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Article type : Research

Water soluble carbohydrates (WSC) during fermentation and baking of composite wheat and lentil flour – Implications for enhanced functionality

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ABSTRACT

Background and objectives: The nutritional benefits of Lentil (*Lens culinaris*. Medik) have been widely acknowledged. Enriching wheat flour using pulses such as lentil flour is a way of increasing both the nutritional and functional content of wheat-based foods. The properties of lentil that may have specific health benefits to the individual are also widely acknowledged. Functional compounds from plant-based foods are commonly attributed to polyphenols, inhibitors, vitamins, as well as soluble and insoluble fiber. Water soluble carbohydrates, (WSC) consisting predominantly of oligosaccharides, make up part of total dietary fiber. The beneficial role of oligosaccharides promoting probiotic health are widely acknowledged. Equally it is recognized that oligosaccharides cause irritable bowel syndrome (IBS) limiting the ability for some individuals in consuming pulses.

Findings: We investigated changes in water soluble carbohydrate (WSC) composition during the dough fermentation phase of bread making and quantified the residual WSC in the bread.

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1002/CCHE.10144](https://doi.org/10.1002/CCHE.10144)

As expected, the addition of lentil flour increased the WSC profile of the resulting composite flours which included raffinose, stachyose, ciceritol and verbascose. A three-fold decrease was observed only for verbascose during the dough mixing phase, however during fermentation raffinose and stachyose decreased but ciceritol was not affected.

Conclusion: The baking process may reduce the effects of IBS suffered by some individuals who consume lentil products prepared by traditional methods. Our study concluded that bread prepared from wheat-lentil flour results in a more complex carbohydrate profile and may potentially enhance the prebiotic functionality compared with bread made from wheat alone.

Significance and novelty: It is now recognized that alternate sources of protein can be gained using plant-based constituents. The functional properties of plant-based compounds are also widely accepted. Processing, such as fermentation and baking can alter the concentration and functionality of plant-based compounds. In this study we identified the changes in carbohydrate composition that occur in a wheat-lentil composite flour under the conditions of fermentation and baking.

KEY WORDS

Oligosaccharide, fermentation, water soluble carbohydrate (WSC)

INTRODUCTION

In developing countries, consuming adequate protein continues to be challenging due to accessibility and environmental practice (Frias et al., 1996). Meeting the nutritional deficits will become a continued challenge (Akhtar, Anjum, & Anjum, 2011), as the world population is projected to exceed 9.8 billion people by 2050 (DESA, 2015). Furthermore, increasing concentration of carbon dioxide (CO₂) is contributing to a reduction in protein of cereals (Panozzo et al., 2014). Therefore the development of alternative food sources ensuring world-food security is imperative (Frias et al., 1996). Lentils (*Lens culinaris*. Medik) are a source of protein, vitamins and a balanced range of minerals (Wang & Daun, 2004). On the other hand, wheat (*Triticum aestivum*. L) continues to be one of the most widely used grains for human consumption (Antle, Cho, Tabatabaie, & Valdivia, 2018). Whilst wheat is an important source of nourishment for humans, its nutritional value is limited, due to its relatively low protein concentration and unbalanced amino acid profile (Nosworthy, Tulbek, & House, 2017). Both lentil and wheat are important food sources for many and unlike meat products, enjoy worldwide acceptance (Savage, 1988). Opportunities exist to develop food alternatives through combining wheat and lentil flours in fermented and baked products such as bread (Bojnanská, Francáková, Lísková, & Tokár, 2012; Dalgetty & Baik, 2006; Kohajdová, Karovičová, & Magala, 2013). Fermentation is a cost-efficient method of food processing

and preparing food, which can enrich both the organoleptic and nutritional qualities of food. During fermentation the interaction of yeast with sugars is fundamentally important to end-product quality, predominantly influencing the flavor profile (Heitmann, Zannini, Axel, & Arendt, 2017). In wheat-lentil composite flour, there is a significantly higher concentration of sucrose and an increase of the disaccharide, maltose. In addition, oligosaccharides (raffinose, stachyose, verbascose and the digalactoside ciceritol), from lentil extend the sugar profile to include non-digestible water-soluble carbohydrates (WSC). It has been reported that WSC which are fermented in the colon display positive functionality. They may assist in stool softening, also concomitantly increasing lactobacilli and bifidobacteria improving gut health while reducing opportunistic pathogens such as enterobacteria (Berrios, Morales, Cámara, & Sánchez-Mata, 2010). Furthermore, WSC may mitigate carcinogenicity through degradation of N-nitroso compounds (NOC) within the gut (Van Loo et al., 1999). The digalactoside ciceritol found in lentil, may also play a prebiotic role in humans (Fernando et al., 2010; Quemener & Brillouet, 1983). A recent *in vitro* study showed that ciceritol extracted from chickpeas increased microflora in the colon and promoted short-chain fatty acid (SCFA) production of acetic, propionic and butyric acids (Zhang et al., 2017). SCFAs have important physiological roles in maintaining body function. Of these, butyric acid is the primary energy source of colonic cells, stimulating growth and maintenance (O'Keefe & Greer, 2011). On the other hand, WSCs can have an adverse effect. Fermentation in the hindgut can cause production of hydrogen, as well as carbon dioxide and methane. These compounds can cause abdominal bloating, excessive gas and in some cases diarrhea (Frias et al., 1996; Gilani, Xiao, & Cockell, 2012). Fleming (1981) reported that ciceritol did not cause production of hydrogen, carbon dioxide, or methane in a rat model, concluding that ciceritol is not involved in negative gastrointestinal symptoms. This study profiled the attenuation of WSC by yeast, contrasting wheat flour to a wheat-lentil blend and quantified the residual WSC in the resulting bread.

MATERIALS AND METHODS

Materials

Lentil (*Lens culinaris*. Medik), cv. Northfield is a commercially grown variety characterized by a gray seed coat and orange-red cotyledon. The wheat variety cv. Elmore (*Triticum aestivum*) is hard-grain, white wheat used for bread making. The wheat flour used in this study was prepared by conditioning wheat to 16% moisture content for 24 hours prior to milling on a Buhler laboratory mill (MLU 202, Buhler, Switzerland). Whole dry lentil seeds were de-hulled mechanically with an abrasive dehuller (Graintec Scientific, QLD, Australia)

and the fractions of cotyledon and seed coat collected separately using an air aspirator (KimSeed, WA, Australia). The cotyledon was milled to flour using a cyclone mill fitted with a 0.5mm screen (Laboratory Mill 120, Perten Instruments, Huddinge, Sweden). An 80-20% wheat-lentil flour composite was prepared by mixing 240g of wheat flour with 60g of lentil flour which was homogenized in a screw mixer (Chopin Technologies, France).

Leavening and Bread Baking

All bread samples were baked in triplicate using the straight dough procedure, AACC Approved Methods 10-09.01. (AACC, 2000). The formula consisted of flour, (110 g) to which 1.1 g of sodium chloride, 1.1 g of sugar, 0.7 g of improver prepared from stock (1g Fungal alpha-amylase (Novozymes, Denmark) of medium-activity (2500 FAU/g) + 200 g corn starch), and 2.2 g of vegetable fat was added. At the commencement of mixing 1.5 g of bakers yeast, 99% purity with a minimum of 2.2×10^5 viable cells per gram (Kerry Pinnacle Bakery Ingredients, NSW, Australia) was added to the flour, followed by 65 mL of RO water and 5 mL of ascorbic acid - ammonium chloride solution which was prepared from a stock (0.28 g of ascorbic acid, 5.5 g of ammonium chloride and in 250 mL RO water). Each sample was mixed in a laboratory pin mixer (National Manufacturing, Nebraska, USA). Dough development (time to peak resistance) was measured using power to mixer P2M software (RAR Software Systems, Winnipeg, CA). Samples were fermented for 3:50 hours with initial humidity and temperature held constant at 80% and 33.2 °C. Samples were mechanically moulded at 2:20 hours then at 2:53 hours (Mono-moulder, Mono Universal, D. Ayres Jones & Co., Ltd, Swansea, UK). Immediately following the second moulding the dough was placed in baking tins and fermented for a final 58 minutes with 85 % relative humidity and a temperature of 33 °C. Each sample was then baked at 205°C for 24 minutes in a pilot-scale electric baking oven (Rotel, APV Inc, QLD, Australia).

Sample Preparation

A sample of dough was taken at each fermentation step, i.e. mixing; 3 minutes, first punch; 2:20 hours, second punch; 2:53 hours, and oven-in; 3:50 hours. Dough samples were placed in air tight bags and immediately placed in a -18°C freezer to terminate fermentation. The crust and crumb for bread samples were separated after baking, 4:11 hours, and stored at -18°C. Both dough and bread samples were freeze-dried for 24 hours (Telstar LyoQuest HT-40, Beijer Electronics, Telstar, Spain), ground to a fine powder in a coffee grinder, and then stored at -18°C.

Water Soluble Carbohydrate Extraction

Carbohydrate extraction was performed using the method described by Maharjan et al., 2018 with modification. Individual sugar was analysed using 0.4 g of ground sample, weighed into Teflon tubes and suspended in 5 mL of reverse osmosis (RO) water. Each sample was then sonicated for a total of 30 minutes at a frequency of 50 hertz (FXP 12, Unisonic Pty Ltd, Australia), and vortexed vigorously (Thermoline Maxi Mix II, Thermoline Scientific, IA, USA), at 10-minute intervals throughout the sonification process. This step was repeated two more times. The extract was then centrifuged at 10,000 g for 10 minutes (Eppendorf Centrifuge 5810, Hamburg, Germany). An aliquot, 0.75 mL of the resulting supernatant was transferred to 2 mL Eppendorf tubes and 0.75 mL of 100% acetonitrile added. Each sample was then centrifuged at 3000 g for 10 minutes (Eppendorf Centrifuge 5430R, Hamburg, Germany), filtered through a 0.2 µm PTFE syringe filter (Grace Davidson Discovery Sciences, IL, USA). The sample was then analysed for WSC using the UPLC/ELSD method outlined by Maharjan et al., 2018.

Water Soluble Carbohydrate UPLC/ELSD Analysis

Water soluble carbohydrate analysis was performed using a Waters ACQUITY Ultra High-Performance Liquid Chromatography (UPLC) system (Waters Corporation, Midford, MA, USA) equipped with UPLC Binary Solvent Manager, UPLC Sample Manager and an Evaporative Light Scattering Detector (ELSD). Separation was performed using a Waters ACQUITY BEH Amide column (2.1×50 mm, 1.7 µm) at 25°C. The mobile phase consisted of 80% acetonitrile with 0.05% ammonia (Solvent A) and 30% acetonitrile with 0.05% ammonia (Solvent B). The injection volume was 2.0 µL for all samples and the flow rate was kept constant at 0.13 mL/min over a 30-minute run time. UPLC/ELSD data was analyzed using Empower 3 software (Waters Corporation, Midford, MA, USA) to identify water soluble carbohydrate compounds and calculate peak area. Identification of individual sugars was performed by internal standards and retention time. All standards were validated by a UPLC/QDa mass detector (Waters Corporation, Midford, MA, USA). Concentration of sugars was determined using power curves constructed from external standards and individual peak area (Maharjan, Jacobs, Deighton, & Panozzo, 2018). The raffinose power curve was used in the quantification of ciceritol, due to a lack of a commercially available ciceritol standard as previously reported by Berrios et al. (2010) and Martín-Cabrejas et al. (2006).

Statistical analysis

All data were subjected to analysis of variance (ANOVA) with GenStat statistical software 17th edition (VSN International, Hemel Hempstead, UK). Means were analyzed for the least significant difference at a probability level of $p < 0.05$. Results are expressed as mean values $\pm$ standard deviation. All analyses were conducted in triplicate.

RESULTS AND DISCUSSION

Wheat and lentil flour were investigated using UPLC/ELSD in an assessment of the extended WSC profile that would occur when using a wheat-lentil composite blend. Table 1 shows the mass to charge ratio and retention times for WSC fructose, glucose, sucrose, maltose, raffinose, ciceritol, stachyose and verbascose.

(Insert Table 1)

The addition of lentil flour extended the WSC profile of the wheat-lentil flour composite with the inclusion of the oligosaccharides raffinose, stachyose, verbascose, and the digalactoside ciceritol (Figure 1c and Table 2). However, during the bread making process, the relative concentration of these sugars was altered.

Figure 2 shows the utilization of simple sugars during fermentation and residual sugars in the resulting bread of 100% wheat, and a composite flour containing 20% lentil flour. Our results show that for both wheat and wheat-lentil flour, fructose and glucose were found in low concentration before dough mixing. Flour contains sucrose that is degraded to fructose and glucose by invertase that is secreted by yeast (Bely, Stoeckle, Masneuf-Pomarède, & Dubourdieu, 2008). Furthermore, the addition of ascorbic acid, a standard component of the straight dough method may cause further hydrolysis of sucrose (Pavlova et al., 2013; Toulouse, 1929). The hydrolyzation of sucrose to fructose and glucose occurs early in the fermentation process and is most likely initiated during dough mixing, as no sucrose was detected in samples obtained after the three-minute dough mixing time (Figure 2c), this coincided with a significant increase ($P < 0.05$) in fructose and glucose being observed during mixing for both wheat and wheat-lentil dough (Figure 2a, 2b, and Table 2).

Maltose concentration also significantly increased ($P < 0.05$) during fermentation in the dough for both the wheat and wheat-lentil composite dough (Figure 2d and Table 2). The increase in maltose as fermentation progressed is most likely due to the enzymatic hydrolysis of starch catalyzed by amylase (Van Der Maarel, Van der Veen, Uitdehaag, Leemhuis, & Dijkhuizen, 2002). Maltose concentration was significantly higher in wheat flour compared to the wheat-lentil composite ($P < 0.05$). Stabilization of maltose concentration was observed during the three hours of fermentation, and a significant decrease in maltose concentration

was only observed in the final 30 minutes of fermentation ($P < 0.05$) (Figure 2c and Table 2). This may indicate that maltose uptake by yeast is regulated by fructose and glucose concentration and occurs concurrently during fermentation.

(Insert Figure 1)
(Insert Figure 2)

Both wheat and the wheat-lentil composite bread contained significantly higher amounts of fructose ($P < 0.05$) in both the crust and crumb in comparison to the original flours, (Figure 2a and Table 2). Glucose was not detected in either the crust or crumb of wheat bread however, wheat lentil composite bread had low concentrations of glucose in both crust and crumb, that were significantly lower ($P < 0.05$) than the originating flour (Figure 2b and Table 2).

Changes in oligosaccharide WSC during each stage of the bread baking process are shown in Figure 3 and Table 2. Raffinose, ciceritol, stachyose and verbascose were present in the wheat-lentil composite flour, these WSCs were not detected in the wheat flour (Figure 1a and Figure 1b).

Water soluble carbohydrates are known to form gas during post-gut fermentation and initiate IBS in sensitive individuals (Frias et al., 1996). This study found that during fermentation, both raffinose and stachyose were significantly reduced ($P < 0.05$), and not detected in the final bread (Figure 3e, Figure 3g, and Table 2).

Conversely, a three-fold reduction was observed for verbascose ($P < 0.05$) and this decrease was only apparent in the dough sampled from the mixing phase. Ciceritol did not change during fermentation and both verbascose and ciceritol were present in the crust and crumb of breads resulting from wheat-lentil blends (Figure 3f, Figure 3h and Table 2). The significant reduction in raffinose and stachyose may partly explain the incomplete attenuation of glucose in the crust and crumb of the wheat-lentil blend (Figure 2 and Table 2). This may be a result of both ease of accessibility, although glucose concentration was substantial at this stage of fermentation, it is possible that yeast was also able to start utilization of raffinose and stachyose early in fermentation, which is supported by our findings (Figure 3e, Figure 3g, and Table 2), where raffinose was not detectable at 2:53 hours of fermentation, (2nd punch) and stachyose was not detected at 3:50 hours of fermentation (oven in). In the latter stages of fermentation, for example beyond 3 hours, yeast can more readily utilize maltose than verbascose, which can be explained by the highly branched structure and α -(1→6) linkages of verbascose resulting in non-fermentable dextran sugars. It is also possible that at this stage of fermentation, all damaged starch granules have been hydrolysed and therefore, there is no

further increase in maltose which may explain the significant decrease ($P < 0.05$) in maltose concentration (Figure 2d).

Overall bread made from a composite wheat-lentil flour had an extended WSC profile when compared to wheat bread. Residual glucose which was detected in wheat-lentil composite bread but was not detected in the wheat bread (Figure 1f, Figure 1h, and Table 2). Maltose concentration in the wheat-lentil composite bread was also significantly higher ($P < 0.05$) compared to maltose in wheat bread (Table 2). Additionally, wheat-lentil blends contained residual

(Insert Figure 3)
concentrations of verbascose and ciceritol, however there was no stachyose or raffinose detected within the composite bread (Table 2), suggesting that both were consumed during the fermentation process. Fermentation reduced the total concentration of WFC for both wheat and wheat-lentil composite breads. This finding aligns with Berrios et al. (2010), who reported a lower total available WSC concentration in extruded pulse flours, and proposed that in part this may be caused by high temperature processing.

(Insert Table 2)

CONCLUSION

In this study we considered the bioactive potential that WSC due to the addition of lentil flour with wheat flour may contribute to confirming a health benefit, and how fermentation and baking processes influenced WSC concentration levels. Our results show that during fermentation both raffinose and stachyose in wheat-lentil dough is completely utilized. Moreover, wheat-lentil breads retained significant levels of verbascose and ciceritol after baking, these WSCs have been reported to act as soluble dietary fiber when fermented in the small intestine. Additionally, both glucose and fructose, important sources for spontaneous short-term energy gain, remain in low concentration in wheat-lentil breads after baking. Overall this study highlights the potential in providing immediate energy requirements through monosaccharides, and potential prebiotic functionality through the introduction of oligosaccharides, achieved by combining lentil with wheat flour in baking.

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TABLE 1 QDa m/z values and retention time observed for carbohydrates

Carbohydrate	[M-H] ⁻	[M+Cl] ⁻	(tR/ELSD)
Fructose	ND	215.0	1.13
Glucose	ND	215.0	1.28
Sucrose	341.0	377.0	1.78
Maltose	ND	377.0	2.11
Raffinose	503.1	539.1	2.89
Ciceritol	517.2	553.2	3.56
Stachyose	665.2	701.1	3.84
Verbascose	827.3	863.3	4.53

Notes. m/z: mass to charge ratio, negative ion: [M-H]⁻, positive ion: [M+Cl]⁻, retention time. electronic light scattering detector: (Tr/ELSD), ND: not detected.

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TABLE 2 Changes in WSC of flour and dough in fermentation and baking (% per 100g)

Wheat Flour- 100%									
Sample	Fructose	Glucose	Sucrose	Maltose	Raffinose	Ciceritol	Stachyose	Verbascose	Total Sugar
Flour (N/A)	0.12 ± 0.01^a	0.02 ± 0.03^a	0.27 ± 0.01	0.65 ± 0.01^a	ND	ND	ND	ND	0.8 ± 0.01^a
Mixing (3 minutes)	1.21 ± 0.01^b	1.45 ± 0.01^b	ND	2.02 ± 0.01^b	ND	ND	ND	ND	4.7 ± 0.01^b
1 st Punch (2:20 hrs)	1.21 ± 0.04^b	1.21 ± 0.04^c	ND	2.61 ± 0.01^c	ND	ND	ND	ND	5.0 ± 0.04^c
2 nd Punch (2:53 hrs)	0.79 ± 0.01^c	0.81 ± 0.01^d	ND	2.49 ± 0.01^c	ND	ND	ND	ND	4.1 ± 0.01^d
Oven In (3:50 hrs)	0.28 ± 0.01^d	0.44 ± 0.01^e	ND	2.08 ± 0.01^d	ND	ND	ND	ND	2.8 ± 0.01^e
Crumb (4:11 hrs)	0.22 ± 0.01^e	ND	ND	2.20 ± 0.03^e	ND	ND	ND	ND	2.4 ± 0.01^f
Crust (4:11 hrs)	0.20 ± 0.01^e	ND	ND	1.66 ± 0.02^f	ND	ND	ND	ND	1.9 ± 0.01^g
Wheat / Lentil 80-20%									
Sample	Fructose	Glucose	Sucrose	Maltose	Raffinose	Ciceritol	Stachyose	Verbascose	Total Sugar
Flour (N/A)	0.09 ± 0.05^a	0.05 ± 0.04^a	0.48 ± 0.01	0.50 ± 0.01^a	0.24 ± 0.01^a	0.41 ± 0.01^a	1.52 ± 0.01^a	0.72 ± 0.02^a	3.8 ± 0.02^a
Mixing (3 minutes)	1.11 ± 0.02^b	1.38 ± 0.03^b	ND	1.88 ± 0.03^b	0.23 ± 0.02^b	0.40 ± 0.01^a	0.30 ± 0.01^b	0.23 ± 0.01^b	5.6 ± 0.02^b
1 st Punch (2:20 hrs)	0.96 ± 0.01^c	1.24 ± 0.02^c	ND	2.63 ± 0.04^c	0.20 ± 0.4^c	0.40 ± 0.01^a	0.23 ± 0.09^c	0.19 ± 0.06^b	5.7 ± 0.01^c
2 nd Punch (2:53 hrs)	0.74 ± 0.01^d	1.03 ± 0.02^d	ND	2.47 ± 0.01^c	ND	0.40 ± 0.02^a	ND	0.19 ± 0.02^b	5.0 ± 0.01^d

Oven In (3:20 hrs)	0.29 ± 0.01^e	0.86 ± 0.01^e	ND	2.29 ± 0.01^d	ND	0.40 ± 0.01^a	ND	0.19 ± 0.07^b	2.1 ± 0.01^e
Crumb (4:11 hrs)	0.24 ± 0.01^f	0.04 ± 0.20^f	ND	2.64 ± 0.04^e	ND	0.30 ± 0.01^a	ND	0.17 ± 0.06^c	3.4 ± 0.01^f
Crust (4:11 hrs)	0.24 ± 0.03^f	0.01 ± 0.52^f	ND	1.72 ± 0.01^f	ND	0.30 ± 0.01^a	ND	0.08 ± 0.01^c	2.4 ± 0.03^g

Notes. ND: not detected. Data are mean $\pm$ SD. Values in the same column with different alphabetical letters differed significantly as determined by ANOVA following a Tukey's HSD test ($p < 0.05$), LSD = 0.76.

FIGURE 1 Ultra High-Performance Liquid Chromatography and evaporative light scattering detection chromatogram of a: 100% wheat at mixing 3 minutes, b: 100% wheat at oven in 4 hours 11 minutes, c: 80% wheat-20% lentil at mixing 3 minutes, d: 80% wheat-20% lentil at oven in after 4 hours 11 minutes, e: 100% wheat crumb, f: 100% wheat crust, g: 80% wheat-20% lentil crumb, h: 80% wheat-20% lentil crust. (peaks: 1; fructose, 2; glucose, 3; maltose, 4; raffinose, 5; ciceritol, 6; stachyose, 7; verbascose).

FIGURE 2 Fermentation profile of mono and disaccharides. Results are the means of three replicates, significance difference at ($P < 0.05$).

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Title:

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Date:

2019-05-01

Citation:

Portman, D., Blanchard, C., Maharjan, P., Naiker, M. & Panozzo, J. F. (2019). Water-soluble carbohydrates during fermentation and baking of composite wheat and lentil flour-Implications for enhanced functionality. CEREAL CHEMISTRY, 96 (3), pp.447-455. <https://doi.org/10.1002/cche.10144>.

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