially increased production but lentil-cultivated area and production in Pakistan declined. Forty percent of the world's lentil production is in North America, where Canada and the United States are major producers and Mexico is a minor producer. In Africa, Ethiopia and Morocco are significant producers, while Algeria, Sudan, Egypt, and Tunisia are only minor producers. In South America, Argentina and Peru are the only significant producers. European lentil production is gradually decreasing, with France and Spain being the only noteworthy producers. In Oceania, Australia has emerged as a global leader with a production of 324,100 tons in 2013.

Year	Area (million ha)	Production (Mt)	Yield (kg/ha)
1961	1.62	0.85	528
1971	1.72	1.05	611
1981	2.27	1.45	640
1991	3.27	2.66	814
2001	3.99	3.25	816
2013	4.33	4.98	1,150

Reproduced from ref [28].

**Table 2.** Trend in world lentil production (1961–2013).

Exports account for approximately one-third of total lentil production, with the remainder eaten locally. International trade in small-seeded, red cotyledon lentil is dominated by Australia, Canada, and Turkey, whereas trade in large-seeded, green lentil is primarily led by Canada and the United States. Countries in the Indian subcontinent and the Middle East are major importers of red lentil, and southern Europe and South America import large-seeded green lentils. Lentils have been a popular food in many countries for thousands of years, likely because of the associated nutritional benefits and preference for vegetable protein over other protein sources.

3.2. Nutritional quality of lentils

Lentils contain a relatively high concentration of protein (~30%) and are a rich source of essential micronutrients (e.g., folates, iron, zinc, selenium, and carotenoids; **Table 3**). Lentils are naturally high in iron, zinc, and selenium in forms that are highly bioavailable to humans [42]. In addition, broad-sense heritability estimates for these minerals are high, indicating it is possible to breed lentil cultivars with enhanced ability to accumulate iron, zinc, and selenium in the seed despite environmental influences [37, 38]. Interestingly, lentils are very low in phytic acid, which enables greater mineral absorption in the human gut [39, 41]. Furthermore, bioavailability studies using both cultured Caco-2 cells and humans clearly show certain lentil varieties are rich in bioavailable iron and selenium [42, 43]. Mineral bioavailability in lentil is relatively high compared with cereals and other legumes because of the presence of high levels of iron absorption promoters (e.g., ascorbic acid) and low levels of Fe absorption inhibitors (e.g., phytic acid, gallic acid, and chlorogenic acid) [39, 42]. Lentil has been used as a model candidate crop for micronutrient biofortification research at the International Centre for Agricultural Research in the Dry Areas (ICARDA) in collaboration with the Clemson University Pulse Quality and Nutrition program. Our recent work has selected a few selenium-enriching lentil accessions to develop high selenium uptake lentil populations with the aim of releasing new lentil cultivars that produce seed with high bioavailable selenium [44]. Further studies are in progress with respect to the release of high iron and zinc lentil cultivars for India and Africa.

Nutrient component (units)	Concentration	Reference
Moisture (%)	1–12	[34]
Protein (%)	20–29	[34]
Ash (%)	1.8–3.3	[34]
Total lipid (fat) (%)	1–2	[35]
Carbohydrate, by difference (%)	60–63	[36]
Total starch (%)	40–70	[10]
Total prebiotic carbohydrates (g/100 g)	12.3–14.1	[10]
Resistant starch	5.5–9.3	[10]
Calcium, Ca (mg/kg)	460–496	[34]
Iron, Fe (mg/kg)	73–90	[37]
Potassium, K (mg/kg)	6,954–7,761	[34]
Zinc, Zn (mg/kg)	44–54	[37]
Selenium (µg/kg)	425–673	[38]
Ascorbic acid (mg/kg)	61.2–84.3	[39]
Gallic acid (mg/kg)	28.2–39.3	[39]
Chlorogenic acid (mg/kg)	10.3–20.3	[39]
Folate, dietary folate equivalent (µg/g)	2.2–2.9	[40]
Phytic acid (mg/g)	2.4–4.4	[41]
Fe bioavailability (ng/mg of protein)	7.2–22	[42]

**Table 3.** Nutritional composition of raw lentils.



4. Prebiotic carbohydrates in lentil

Prebiotics, also known as low digestible carbohydrates, are defined as *a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health* [45]. A prebiotic is a specific colonic nutrient that provides a metabolic substrate, and/or acts as a biosynthetic precursor or cofactor for human microbiota. Classification of a food as a prebiotic requires scientific demonstration that the ingredient (1) resists digestive processes in the upper part of the gastrointestinal tract, (2) is fermented by intestinal microbiota, and (3) selectively stimulates growth and/or activity of health promoting bacteria in that microbiotic population [45].

Food	Prebiotic type	Range (g/100 g)	Reference
Lentil	Sugar alcohol		
	Sorbitol	1.04–1.35	[10]
	Mannitol	0.16–0.29	[10]
	Galactinol	0.03–0.13	[10]
	Non-starch polysaccharides		
	RFO	2–4	[10]
	Nystose	0.06–0.07	[10]
	FOS	0.20–0.10	[46]
	Starch polysaccharides		
	Total starch	44–49	[10]
Common bean	Resistant starch	3.4–9.3	[10, 47]
	Non-starch polysaccharides		
	RFO	0–1.4	[46]
	FOS	0–0.5	[46]
	Starch polysaccharides		
White bread	Resistant starch	1.8–1.9	[47]
	Non-starch polysaccharides		
	RFO	0–0.2	[46]
	FOS	0–0.7	[46]
	Starch polysaccharides		
Jerusalem artichoke	Resistant starch	0.1–1.2	[47]
	Non-starch polysaccharides		
	FOS	12.2	[48]

Table 4. Types and concentrations of prebiotic carbohydrates in foods.

Lentils, on average, contain a total of 63% carbohydrates [36] and support healthful hindgut microflora [10]. Naturally occurring prebiotic carbohydrates in lentils are categorized into two major groups: (1) dietary fiber and (2) sugar alcohols [10, 45]. Dietary fiber is comprised of starch polysaccharides, including resistant starch (RS), and non-starch polysaccharides, including raffinose family oligosaccharides (RFO), fructooligosaccharides (FOS), galactooligosaccharide (GOS), xylooligosaccharide (XOS), and insulin. Sugar alcohols include

sorbitol, mannitol, and galactinol. Types of prebiotic carbohydrates present in lentils and other foods are shown in **Table 4**. Concentration of prebiotics in foods range from trace levels, as seen in white bread, to relatively high amounts in Jerusalem artichoke. Many of these naturally occurring prebiotic carbohydrates are found in vegetables, pulses, and fruits at concentrations ranging from 35.7 to 47.6 g/100 g in chicory root to trace amounts in many vegetables [49]. In addition to vegetables and legumes, wheat, onion, and green bananas are other major sources of these carbohydrates. A few studies have reported RFO [50, 51] and RS [52] concentrations in lentil but did not assess genetic and environmental influences to determine the baseline concentration of these prebiotic carbohydrates in current lentil cultivars in production. Therefore, the ICARDA-Clemson University research program is working toward answering the following questions with respect to lentil prebiotic carbohydrates: (1) *What is the profile of lentil prebiotic carbohydrates?* [10] (2) *Is there any genetic or environmental variation in prebiotic carbohydrates in lentil?* [10, 53] (3) *What is the effect of dehulling, cooking, and cooling on lentil prebiotic carbohydrate concentrations?* [54] and (4) *What is the effect of lentil prebiotics on obesity?* The remainder of this chapter will provide an overview of research findings to date with respect to the first three questions as well as research currently in progress to address question four.

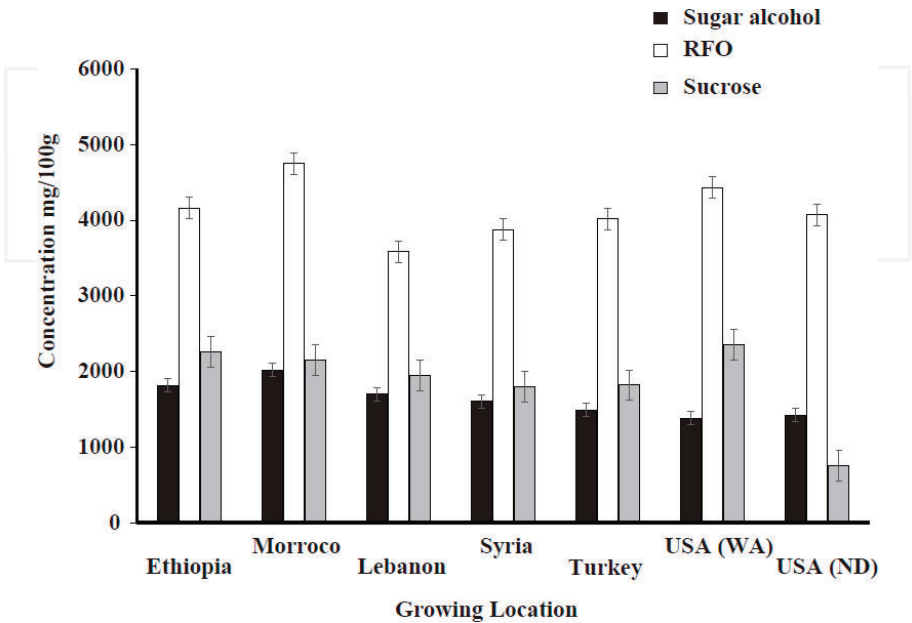
#### 4.1. What is the profile of lentil prebiotic carbohydrates?

To understand the profile of prebiotic carbohydrates, we analyzed 10 commercial lentil genotypes grown in North Dakota, USA, in 2010 and 2011. Study results clearly characterized the following lentil prebiotics: FOS (kestose and nystose), RFO (raffinose, stachyose, and verbascose), sugar alcohols (sorbitol and mannitol), and total and resistant starch [10]. Mean concentrations of RFO, sugar alcohols, FOS, and resistant starch were 4071 mg, 1423 mg, 62 mg, and 7.5 g 100 g<sup>-1</sup>, respectively. Total starch ranged from 45 to 48 g 100 g<sup>-1</sup>. This means a 100 g serving of lentil could provide over 13 g of total prebiotic carbohydrates (**Table 3**) [10]. Overall, these results indicate that lentil contains nutritionally significant amounts of prebiotic carbohydrates, the levels of which could potentially be further increased through genetic selection and location sourcing.

#### 4.2. Is there any genetic or environmental variation in prebiotic carbohydrates?

Work to date clearly demonstrates that prebiotic concentrations in lentils vary with genetic and environmental factors. Growing location/country significantly influences the concentration of various prebiotic carbohydrates (**Figure 2**). We completed a global survey of 335 lentil samples from 10 locations in 6 countries for sugar alcohols and various mono-, di-, and oligosaccharides, including RFO and FOS [54]. Mean LMWC concentrations varied widely: sorbitol, 1250–1824 mg/100 g; mannitol, 57–132 mg/100 g; galactinol, 46–89 mg/100 g; sucrose, 1,750–2,355 mg/100 g; raffinose + stachyose, 3,314–4,802 mg/100 g; verbascose, 1,907–2,453 mg/100 g; nystose, 8–450 mg/100 g; and kestose, from not detected to 244 mg/100 g. In addition, the concentrations of these prebiotics varied with average temperature and precipitation of the region/country of origin, which was expected because of the fact that sugar alcohols, RFOs, and sucrose are primarily stored in plants as reserves for survival during abiotic stress [55].

Moroccan lentils had consistently higher concentrations of RFOs and sugar alcohols (**Figure 2**) than those grown in other regions. Likewise, concentrations in Ethiopian and the American (WA) lentils were also high in RFOs. Regions with less precipitation and higher temperatures during the growing season showed higher concentrations of prebiotic carbohydrates, reflecting the existence of a response mechanism to water-deficit stress.



**Figure 2.** Sugar alcohol, raffinose family oligosaccharides (RFO), and sucrose concentration of lentils grown in seven different locations [10, 54].

Genotypic variation with respect to prebiotic carbohydrates was also evident. In lentils from the United StatesA [10], for example, total sugar alcohol concentrations were highest in CDC Riveland ( $1,598 \text{ mg } 100 \text{ g}^{-1}$ ), RFO concentrations ranged from  $3,508 \text{ mg } 100 \text{ g}^{-1}$  in CDC Rosetown to  $4,652 \text{ mg } 100 \text{ g}^{-1}$  in Pennell, and FOS ranged from  $52 \text{ mg } 100 \text{ g}^{-1}$  in CDC Red Rider to  $79 \text{ mg } 100 \text{ g}^{-1}$  in CDC Viceroy. Resistant starch ranged from 6.0 to  $8.9 \text{ g } 100 \text{ g}^{-1}$  and total starch ranged from 45 to  $48 \text{ g } 100 \text{ g}^{-1}$ .

#### 4.3. What is the effect of dehulling, cooking, and cooling on lentil prebiotic carbohydrate concentrations?

Food processing is an important component of food production. Generally, processing involves separation of non-edible parts, making foods more storage stable, and converting foods into easily cooked forms. Lentil processing involves three steps: cleaning, dehulling, and splitting. Cleaning removes other seeds, soils, and physical contaminants from the harvested lentils, dehulling involves removal of the lentil seed coats, and splitting breaks the lentil cotyledons

into two halves. The last two steps in lentil processing are determined by consumer preference: some lentil consumers favor lentils with the seed coat intact while others choose non-split and split lentils without seed coats. Lentils can also be further processed to separate protein, carbohydrate, and other fractions, but this is not typical; more than 90% of lentil produced in the world is consumed in either whole seed with seed coat, football (dehulled but whole), or split form.

Lentil is a fast cooking food (~10 minutes), and the majority of consumers eat lentils as a soup or curry as a nutritional complement to accompanying rice or bread. Their short cooking time makes them a popular whole food for millions of people. Unlike other legumes, cooked lentils retain all of their nutritional value as there are no cooking water nutrient losses. As noted above, lentils can be processed into various fractions, with the carbohydrates and proteins contributing unique physical and chemical properties to foods when added as an ingredient. However, separation of lentil into different fractions requires higher energy and labor inputs that increase in cost of production. Such further lentil processing may also lead to a loss of essential nutrients found only in the whole seeds. Current lentil supply and demand, the increased focus on micronutrient delivery from whole foods, and consumer preferences suggest lentil will continue to be produced and marketed primarily as a whole food.

Few studies have indicated the effects of processing and cooking on lentil prebiotic carbohydrate concentrations. Two commercially available lentil market classes (medium green and small red) showed some RFO reductions with cooking, cooling, and reheating [54]. Mean RS concentrations in raw, cooked, cooled, and reheated lentil were measured at 3.0, 3.0, 5.1, and 5.1% (w/w), respectively, indicating cooling-induced synthesis of RS from gelatinized starch. These results highlight the impact of temperature on lentil nutritional quality and shows lentil are more nutritious after cooling. Similar increases (400%) in RS from raw to cooked then cooled potatoes have also been demonstrated [56].

#### **4.4. What is the effect of lentil prebiotics on obesity?**

Recent discoveries suggest the intestinal microbiome and a prebiotic-rich, low-caloric diet can play important roles in combating obesity and related diseases. Three dominant phyla have been identified in human fecal flora: Firmicutes, Bacteroides, and Actinobacteria. Subdominant groups are enterobacteria, streptococci, and lactobacilli [57]. The relative proportion of Bacteroidetes is decreased in obese individuals compared with lean individuals; however, this relative proportion rebounds with weight loss on a prebiotic-rich, low-caloric diet. Furthermore, consumption of non-digestible, fermentable carbohydrates (or prebiotics) may stimulate the growth and activity of hind gut bacteria by producing short-chain fatty acids that provide energy source for colonocytes, strengthen the gut mucosal barrier, and suppress colonization of pathogens [43]. As clearly shown by our research, lentil is a rich source of prebiotic carbohydrates, therefore, lentils offer new opportunities in this regard. To date, no research has been carried out to understand the true effect of lentil prebiotics on obesity. We are expecting to finish preliminary research related to this question in summer 2016. Lentil prebiotic carbohydrates provide numerous positive benefits to human health. However, accurate prebiotic carbohydrate characterization and quantification is important not only to

determine types and levels but also inform consumers of food sources that are rich in these important compounds.

#### 4.5. Accurate measurement of prebiotics

Human intestinal enzymes are able to digest carbohydrates based on their molecular structure. Carbohydrates can be categorized into readily-, slow- and non-digestible carbohydrates, which are known chemically as mono-, di-, and polysaccharides. Glucose, a monosaccharide, is a readily digestible carbohydrate; however, starch is a glucose polysaccharide with a digestibility varying from fully digestible to non-digestible. Some prebiotic carbohydrates resist the activity of human digestive enzymes and pass to the large intestine where they are acted upon by bacterial enzymes. The use of both modern analytical instrumentation and enzymatic procedures is required to accurately characterize the true levels of prebiotic carbohydrates.

Carbohydrate analysis involves two main steps: carbohydrate isolation from a sample and analysis of those isolated compounds. Hot water extraction or a combination of hot water and ethanol is used to isolate most water-soluble carbohydrates. These water-ethanol soluble carbohydrates are small in molecular size. The most accurate analytical method to quantify these smaller carbohydrates is high performance anion exchange chromatography with pulsed amperometric detection (HPAE-PAD). Sugar alcohols of monosaccharides represent two groups of water-soluble carbohydrates that can be accurately identified and quantified by HPAE-PAD. Other carbohydrates such as disaccharides (two monosaccharide units) and oligosaccharides (3–10 monosaccharide units) are also water or water-ethanol mixture soluble carbohydrates and can be successfully be quantified using HPAE-PAD. However, HPAE-PAD and other analytical methods do not provide accurate identification and quantification of polysaccharides comprised of 10 or more monosaccharides. These carbohydrates can only be quantified only after hydrolyzing them into monosaccharides by enzymatic hydrolysis.

Enzyme hydrolysis of prebiotic carbohydrates has two objectives: (1) to simulate human intestinal environment to isolate digestible and non-digestible carbohydrate fractions and (2) to selectively hydrolyze the glycosidic linkage of carbohydrates for accurate quantification. For example, amylases are a type of human digestive enzyme that can be used to separate starches from resistant starches during sample preparation/isolation procedures. The same type of enzyme can also be used to completely hydrolyze starch-like macromolecules into their simpler glucose units, thus enabling quantification of larger carbohydrate molecules.

Sugar alcohols such as sorbitol have the simplest molecular structure of all prebiotic carbohydrates; however, resistant starches are large, complex macromolecules. Well-established HPAE-PAD and enzymatic procedures are available to accurately quantify some but not all prebiotic carbohydrates. However, work is ongoing to combine good understanding of prebiotic carbohydrate molecular structures, HPAE-PAD instrumentation, and enzymatic hydrolysis procedures to accurately quantify more prebiotic carbohydrates.

## 5. Lentil breeding at the ICARDA

Within the biofortification framework, the ICARDA lentil breeding program is working together with Clemson University to increase mineral concentration and bioavailability to combat global micronutrient malnutrition. In addition, future lentil selections will be carried out by selecting cultivars with higher micronutrients, prebiotic carbohydrates, and low phytate. Biofortification can improve crop nutritional value with minimal impact on consumer cost. The ICARDA has also created a composite collection of more than 1,000 lentil lines to understand the genetic diversity with respect to different nutritional traits, including iron and zinc accumulation. Knowledge of the patterns of variation in the world germplasm collection for yield, disease resistance, and nutrition is the key to understand factors affecting lentil adaptation that can then be applied to lentil breeding. The geographic distribution of these landraces in the world lentil collection for various morphological characteristics, responses in flowering to temperature and photoperiod, winter hardiness, iron-deficiency chlorosis, and boron imbalances collectively illustrate the specificity of adaptation in lentil. Additional information on the specificity of adaptation within the crop has come from collaborative multi-environment yield trials of common entries selected in different locations.

Understanding genotypes and environmental factors, local constraints to production, and the various consumer requirements of different geographic areas has led the breeding program at the ICARDA to develop new genetic materials for a series of separate but finely targeted geographical streams, linked closely to national breeding programs. The major agro-ecological regions of production of lentil being targeted include (1) South Asia, East Africa, and Yemen, (2) low-to-medium elevation Mediterranean regions, and (3) high-elevation areas of West Asia and North Africa. These regions correspond to early, medium, and late maturity groups, respectively. Additionally, lentil improvement activities have also been extended to the Central Asia and Caucasus (CAC) region, where an initial thrust has been to study the adaptation of diverse material suitable to their agro-climatic conditions.

## 6. Final thoughts

Recent studies have focused on health-beneficial bioactive components in commonly eaten foods to understand their impact on human health and disease. Among these bioactive compounds, prebiotic carbohydrates are important food constituents to reduce obesity-related non-communicable diseases through interactions with the hindgut microbiome. Lentils induce a low-glycemic response, and this has been attributed to their prebiotic content that has a high resistance to human enzyme hydrolysis. In addition to human health benefits, prebiotic carbohydrates are important for plant survival, e.g., with respect to water-deficit or cold stress. Overall, lentil is a highly nutritious pulse crop that has supplied essential nutrients including proteins, dietary fiber, macro, and micronutrients to various populations over centuries.

Despite current research evidence on prebiotic health effects, lentil breeding programs continue to work toward lentil cultivars with reduced levels of RFOs in response to consumer preference in certain markets [51]. RFOs have long been known as antinutrients and linked to the flatulence and gastrointestinal discomfort that occur in some consumers [58]. Conventional plant breeding programs have reduced the RFO concentration in seeds [59]; however, human health benefits associated with RFOs have begun to be documented. RFO and other prebiotic carbohydrates are important dietary components for preventing overweight and obesity and associated diseases. Therefore, the aim of future plant-breeding efforts may instead be to enhance all types of prebiotic carbohydrates.

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