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Activation and Inhibition of Nuclear Factor Kappa B Activity by Cereal Extracts: Role of Dietary Phenolic Acids

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The transcription factor nuclear factor kappa B (NF-κB) plays a critical role in stress, immune, and inflammatory responses, and the modulation of its activity can be a potentially effective preventive strategy for controlling certain diseases. Cereal grains contain phenolic compounds in concentrations comparable to those in fruits and vegetables, well-known for their beneficial effect on human health. In this study we aimed to examine the effect of different phenolic extracts from barley, oat, wheat, and buckwheat on the modulation of basal and lipopolysaccharide (LPS)-induced NF-κB activity and elucidate the role of phenolic acids in this modulation. Three extracts were prepared: extracts of free phenolic compounds (M1), extracts of free phenolic acids (M2), and extracts of bound phenolic acids (HY). Generally, extracts M2 showed the highest effect on modulation of NF-κB activity with strong inhibition of LPS-induced NF-κB activity at all concentrations and of the basal NF-κB activity at concentrations equal to or lower than 3 mg/mL. Most of extracts M1 and HY slightly increased both the basal and the LPS-induced NF-κB activation. However, at the highest concentrations (3 or 15 mg/mL) extracts HY inhibited LPS-induced NF-κB activation. Similar experiments with standard solutions of phenolic acids indicated their ability to modulate the NF-κB activity.

KEYWORDS: Nuclear factor kappa B; cereal extract; phenolic acids

INTRODUCTION

It has been shown that consumption of grains lowers the risk of diseases such as cardiovascular disease, ischemic stroke, diabetes, metabolic syndrome, and gastrointestinal cancers (*1*−*3*). The focus of such studies has been directed toward finding a connection between a grain diet, with its content of different dietary fibers, and its positive effect on human health (*4*−*6*). In addition to dietary fiber, whole grain contains vitamins, minerals, and phytochemicals, including phenolic acids. The interest in phenolic compounds in grains (dietary phytochemicals (DP)) has increased in the last few years, resulting in several studies of the health-promoting effects of whole-grain consumption (*7*−*10*). DP possess antioxidant properties that may protect against reactive oxygen species (ROS) involved in different diseases (*11*−*13*).

The content and diversity of antioxidants in fruits and vegetables are high; however, it has been shown that the phenolic content in cereal grains and their antioxidant capacity levels are comparable to those of fruits and berries (*14*, *15*). In addition, some cereals contain unique types of phenols, such as avenanthramides in oat, that are proposed to have health-promoting effects (*16*, *17*).

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The effect on human health of bioactive compounds, natural vitamins, tannins, and flavonoids has been widely studied (*18*−*20*). Less is known about the effect of phenolic acids present in grains. These acids are present in both free and bound form and possess antioxidant activity comparable with that of other well-known phenolic compounds such as tocopherols, including vitamin E (*21*). Free phenolic acids are located in the outer layer of the pericarp of the cereal kernel, while the majority of phenolic acids is found in bound form esterified to the cell walls. *p*-Coumaric and ferulic acids are the major phenolic acids found in cereals, although their exact level may vary among cereal varieties (*9*, *14*, *21*).

Nuclear factor kappa B (NF-κB) is a family of transcription factors that play a critical role in immune, cellular stress, and inflammatory responses and controls the gene expression of proteins involved in innate immunity, apoptosis, cell cycle, proliferation, and cell survival. Activation of NF-κB promotes cell survival, while suppression of its activity induced by various stimuli is important for apoptosis. Several human disorders involve inappropriate regulation of NF-κB. Aberrant regulation of factors in the NF-κB pathway has been noted in several human cancers (*43*), and also atherosclerosis (*44*), neurodegenerative diseases (*45*), rheumatoid arthritis (*46*), asthma (*47*), and inflammatory bowel disease (*48*) have been associated with a persistent increase in NF-κB activity. It has, however, also been

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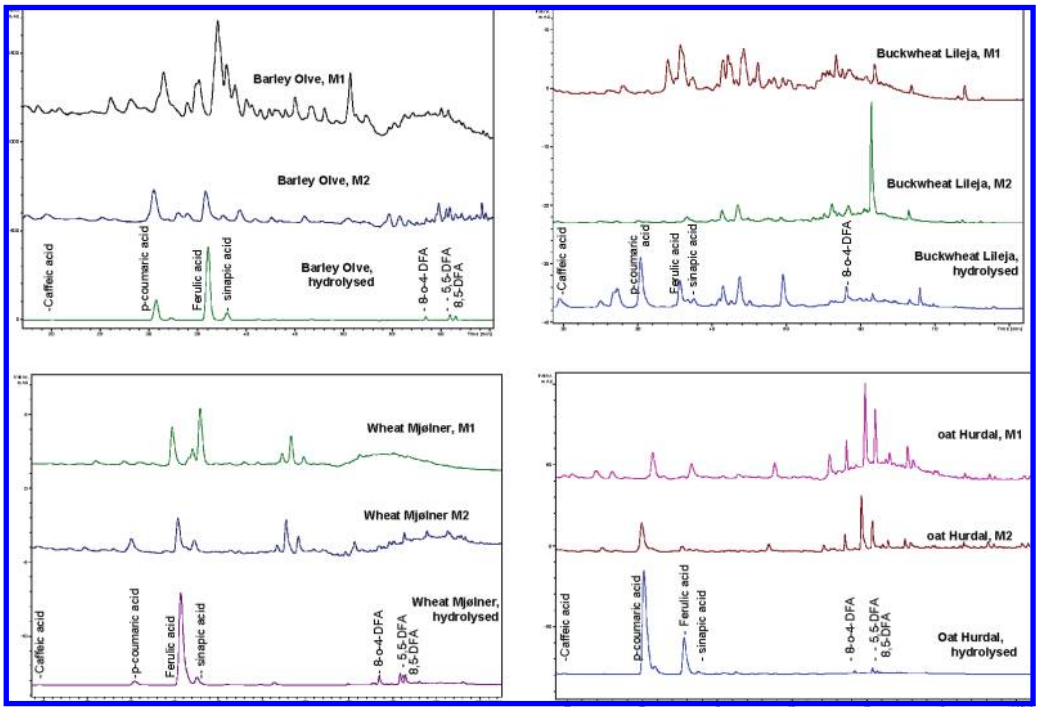

Figure 1. Chromatograms of extracts M1, M2, and HY. The chromatograms were obtained using the LC-MS system with the analytical column Phenomenex 150 × 2.0 mm I.D 3 μm and wavelength detection at 320 nm. The gradient elution program used involved 1% acetic acid as eluent A and 1% acetic acid in acetonitrile as eluent B with a flow rate of 0.2 mL/min at 25 °C. The M1, M2, and HY extracts contain free phenolic compounds, free phenolic acids, and bound phenolic acids, respectively.

demonstrated that small repetitive activations of basal NF-κB can be protective against diseases such as sepsis, coronary heart disease, hepatic ischemia injury, and epilepsy (*22*−*25*). Lipopolysaccharide (LPS) is often used in similar studies for the simulation of bacterial infection that leads to the strongest activation of NF-κB activity. Numerous plant/berry extracts and antioxidants have been shown to be able to significantly decrease LPS-induced NF-κB activity (*27*, *35*), thereby showing promising pharmacological effect. Thus, modulation of NF-κB activity can be a potentially effective preventive strategy for controlling certain inflammation-, immune-, or apoptosis-related diseases.

The aim of the present study was to examine the effect of different phenolic extracts of barley, oat, wheat, and buckwheat on the activation of basal and LPS-induced NF-κB activity.

MATERIALS AND METHODS

Chemicals. Ferulic acid, *p*-coumaric acid, lipopolysaccharide, and hygromycin were obtained from Sigma-Aldrich (St. Louis, MO). Caffeic acid and sinapic acid were obtained from Fluka (Buchs, Switzerland). Methanol and acetonitrile of chromatographic grade, ethyl acetate, and acetic acid (GR for analysis) were putchased from Merck (Darmstadt, Germany). RPMI-1640 medium, fetal bovine serum, L-glutamine, trypan blue solution, and penicillin−streptomycin solution were obtained from Invitrogen (Carlstad, CA).

Cereal Samples. Whole grains of barley Olve, oat Hurdal, buckwheat Lileja, and wheat Mjølner were milled using a Retch Morter Grinder, Model RM100. Commercial wheat flour was obtained from Lantmannen Cerealia, Stockholm, Sweden.

Extraction of Free Phenolic Compounds, Methanol Extract 1 (M1). To extract free phenolic compounds, we used the method described in ref *35*: 40 mL of 50% methanol in distilled water (v/v) was added to 10 g of the sample, and the mixture was sonicated in a water bath for 30 min at 0 °C using a high-power ultrasonic cleaner (Bandelin Sonorex RK 106, Germany). The samples were then centrifuged at 3000*g* for 15 min (Multifuge 4 KR Heraeus, U.K.), and the liquid phase was concentrated under nitrogen gas to a viscous fluid using a Reacti-Therm III heating/stirring module (Pierce). The fluid was diluted to 5 mL in dimethyl sulfoxide (DMSO).

Extraction of Free Phenolic Acids, Methanol Extract 2 (M2). To extract free phenolic acids, we used the method described in ref *21* with some modifications: 10 mL of 50% methanol (v/v) was added to 1 g of the sample, and the mixture was sonicated in a water bath for 30 min at 0 °C using a high-power ultrasonic cleaner (Bandelin Sonorex RK 106, Germany). The sample was centrifuged at 4000*g* for 15 min (Multifuge 4 KR Heraeus, U.K.), and the liquid phase was collected. The residue was subjected to the same treatment twice using 5 mL of 50% methanol (v/v). The collected extract was then evaporated to dryness using a rotavap (BUCHI EL 131, Laboratoriums-technik AG, Switzerland). The dry residue was transferred to a centrifuge tube with a screw cap using 10 mL of distilled water acidified to pH 2.0 (±0.2) with 6 M HCl and then extracted with ethyl acetate for 10 min using an OS-10 Orbital Shaker (Biosan, Latvia), followed by centrifugation for 10 min at 2800*g* (Multifuge 4 KR Heraeus, U.K.). The extraction with ethyl acetate was repeated four times. The collected ethyl acetate phase was evaporated to dryness using a Savant SPD131DDA SpeedVac Concentrator coupled with a UVS 800 DDA Universal Vacuum System (Thermo Electron Corp.), and the dry residue was dissolved in 2 mL of 25% methanol in distilled water (v/v).

Extraction of Bound Phenolic Acids, Hydrolyzed Extract (HY). To extract bound phenolic acids, we used the method described in ref *9* with some modifications: 10 mL of 50% methanol (v/v) was added to 0.2 g of the sample, and the mixture was sonicated in a water bath for 30 min at 0 °C using a high-power ultrasonic cleaner (Bandelin Sonorex RK 106, Germany). The sample was then centrifuged at 4000*g* for 15 min (Multifuge 4 KR Heraeus, U.K.), and the supernatant was discarded. The pellet was washed twice with 5 mL of 50% methanol (v/v) and centrifuged after each treatment at 4000*g* for 15 min (Multifuge 4 KR Heraeus, U.K.). To the pellet was added 10 mL of 2 M NaOH, and this mixture was stirred manually and left at room temperature for hydrolysis for 18 h. After hydrolysis the pH was adjusted to 1.3−1.5 with 6 M hydrochloric acid. Released phenolic acids were extracted from the acidic solution by liquid−liquid extraction with ethyl acetate for 10 min using an OS-10 Orbital Shaker (Biosan, Latvia), followed by centrifugation for 10 min at 2800 *g* (Multifuge 4 KR Heraeus, U.K.). The liquid−liquid

Table 1. Content of Phenolic Acids in Free and Bound Form in Cereal Samples, Determined by LC-DAD-MS Analysis from the M2 and HY Extracts, Respectively

	caffeic acid, μg/g			*p*-coumaric acid, μg/g			ferulic acid, μg/g			sinapic acid, μg/g			8-o-4-DFA, bound,μg/g		5,5′-DFA, bound, μg/g		8,5-DFA, bound, μg/g		amt, μg/g	
sample	free	σ	bound	free	σ	bound	free	σ	bound	free	σ	bound	amt	σ	amt	σ	amt	σ	free	bound	total
oat Hurdal	**0.62**	0.160	**21.37**	**4.07**	0.398	**2402.76**	**1.55**	0.467	**1038.41**	**0.81**	0.008	**86.97**	**58.71**	4.433	**15.19**	0.358	**23.42**	1.795	7.05	3646.84	3653.88
wheat Mjølner	**0.28**	0.109	**4.20**	**0.25**	0.021	**24.11**	**0.85**	0.193	**872.77**	**0.27**	0.010	**76.79**	**42.06**	1.713	**31.09**	0.812	**35.29**	1.268	1.65	1086.31	1087.96
buckwheat Lileja	**0.69**	0.045	**8.45**	**0.84**	0.148	**27.04**	**0.23**	0.011	**19.96**	**4.08**	0.263	**6.59**	**0.92**	0.069	**0.00**		**0.00**		5.84	62.96	68.80
wheat standard	**0.10**	0.037	**0.00**	**0.21**	0.028	**2.38**	**0.48**	0.004	**89.20**	**0.15**	0.030	**13.86**	**5.33**	0.151	**2.99**	0.327	**3.56**	0.066	0.94	117.32	118.26
barley Olve	**0.18**	0.027	**16.01**	**0.82**	0.095	**195.90**	**1.28**	0.075	**960.32**	**0.18**	0.021	**61.24**	**72.21**	5.999	**56.16**	3.319	**49.26**	4.865	2.45	1411.09	1413.54

extraction was repeated four times. The collected ethyl acetate phase was evaporated to dryness using a Savant SPD131DDA SpeedVac Concentrator coupled with a UVS 800 DDA Universal Vacuum System (Thermo Electron Corp.), and the dry residue was dissolved in 2 mL of 25% methanol (v/v).

Standard Solutions of Phenolic Acids. Individual stock solutions of ferulic, caffeic, *p*-coumaric, and sinapic acids (500 mg/L) were prepared by dissolving the compounds in 25% methanol (v/v). Standard calibration solutions of the phenolic acids with concentrations of 2.5, 5, 7.5, and 10 mg/L were prepared by mixing appropriate volumes of the stock solution with distilled water. Solutions were kept at −4 °C.

Quantitative Analysis of Phenolic Acids. The identification and quantification of phenolic acids were carried out using an Agilent 1100 series HPLC system with an LC/MSD trap (Agilent) using negative electrospray ionization as previously described (*32*). The identification of acids was carried out using caffeic, sinapic, *p*-coumaric, and ferulic acids as external standards, UV spectrum characteristics, and fragmentation patterns from mass spectrometry.

Cell Culture and NF-κB Activity Assay. The U937-3xkB-LUC cell line was maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 50 U/mL penicillin, 50 mg/mL streptomycin, and 75 μg/mL hygromycin. In order to measure NF-κB activity, cells were transferred to the medium with 2% fetal bovin serum. To measure basal NF-κB activity, cells were incubated with extracts, standard solutions of phenolic acids, or vehicle control for 6.5 h. To measure lipopolysaccharide (LPS)-induced NF-κB activity, cells were preincubated with extracts, standard solutions of phenolic acids, or vehicle control for 30 min, and then 1 μg/mL lipopolysaccharide (bought as a purified material isolated from *E. coli* 0111:B4 from Sigma-Aldrich, St. Louis, MO, Catalog No. L4391) was added to the cells and the incubation continued for 6 h. Cell viability was determined by trypan blue exclusion with a cutoff value of 10% nonviable cells. The NF-κB activity was determined by measuring the luciferase activity after addition of Bright-Glo Reagent (Pomega, Madison, WI) in accordance with the manufacturer's instructions. Luminescence was detected for 1 min using a Glomax96 Microplate Luminometer (Promega, Madison, WI).

Analysis of Antioxidant Capacity of Extracts. Extracts of free and bound phenolic compounds were measured for total antioxidant activity by FRAP (ferric reducing/antioxidant power) as previously described (*31*). The working FRAP solution was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl-*s*-triazine) solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ solution (Fe(III)) in a ratio of 10:1:1. Extracts were mixed with fresh FRAP solution for 30 min in darkness. Reading of the colored solutions was done at 593 nm in a UVmini 1240 UV−vis spectrophotometer (Shimadzu). Results are expressed in mmol of Fe (Fe(II) equiv)/100 g of cereal sample, DM.

Statistical Analysis. All experiments were performed at least in duplicate, and data are expressed as means ±SE (cereal extracts and standard solutions of phenolic acids were performed in duplicate; effects from each extract were tested in triplicate when the variation in data was ≤10% and more than triplicate when the variation in data was >10%). Statistical analysis of the data was performed by two-way analysis of variance (ANOWA, Minitab 13.30, the Statistical Analysis System (SAS, 1998)) F-test to examine possible effects of cereal extracts or phenolic acids on NF-κB activity in U937 cells. Dunnett comparisons were used for the identification of differences. The Pearson coefficient was calculated for correlation analysis. $P < 0.05$ was considered statistically significant.

RESULTS

Phenolic Extracts of Five Cereal Varieties. Three phenolic extracts were prepared from each of five cereal varieties: oat Hurdal, barley Olve, commercial standard wheat flour, wheat Mjølner, and buckwheat Lileja. Chromatograms of these extracts are shown in **Figure 1**. The unfractionated methanol extract (M1) contained a high variety of free phenolic compounds. Acidification of M1 followed by extraction with ethyl acetate to obtain an extract of free phenolic acids (M2) resulted in a cleaner chromatogram (**Figure 1**). The hydrolyzed extract (HY) was prepared by basic hydrolysis of the pellet from the methanol extract followed

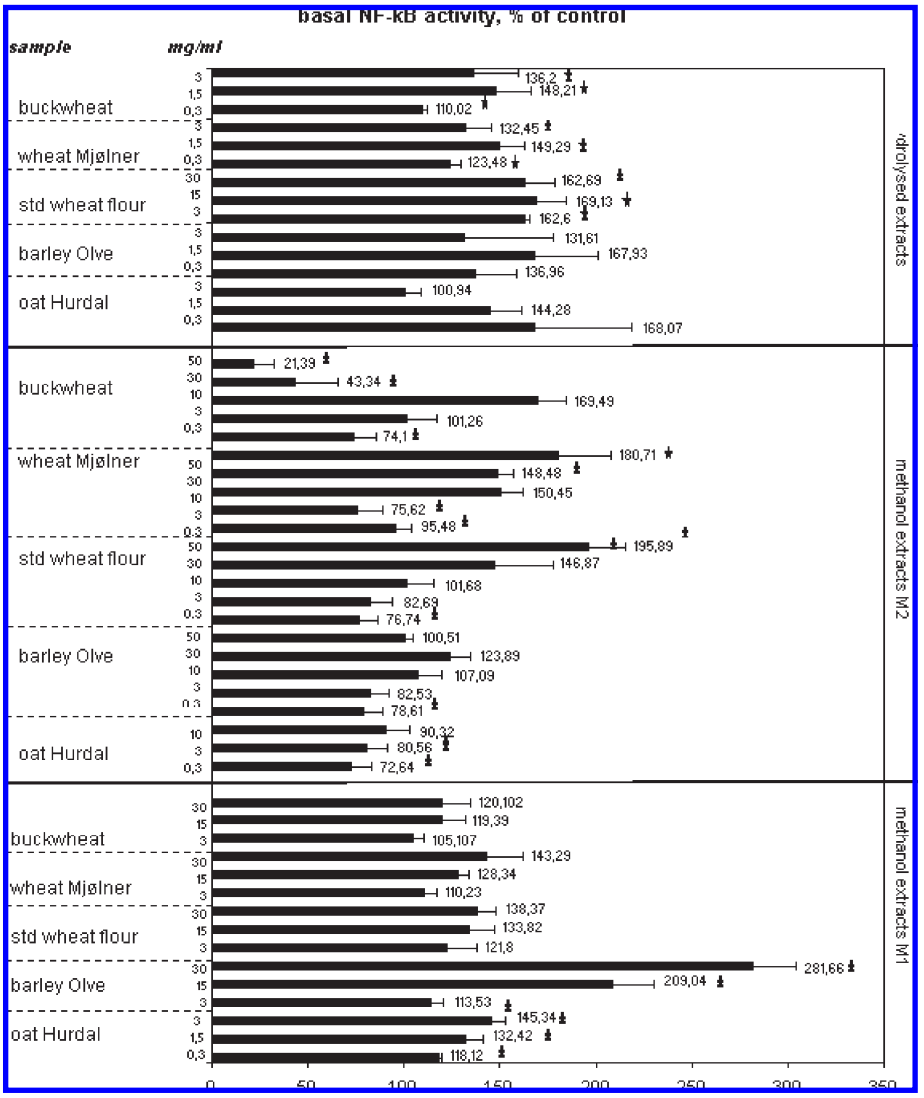

Figure 2. Effect of cereal extracts on basal NF-κB activity. U937-3xκB-LUC cells were incubated with 50, 30, 15, 10, 3, 1.5, or 0.3 mg/mL as indicated, of the respective extract in cell culture medium for 6.5 h before luciferase activity was measured. The M1, M2, and HY extracts contain free phenolic compounds, free phenolic acids, and bound phenolic acids, respectively. Each bar represents the mean of at least three experiments performed in triplicate $\pm$SEM. The asterisk indicates $p < 0.05$.

by ethyl acetate extraction, giving a solution containing only bound phenolic acids released from the cell walls in cereal grains. This gave a very clean chromatogram with only a few peaks.

The content of different phenolic acids in the M2 and HY extracts (free and bound phenolic acids, respectively) was determined with LC-DAD-MS analysis (**Table 1**). Phenolic acids are mostly found in the bound form in cereals, giving a high ratio of bound to free phenolic acids (100−1000). The content of each phenolic acid differed greatly among the cereal varieties. The dominant phenolic acid in the M2 extracts was different for each sample: *p*-coumaric acid for oat, ferulic acid for standard wheat flour, barley, and wheat Mjølner, and sinapic acid for buckwheat (**Table 1**). In the hydrolyzed extracts ferulic acid was the dominant phenolic acid for all cereals, except for oat Hurdal, where *p*-coumaric acid had the highest concentration.

Effect of Extracts on Basal and LPS-Induced NF-κB Activity. The ability of the different extracts to modulate basal and LPS-induced NF-κB activity was tested using the U937-3kB-LUC cell line stably transfected with a luciferase reporter containing three NF-κB binding sites. The extracts were tested in concentrations in the range of 0.3−50 mg of dry sample per mL of cell media, where the exact concentrations used for each sample depended on the toxic effect (cutoff value of 10% nonviable cells). As shown in **Figure 2**, increasing concentrations of methanol extracts M1 (containing free phenolic compounds) from oat Hurdal and barley Olve resulted in an increasing stimulation of the basal NF-κB activity. The highest concentrations of oat Hurdal (3 mg/mL) and barley Olve (30 mg/mL) increased the basal NF-κB activity by 45.3% and 181.7%, respectively. At concentrations above 3 mg/mL the methanol extract M1 of oat Hurdal became toxic (cell viability was less than 90%). The methanol extracts M1 from standard wheat flour, wheat Mjølner, and buckwheat Lileja did not have any significant effect on the basal NF-κB activity. Low concentrations (0.3−3 mg/mL) of the methanol extract M2 (containing free phenolic acids) of all cereal samples inhibited the basal NF-κB activity by around 20−25% (**Figure 2**). Interestingly, high concentrations (30 and 50 mg/mL) of M2 extracts of wheat Mjølner and standard wheat flour stimulated the basal NF-κB activity. The HY extracts (containing bound phenolic acids) of standard wheat flour, wheat Mjølner, and buckwheat Lileja gave a small increase of the basal NF-κB activity at all concentrations, while the HY extracts of barley Olve and oat Hurdal did not have any significant effect.

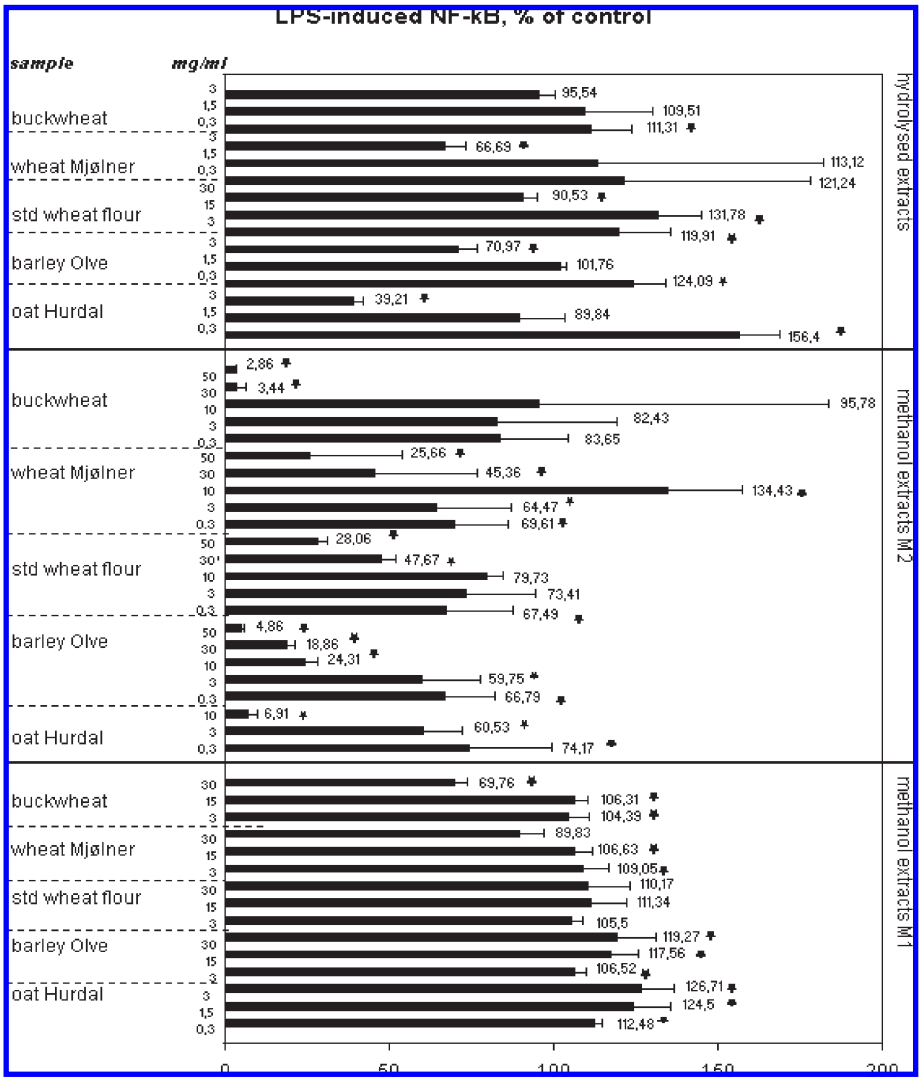

Figure 3. Effect of cereal extracts on LPS-induced NF-κB activity. U937-3xκB-LUC cells were incubated with 50, 30, 15, 10, 3, 1.5, or 0.3 mg/mL, as indicated, of the respective extract in cell culture medium for 30 min before 1 mg/mL LPS was added. The cells were incubated further for 6 h before luciferase activity was measured. The M1, M2, and HY extracts contain free phenolic compounds, free phenolic acids, and bound phenolic acids, respectively. Each bar represents the mean of at least three experiments performed in triplicate $\pm$ SEM. The asterisk indicates $p < 0.05$.

Addition of 1 μg/mL LPS to the U937-3kB-LUC cells leads to a high increase in the NF-κB activity, simulating a bacterial infection. This can be used to test the extracts' ability to modulate the cellular response to a bacterial infection. As shown in **Figure 3**, most of the methanol extracts M1 gave a small but significant (between 4 and 26%) increase of the LPS-induced NF-κB activity. However, 30 mg/mL of M1 extract of buckwheat Lileja decreased the LPS-induced NF-κB activity by 30% compared to control. Interestingly, all methanol extracts M2 significantly decreased the LPS-induced NF-κB activity (**Figure 3**). The difference in effects among the various methanol extracts M2 was only in the concentration needed to significantly inhibit the LPS-induced NF-κB activity. At 50 mg/mL, barley Olve and buckwheat Lileja almost completely inhibited the LPS-induced NF-κB activity, while wheat Mjølner and standard wheat flour gave around 70% inhibition. Extracts M1 and M2 for oat Hurdal were toxic at concentrations above 3 and 10 mg/mL, respectively. The highest concentrations of the HY extracts also reduced the LPS-induced NF-κB activity (**Figure 3**), although the effect was less than for M2 extracts (wheat standard 10% (30 mg/mL), barley 29% (3 mg/mL), wheat Mjølner 33% (3 mg/mL), oat 61% (3 mg/mL)). At lower concentrations the HY extracts gave a small but significant increase of the LPS-induced NF-κB activity (standard wheat flour (131.78%, 15 mg/mL), wheat Mjølner (121.24%, 0.3 mg/mL), barley Olve (124.09%, 0.3 mg/mL), oat Hurdal (156.39, 0.3 mg/mL), buckwheat Lileja (111.3%, 0.3 mg/mL)).

Effect of Individual Standard Solutions of Phenolic Acids on Basal and LPS-Induced NF-κB Activity. In order to understand the effect of the cereal extracts on the U937 cell line and to elucidate the ability of phenolic acids to modulate the NF-κB activity, the effect of standard solutions of individual phenolic acids were also tested on the same cell line. Concentrations were chosen on the content of the various phenolic acids in the extracts (**Table 1**). The effect of the phenolic acids on the basal and LPS-induced NF-κB activity is shown in **Figure 4**. *p*-Coumaric, ferulic, sinapic, and 8,5-diferulic acids significantly increased the basal NF-κB activity. As seen in **Figure 4**, low concentrations of 8,5-diferulic acid (10 mg/L) gave the same increase in basal NF-κB activity (80−90%) as 25 and 70 mg/L for ferulic and *p*-coumaric acids, respectively. High concentrations of ferulic, *p*-coumaric (70 mg/L), and 8,5-diferulic (10 mg/L) acids reduced the LPS-induced NF-κB activity, while lower concentrations of *p*-coumaric and ferulic acids (25 mg/L) gave a small but significant increase of the LPS-induced NF-κB activity.

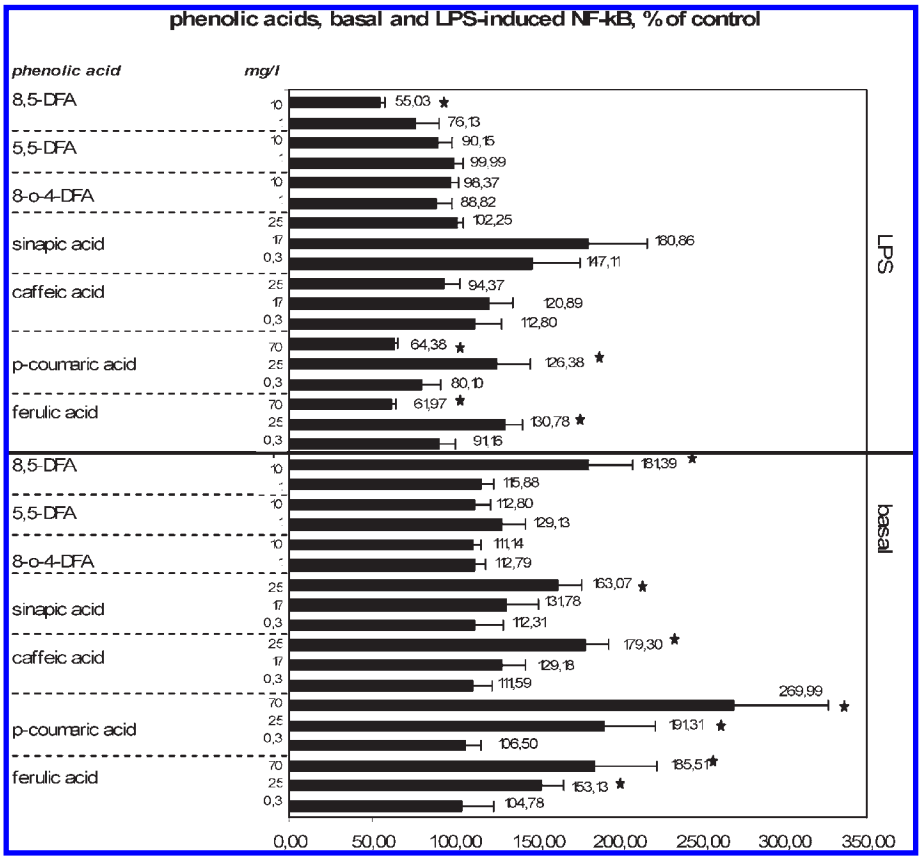

Figure 4. Effect of standard solutions of phenolic acids on basal and LPS-induced NF-κB activity. U937-3xκB-LUC cells were incubated with 0.3, 1, 10, 17, 25, or 70 mg/L, as indicated, of the respective phenolic acid in cell culture medium for 6.5 h before luciferase activity was measured. For LPS induction, 1 mg/mL of LPS was added after 30 min, and the cells were incubated further for 6 h before the luciferase activity was measured. Each bar represents the mean of at least three experiments performed in triplicates ±SEM. The asterisk indicates $p < 0.05$.

Generally, 8,5-diferulic, ferulic, and *p*-coumaric acids had the largest effect on both basal and LPS-induced activity.

DISCUSSION

A large amount of data has demonstrated the ability of different fruit and plant extracts to inhibit NF-κB activity (*26*−*30*, *33*−*35*, *39*). Cereal grains contain phytochemicals in concentrations comparable with those in fruits and vegetables (*14*). Phenolic acids are abundant dietary antioxidants present in cereals both in free and bound forms. These compounds possess potential health-promoting properties, possibly explained by their antioxidative action (*8*). However, little is known about the activity of phenolic acids from cereals on the modulation of immune cells, signaling molecules, or transcription factors. In the present study we aimed to screen extracts from five cereal grains (oat Hurdal, barley Olve, buckwheat Lileja, wheat Mjølner, and commercial standard wheat flour) for their ability to modulate basal and LPS-induced NF-κB activity. Three extracts differing in the content of phenolic compounds were prepared from each grain variety. The methanol extract M1 contained free phenolic compounds, while the methanol extract M2 contained free phenolic acids. The hydrolyzed extract contained bound phenolic acids mostly released from the arabinoxylan chain.

Our results show that cereal extracts can be potent modulators of NF-κB activity and that the difference in this modulation of NF-κB activity depends on the type of extract and cereal sample. Generally, extracts M1 were not significant modulators for basal or LPS-induced NF-κB activity, with the exception of buckwheat, which gave significant inhibition of LPS-induced NF-κB in a concentration of 30 mg/mL, and of barley Olve, which gave significant stimulation of basal NF-κB activity in concentrations over 15 mg/mL. Extracts M2, on the other hand, displayed strong inhibitory effects on basal and LPS-induced NF-κB activity in concentrations over 10 mg/mL for oat Hurdal, barley Olve, and buckwheat Lileja. Furthermore, M2 extracts from commercial standard wheat flour and wheat Mjølner gave a significant stimulation of basal NF-κB activity for all tested concentrations and a significant inhibition of LPS-induced NF-κB activity in concentrations over 10 mg/mL. Interestingly, the methanol extract M2, containing free phenolic acids, had a comparable or even stronger effect on LPS-induced NF-κB activity than similar extracts from strawberries, which are known as a good source of natural antioxidants and phenolic acids (*33*, *35*). Hydrolyzed extracts from standard wheat flour and wheat Mjølner showed dual effects, increasing basal NF-κB activity at all tested concentrations while stimulating LPS-induced NF-κB activity at low concentrations and inhibiting it at higher concentrations. Such dual effects could be useful in studies of prevention of diseases. HY extracts from oat and barley did not exhibit any significant modulation of basal NF-κB activity but significantly suppressed LPS-induced activation of nuclear factor at higher concentrations and stimulated it at lower concentrations.

Our experiments with standard solutions of phenolic acids revealed that ferulic, *p*-coumaric, and 8,5-diferulic acids were significant inducers of basal NF-κB activity and inhibitors of LPS-induced activity especially in higher concentrations. Interestingly, a correlation between the content of phenolic acids in the hydrolyzed extracts and their ability to modulate NF-κB activation was found. Hydrolyzed extracts of oat Hurdal, barley Olve,

and wheat Mjølner, which contained the highest total levels of phenolic acids with significant amounts of ferulic, *p*-coumaric, and 8,5-diferulic acids (**Table 1**), had the highest inhibition of LPS-induced NF-κB activity. In contrast, the hydrolyzed extracts of buckwheat Lileja had a low concentration of phenolic acids and had no effect on the LPS-induced NF-κB activity (**Figure 3**). The ability to modulate NF-κB activation does not correlate with the antioxidant capacity of the phenolic acids, as the order of decreasing antioxidant strength for phenolic acids is DFA > CA > FA > SA > pCA (*36*, *37*). Moreover, 8,5-diferulic acid was more active than other well studied NF-κB inhibitors such as epicatechin (*40*), resorcinol (*27*), caffeic acid phenethyl ester (*41*), resveratrol, and quercetin (*42*).

The strong inhibitory effect of extracts M2, containing much lower concentrations of phenolic acids compared with HY, is not fully understood. Most studies report screening of standard solutions of individual phenolic antioxidants for their ability to modulate the activity of transcription factors, but their combinatorial effect has been scarcely studied. Phytochemicals in plant extracts may act synergistically, suppressively, or separately on the modulation of NF-κB activity. Synergic effects can be demonstrated by agents acting on different pathways, steps of activation, or different targets involved in signaling events (*38*). Inhibitors of NF-κB activation with similar modes of action may suppress the effects from each other. To explain the effects of different plant extracts, it is necessary to know the various kinds of NF-κB inhibitors and their mode of action as well as the concentration of phytochemicals contained therein. Although several natural products, synthetic compounds, and antioxidants with effective inhibitory effects have been identified, the lack of detailed information about their combinatorial action makes it difficult to explain and predict possible chemopreventive effects from plant/fruit/vegetable/cereal extracts. A synergic effect of different phenolic acids in low concentrations may explain the significant inhibitory effect demonstrated in our study of extracts M2 (**Figures 2** and **3**). Combinatorial actions from other phytochemicals and phenolic acids might explain the weak modulation of NF-κB activity by extracts M1.

In the present study we report the ability of cereal extracts of free and bound phenolic acids to modulate NF-κB in a monocytic cell line. The strongest effect was registered for oat, barley, and buckwheat. No correlation between the antioxidant activity of extracts and individual phenolic acids with effects on both basal and LPS-induced NF-κB was found. A possible synergic action of cereal phytochemicals gave a much stronger effect for extracts of free phenolics (M2) than for hydrolyzed extracts (**Figures 2** and **3**). Studies on the effects of a range of cereal phytochemicals and their combinatorial action is lacking: determinations of phenolics in tested extracts and cell line experiments studying synergic effects of cereal antioxidants should be performed.

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