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Pilot-Scale Production and Application of Microparticulated Plant Proteins

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Abstract

New Carbon Dioxide Assisted Spray Nebulisation Drying (CASND) technology has been used to produce microparticulated protein concentrates for human nutrition from alternative plant sources - hemp and canola seed filtration cakes. Alkali extraction was used to extract the proteins from the filtration cakes. The protein solutions after the alkali extractions were dried with the CASND demonstrator ATOMIZER. Aerosol particle size distribution and concentration in the draying chamber were determined by two different on-line aerosol spectrometers SMPS (Scaning Mobility Particle Sizer) and APS (Aerodynamic Particle Sizer). The protein powders were in form of hollow spheres with average particle diameter about 600 nm. The particles were characterized by the SEM method. The functional properties of the microparticulated protein concentrates were compared with the same protein concentrates dried by the convenctional spray drying process. The protein microparticulatin resulted in improved foaming and emulsifying properties and formation of long-term stable water dispersions. Gluten-free baguettes were prepared with the microparticulated protein concentrates as only protein source and evaluated by sensory analysis.

Keywords: Carbon dioxide-assisted spray nebulization drying; Microparticulated proteins; Hemp seed; Canola seed; Functional properties

Introduction

Protein in food is an essential nutrient which comes from animal and plant sources. A protein usually contains various amounts of 20 different amino acids linked via peptide bonds. The content, digestibility coefficients, and relative proportions of amino acids in dietary protein are the determinants of its nutritional value. The WHO/FAO/UNU (2007) report examines dietary protein and amino acid requirements for all age groups, protein requirements during pregnancy, lactation and catch-up growth in children, the implications of these requirements for developing countries and protein quality evaluation [1]. An essential amino acid, or indispensable amino acid, is an amino acid that cannot be synthesized *de novo* (from scratch) by the organism, and thus must be supplied in its diet. The nine amino acids humans cannot synthesize are phenylalanine, valine, threonine, tryptophan, methionine, leucine, isoleucine, lysine, and histidine. Six other amino acids are considered conditionally essential in the human diet, meaning their synthesis can be limited under special pathophysiological conditions, such as prematurity in the infant or individuals in severe catabolic distress. These six are arginine, cysteine, glycine, glutamine, proline, and tyrosine [2]. Besides the nutritional value, the functional properties of food proteins affect behavior in food systems and influence the quality attributes, structure, texture, mouth-feel, and flavor of the final product.

Plant proteins can differ from animal proteins in terms of digestibility, amino acid composition, the presence of antinutritional factors which influence digestibility and safety, and the presence of phytoprotectant factors which mediate disease protection [3].

Most evidence suggests that a shift to largely plant-based protein diets would reduce chronic disease risks among industrialized and rapidly-industrializing populations. High intake of animal protein increases total blood cholesterol, low-density lipoprotein [LDL] cholesterol, obesity, and risks of atherosclerosis and coronary heart disease. Many studies report that vegetable protein is associated with low blood cholesterol and the low risk of the diseases aforementioned. The negative association between excessive intake of animal protein and diseases is possibly due to the fact that animal food products are also high in fat content, particularly, saturated fat. Studies have linked red meat - particularly processed meats such as hot dogs, bacon, and deli meats - to cardiovascular diseases such as heart attack and stroke, even cancer while promoting the health benefits of plant-based proteins. A recent study [4] directly compared animal protein with plant protein. The study re-analyzed data from studies previous done on the health impact of consuming red meat. Those studies offered 32 years of data (collected during 1980-2012 and 1986-2012) on the diet, health history, and cause of death of 131,342 participants. The researchers concluded that people with a high percentage (>3%) of plant protein per daily energy intake had a significant drop in risk of death from CVD and other causes. People with a high percentage (>10%) of animal protein per daily calorie intake had a non-significant increase in the risk of death from cardiovascular and other causes. When people switched 3% of their daily calories from animal to plant protein, mortality risk declined significantly (34%) in those giving up processed red meat and, to a lesser extent, unprocessed red meat (12%) [4].

Animal protein has a balanced combination of all the essential amino acids, hence it is called complete protein. To the contrary, plant (vegetable) protein is usually incomplete, regarding to the essential amino acids composition. There are several exceptions, such as soya, hemp or amaranth proteins, which approximate the recommended optimal essential amino acids profile. However, even soya protein is

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deficient in one of the essential amino acids - methionine. Therefore, to achieve a balanced amino acids intake with vegetarian diet, a variety of plant protein sources need to be complemented with each other in the diet. Plant protein accounted for 24-39% of the total protein in European countries. Cereals contributed to the highest proportion of plant protein, followed by potatoes, vegetables, legumes and fruits [5]. Nuts and oil seeds are other important sources of plant proteins [6]. Cereal-based diets is inadequate for lysine [1]. Recently, alarming increase of frequency of intolerant and allergic reactions to the cereal and soya proteins has been observed. Moreover, interest in vegetarianism and plant-based diets in developed countries is on the rise and vegetarians and vegans constitute a significant population group. That is why enlargement of market supply of high-quality food plant-based proteins is very desirable. To accomplish this shift, it will be necessary to overcome market-place barriers and to develop new technologies and policies that will encourage greater consumption of the vegetable proteins. Filter cakes from canola / rapeseed, sunflower and hemp seeds obtained after oil pressing and extraction, rice, cell fluids obtained from potatoes, or pea seeds belong among the main alternative sources of commercially available plant protein concentrates and isolates for human nutrition. Seeds of amaranth or legumes lupin and chickpea should be considered as excelent plant source of protein for animal and human nutrition, too. As yet, the chickpea, lupin and amaranth protein concentrates are not available for industrial use. Canola protein isolate has been suggested as an alternative to other proteins for human food use due to a balanced amino acid profile and potential functional properties such as emulsifying, foaming, and gelling abilities. Tan et al. [7] reviewed the studies on the utilization of canola protein in human food, comprising the extraction processes for protein isolates and fractions, the molecular character of the extracted proteins, as well as their food functional properties. Canola protein has a well-balanced amino acid profile, with a protein digestibility-corrected amino acid score that is very competitive with other plant proteins currently on the market [8]. Seed storage proteins that include cruciferin [11S] and napin [2S] dominate the protein complement of canola while oleosins, lipid transfer proteins and other minor proteins of non-storage nature are also found [9]. Canola / rapeseed concentrates and isolates showed excellent water-and fat-holding capacity and the isolate was high in oil emulsification and whipping characteristics, superior to soybean products in most functional tests [10]. Limited rapeseed protein hydrolysis resulted in better functional properties than the original protein isolate. These improved functional properties make rapeseed protein hydrolysates a useful product to be used in foods such as breads, cakes, ice creams, meat products, desserts, and salad dressings [11]. The commercial cruciferin-rich and napin-rich protein isolates derived from canola (Puratein® and Supertein®) can be used as food ingredients in powdered egg and egg substitutes, dairy products, processed meats, grain products, vegetable/fruit juices and beverages, salad dressings, protein supplement powders and meal replacement/nutritional bars [12]. Hemp seed contains the salt-soluble globulins or edestin (~ 75%) and the water-soluble albumin (~ 25%) as the main storage proteins. Hemp seed proteins have a high level of arginine and a sulfur-rich protein fraction, two unique features that impart high nutritional values [13]. Hemp proteins have a protein digestibility-corrected amino acid score equal to or greater than certain grains, nuts, and some pulses [14]. Peptide mixtures and hydrolysates derived from canola proteins have been reported to possess a range of biological activities that could have beneficial health effects in humans. Pepsin-hydrolysed protein concentrates showed significant angiotensin I-converting enzyme inhibition and radical scavenging activity [15]. In addition, antioxidant, antidiabetic, anorexigenic, anticancer, antiviral, hypercholesterolemic and bile acid binding activities have been reported for peptides and hydrolysate fractions generated from canola proteins [8,16]. Hemp seed enzymatic hydrolysates have also proven effective during *in vitro* and *in vivo* tests as antioxidant and antihypertensive agents. Therefore, hemp seed proteins and hydrolysates have the potential to be used as ingredients to formulate functional foods [13,17]. Alkaline and/or high-salt extraction, followed by isoelectric precipitation or ultrafiltration to separate and purify proteins form the solutions are most frequently used industrial processes of production of the protein concentrates or isolates. Osborn fractionation and sonication [18,19] and micellisation [20] are alternative technologies to extract and purify proteins. Enzymes with specific activities towards the ballast compounds can be also used to purify proteins. Protein concentrates may also be prepared by acid or ethanol leaching, and this is the typical wet process for soybean protein concentrates [8]. Spray drying is usually used to produce powders from the protein rich solutions. Freeze drying is alternative drying technique, which is used less frequently because of higher price and limited capacity. Functional properties of proteins in food matrices are determined by the physicochemical properties of the proteins, degrese of denaturation, type of processing, and particle morphology and size, in the case of powdered proteins with limited solubility. The design of protein particles with tailored properties has received an increased attention recently. Control of particle size, morphology, surface and internal properties is crucial for obtaining protein particles with the necessary properties for emerging applications. Singer et al. [21] originally patented the use of whey protein microparticles, formed by thermal aggregation at high shear and low p^H. A commercial fat replacer SIMPLESSE®, based on this process, was launched by NutraSweet in 1988 in form of spherical particles in the size range of 0.1-2.5 μm. The initial patent was later extended to other proteins, e.g., bovine serum albumin, egg white albumin and soy protein [22]. SIMPLESSE® has been applied in dressings and mayonnaise, frozen desserts, cheese and other dairy products since that time [23]. The improved creaminess obtained by use of microparticulated protein products was due to particles <5 μm in size, mimicking emulsion droplets [24,25]. The controlled microparticulation process produces uniform microparticles averaging one micron in diameter, and prevents the formation of large protein agglomerates. Microparticulated protein has been proven to be more digestible than other proteins. As a result of their consistently small size and uniform spherical shape, SIMPLESSE microparticles, in suspension, behave like a creamy fluid and provide a unique set of functionalities. The preparation provides emulsion and foam stabilization, heat and p^H stability, texture, creaminess and smoothness in a wide range of full-fat and low-fat applications (www.cpkelco.com). The potential of using microparticulated whey protein (MWP) as a fat replacer in dairy products has been extensively explored and recently reviewed by Ipsen [25]. Liu et al. [26] described the sensory properties of microparticulated whey protein (MWP) particles in relation to their rheological and tribological properties in liquid and semi-solid model foods. MPW is known to provide fat-related mouthfeel in specific liquid and semi-solid foods, such as yoghurts and cheeses, because of friction reduction due to a ball-bearing mechanism [27]. The potential utilization of MWP as a fat mimetic in reduced calorie model sauces and dressings was examined by Chung et al. [28]. MWP increases the lightness and viscosity of products, thereby mimicking some of the desirable characteristics of fat droplets. The microstructure of low-fat stirred yoghurt manufactured with microparticulated whey protein was analysed using fractal image analysis. The microparticles had a positive influence on the structure of the formed gel [29]. An enhanced surface

reactivity was shown in small microparticles, resulting in improved gelling behaviour in a non-fat milk system [30]. Effect of microparticulated whey proteins on milk coagulation properties has been studied by Sturaro et al. [31]. Increasing the amount of MWP added to milk led to a longer rennet coagulation time. Effect of microparticulated whey protein concentration and protein-to-fat ratio on Caciotta cheese yield and composition examined Sturaro et al. [32]. The increment of protein-to-fat ratio affected rennet coagulation time. The stable composition of low-fat Caciotta suggests the possibility to include MWP as fat replacer. Microparticulated whey protein concentrate (WPC) emulsions showed significantly enhanced heat stability compared with standard WPC emulsions. With this specific technology, high protein whey-based nutritional beverages can be produced using conventional thermal treatments [33]. Interactions in heated milk model systems with different ratios of nanoparticulated whey protein at varying p^H were examined by Liu et al. [34]. Chang et al. [35] investigated the potential of egg white protein (EWP) to be developed into a kind of Pickering stabilizer for oil-in-water emulsions. The EWP microparticles were formed by heating at 90°C for 45 mins, followed by homogenization under low pressure. These results have important implications for the formulation and production of emulsion based semi-solid products, using egg white protein as emulsifier and fat substitute. Additions of egg white protein microparticles and pectin sol resulted in blocking flow behavior of the light mayonnaise [36]. Other applications of protein nanoparticles and microparticles include delivery biologically active compounds, too [37]. When the original patent ran out in 2004, the interest in using MWP in dairy products, as well as in developing whey protein particles with tailored functionality for specific end uses, increased and currently a number of such products and/or processes are available and used commercially [25]. Two approaches, namely, isoelectro-mechanical and thermo-mechanical processes, are most often used in microparticulation of protein. In these processes, conditions that denature (i.e. heat) or induce a complex or precipitate formation (i.e. isoelectric precipitation) are combined with a strong mechanical treatment, e.g. high share rates or turbulence and cavitation to produce nanoaggregated protein microparticles of desired size and distribution. Several methods, from simple heat treatment in dilute systems to the combination of heat and mechanical treatments in concentrated protein solutions, have been used to obtain protein particles with varying functional properties [37]. Turbulation, cavitation and shear are also the main phenomena encountered in high-pressure homogenizers, such as microfluidizers. The microfluidization technology is an unique type of high-pressure homogenization [38,39]. For example, the SPX Flow Technology combines high heat treatment with controlled shear force to produce LEANCREME™ micronised whey proteins with improved functional properties [40]. Alternative processes may also be implemented, such as extrusion cooking at acid p^H [41], or two-step emulsification of whey protein isolate in sunflower oil containing polyglycerol polyricinoleate with subsequent heating and oil removal [42]. Supercritical fluid assisted atomization processes are other technologies of choise to produce microparticalated proteins [43]. In this study we have developed a cheap, simple, and effective method of simultaneous drying and micronisation of proteins from solutions. Engineering efforts of researches of the Food research institute Prague and the Czech Technical University in spray drying technologies led to introduction of a demonstrator ATOMIZER and Carbon-Dioxide Assisted Spray Nebulisation Drying (CASND) technology. The ATOMIZER demonstrator combines the spray drying technology, when the liquid to be dried is atomized by a rotary atomizer, with carbon dioxide assisted nebulisation process in an original way. A wide range of application forms - low density particles, composite particles, sterically stabilized liposomes, phytosomes, microencapsulated particles or microbial cells, solid dispersions, dried single and multiple emulsions, nano- and microfibers and other - can be produced by this process.

Materials and Methods

Material

The dried filter cake produced by cold pressing canola seeds (variety LIRAJE) was obtained from Fabio Product (Holin, Czech Republic).

The dried filter cake produced by cold pressing hemp seeds (variety BENIKO) was obtained from Hemp production company (Chrastice, Czech Republic).

A commercial potato protein concentrate SOLANIC® 200, containing 90, 5% (w/w) of protein, was purchased from AVEBE U.A. (Veendam, The Netherlands).

All the chemicals were purchased from Sigma-Aldrich (Prague, Czech Republic).

Protein extraction from the oil seed filter cakes

Extraction of canola proteins from the dried filter cake: The dried filter cake of canola seeds was pulverized with a knife blender Catler 800 series (Breville Group Limited, Sydney, Australia). Canola proteins were extracted from the pulverized filter cake after addition of six-time bigger weight of a potable water containing 1, 2% (w/w) NaCl and p^H adjustment to 11, 0 by NaOH addition. The extraction takes place at 5°C for 7 hours. The insoluble ballast was removed by centrifugation with Westfalia centrifugal separator SC 6-06-076 (GEA Westfalia Separator, Oelde, Germany), 9000 rpm. NaCl was removed from the obtained solution by ultrafiltration using Alfa Laval module 3838/48 and UFX 10 pHt spiral membrane (Alfa Laval Corporate AB, Lund, Sweden).

Extraction of hemp proteins from the dried filter cake: The dried filter cake of canola seeds was pulverized with a knife blender Catler 800 series (Catler, Australia). Hemp proteins were extracted from the pulverized filter cake after addition of ten times bigger weight of a potable water and p^H adjustment to 12, 1 by KOH addition. The mixture was left for 5 days at 5°C. The p^H value dropped to 9, 1 at the end of the extraction process. The insoluble ballast was removed by centrifugation with Rousselet Robatel RA20 Vx centrifuge (Rousselet Robatel, Annonay, France), 4000 rpm.

Drying and microparticulation of the plant protein extracts

The solutions of the canola and hemp protein concentrates were dried by the CASND process using the ATOMIZER demonstrator (Figure 1) under following conditions: Liquid flow rate, 20 $ml.min^{-1}$; Carbon dioxide flow rate, 1,8 $ml.min^{-1}$; Pressure, 6 MPa; Inlet air teperature, 60°C; Outlet air temperature, 48°C; Air flow rate: 1000 $m^3.h^{-1}$.

Determination of the aerosol particle size distribution and concentration inside the drying chamber

The drying chamber was equipped with openings and fittings for sample probe tubes of changeable length. The fittings were equipped with a mechanism allowing for the precise estimation of the position of the sampling inlet inside the drying chamber. Five of the openings were positioned along the height of the drying chamber. In this way, aerosol samples were collected from many different places of the drying chamber under the conditions described in the previous paragraph.

Figure 1: Demonstrator atomizer.

Aerosol particle size distribution and concentration were determined by two different on-line aerosol spectrometers-SMPS (Scanning Mobility Particle Sizer) and APS (Aerodynamic Particle Sizer). SMPS spectrometer (TSI Inc., USA) is an aerosol instrument measuring particle number and size distribution in size range starting at units of nanometers up to approximately 1 micron (based on the operating parameters). This instrument sizes the particles according to their mobility in electrostatic field and counts their number in individual size bins using Condensation Particle Counter (CPC). APS spectrometer (TSI Inc., USA) is an optical aerosol spectrometer measuring particle number size distribution in the range 0.5-20 μm. Size of the particles in the APS spectrometer is based on their inertial behavior. After being accelerated, the aerosol particles pass two parallel laser beams perpendicular to the air flow and the time of the passage of aerosol particle between the two beams is measured. The measured time, after the calibration of the instrument, is directly proportional to the particle size. APS spectrometer represents useful extension of SMPS measurement range allowing to measure number size distribution of aerosol particles in the merged size range from units of nanometers up to 20 μm.

Determination of filtration efficiencies of the cyclone and electrofilter separators

The ATOMIZER was originally equipped with a cyclone powder separator. The cyclone separator proved to be highly ineffective to separate the fine submicron particles produced by the CASND process. In order to increase the fine powder separation efficiency, we have also tested AEROFOG KE1 electrofilter, which has been kindly lent us by VZDUCHOTECHNIK company (Chrastava, Czech Republic). To compare the filtration efficiencies of the cyclone and electrofilter separators, we carried out the following experiment. Portions and particle size distributions in the inlet to the separators and outlet drying air escaping the separators were determined using the sample probe tubes placed prior and after the separator. The samples were collected alternately from both the sampling positions and analysed by the SMPS (Scaning Mobility Particle Sizer) and APS (Aerodynamic Particle Sizer) aerosol spectrometers. The filtration efficiencies were calculated as differences in the concentrations of particles in the separator inlet and outlet in dependence on the particle size. The conditions of the CASND process were the same as described in the previous paragraphs. The solution of the canola protein concentrate was used for the experiment.

Characterisation of the protein microparticles by scanning electron microscopy

Scanning Electron Microscopy (SEM) was used to analyze the particle sizes and morphologies. The samples of the fibres were fixed with a double-faced adhesive tape to the holders and evaluated in a Phenom G2 scanning electron microscope (Phenom-World BV, Eindhoven, Netherlands).

Determination of functional properties of the canola protein concentrate

Foaming properties of the canola protein concentrate samples dried by a conventional spray dryer and by the ATOMIZER demonstrator using the CASND technology were determined according to the method of Okaka and Potter [44]. Emulsifying properties were determined by the method of Yasumatsu et al. [45].

Production of the gluten-free baguettes containing the microparticulated plant protein concentrates as only protein source

To evaluate consumer sensory acceptance of the products, gluten-free baguettes containing the microparticulated canola and hemp protein concentrates as only protein source were prepared in the Perník Company Ltd. (Techlovice, Czech Republic). The baking process and the composition of the baguettes were exactly the same, except the protein concentrates. A commercial potato protein was used as a control sample. The exact composition of the baguettes is a secret know-how of the company. A commercial potato protein concentrate SOLANIC® 200 (1, 25% w/w), or the microparticulated canola protein concentrate (1, 3% w/w), or the microparticulated hemp protein concentrate (5% w/w) were used as only protein sources.

Baking temperature profile was in the range 180-230°C.

Sensory analysis of the gluten-free baguettes

The samples of the gluten-free baguettes with different protein concentrates were evaluated by the graphic rating scale method with 11 trained assessors. The selected sensory descriptors (Figure 2) were evaluated at the scale from 0 (the worst evaluation of the descriptor) to 100 points (the best evaluation of the descriptor). Shapiro-Wilk normality test and Dean-Dixon test ($\alpha = 0.05$) were used to check

normality of data and identify outliers. The ordinal classification evaluation method was used to determine the effect of additions of the potato, hemp and canola protein concentrates on the selected sensory descriptors. Statistical significance of differences between the sensory descriptors of the three samples was calculated by the Student´s T-test ($\alpha = 0.05$).

Results and Discussion

Microparticles and nanoparticles in pharmaceutical and food industry can be manufactured by many different processing methods. Wet chemistry, phase separation processes, spray drying and alternative drying processes such as spray freeze drying, or supercritical fluid technologies, have been used widely for particle engineering purposes. The spray-drying process is a well established operation in the pharmaceutical and food industry, especially. The solution or suspension to be dried is atomized into droplets using a spray nozzle. Many different types of spray nozzles can be used including two-fluid, ultrasonic, rotary, and pressure (or hydraulic) nozzles. When the spray-solution droplets contact the hot drying gas, the solvent in the droplets evaporates, leaving dried particles entrained in the drying gas that exits the drying chamber. These particles are separated from the gas stream, usually by a cyclone separator. The last two decades has seen a shift from empirical formulation efforts to an engineering approach based on a better understanding of particle formation in the spray drying process. Microparticles with nanoscale substructures can now be designed and their functionality has contributed significantly to stability and efficacy of the particulate dosage form.

CAN-BD is Aktiv-Dry's proprietary process for making fine, dry powders of pharmaceutical, vaccines, biotech, and other compounds. The heart of CAN-BD is high pressure nebulization assisted by supercritical fluids, usually CO_2, but N_2 has also been used. CAN-BD is a continuous drying technology that produces uniform particles. Nebulization occurs when the supercritical, or near-critical, fluid mixes with a solution or suspension of the pharmaceutical product to form an emulsion that expands abruptly from 80 bar to atmospheric pressure through a capillary flow restrictor. Microbubbles that form during the expansion burst almost instantaneously at atmospheric pressure to create a plume of millions of micro- and nano-meter daughter droplets, which rapidly dry in a warm carrier gas such as nitrogen. When the plume is directed into a drying chamber, similar to that used in conventional spray drying, the liquid solvent evaporates to leave behind fine particles that may be collected in a proper separator. The drying process takes place at low temperature in the range 25°C to 65°C. Particle size distributions may be engineered by changing process variables [46].

The CANBD combines the spray drying technology, when the liquid to be dried is atomized by a rotary atomizer, with carbon dioxide assisted nebulization process in an original way. The rotary atomizer used for the CANBD technology has an unique contruction allowing to regulate and keep the outlet pressure at an exactly defined level. A solution, emulsion or suspension is saturated by carbon dioxide at pressure 4-8 MPa before the drying process. The atomization process takes place in two steps. In the first step, primary droplets are produced by the centrifugal force at the outlet of the rotary atomizer of special construction. In the second step, the primary droplets are divided into secondary droplets by the CO_2 expansion from the inside of primary droplets. The secondary droplets, usually in the form of microbubbles, are rapidly dried by warm air stream at temperatures up to 60°C and solid particles are formed in a drying chamber. The CANBD process allows to regulate precisely the concentration of carbon dioxide in the liquid to be dried and is more suitable for industrial scale-up than the CAN-BD technology.

The ATOMIZER was originally equipped with a cyclone powder separator. The cyclone separator proved to be highly ineffective to separate the fine submicron particles produced by the CASND process (Figure 3). Cyclonic separation is a method of removing particulates from an air, gas or liquid stream, without the use of filters, through vortex separation. Rotational effects and gravity are used to separate mixtures of solids and fluids. A high speed rotating (air) flow is established within a cylindrical or conical container called a cyclone. Air flows in a helical pattern, beginning at the top (wide end) of the cyclone and ending at the bottom (narrow) end before exiting the cyclone in a straight stream through the center of the cyclone and out the top. Larger (denser) particles in the rotating stream have too much inertia to follow the tight curve of the stream, and strike the outside wall, then falling to the bottom of the cyclone where they can be removed [47]. In order to increase the fine powder separation efficiency, we have also tested AEROFOG KE1 electrofilter. The electrofilter was constructed as an air purifier to remove aerosol particles from air stream. Our experiments proved high filtratition efficiency of the electrofilter in the whole interval of the protein concentrate particles produced by the CASND process (Figure 3). However, the product yield of the CASND process could not be calculated. The reason was that the AEROFOG KE1 electrofilter is not suitable for quantitative recovery of powdered products, because of the small gaps in between the collecting electrodes (Figure 4). The results have already inspired a development of a new electrostatic fine powder precipitator which will be constructed with the aim to facilitate the maximal recovery of the products powdered by the CASND technology.

The resulting solution of the canola protein concentrate contained 8.3% DM, of which 5.8% constituted protein and 0.19% NaCl. The

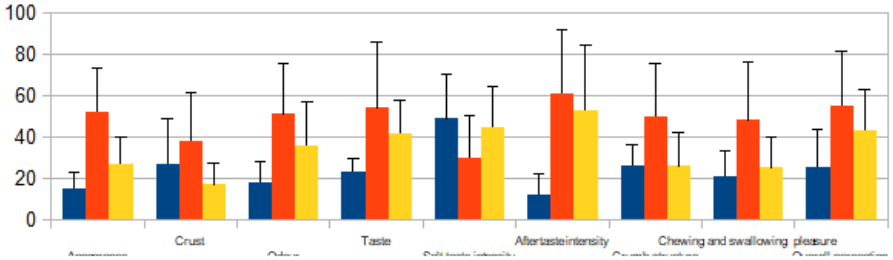

Figure 2: Sensory evaluation of the gluten-free baguettes (Aritmetic Average ± Standard Deviation). Blue colour: potato protein; Orange colour: canola protein, Yellow colour: hemp protein.

dried microparticulated canola protein concentrate contained 96.4% DM of which 70.0% constituted protein. The resulting solution of the hemp protein concentrate contained 7.73% DM, of which 4.16% constituted protein. The dried microparticulated hemp protein concentrate contained 96.8% DM of which 53.8% constituted protein. Exept the protein fraction, the concentrates contained mainly fiber and mineral compounds. The oil content in the samples was up to 1% on dry matter basis. Figure 5 shows a photography of the canola protein concentrate. Comparison of microstructures of the hemp protein concentrates dried with the ATOMIZER demonstrator and the same sample dried with a conventional spray dryer is shown in Figure 6**.** There is a visible difference between both the samples perceptible in the SEM images. Both the samples consist of hollow nanospheres or microspheres. Figures 7 and 8 show results of the measurement of concentration and particle size distribution in the drying chamber. Suprisingly, we have found almost the same size distrubution and particle concentrations in different places of the drying chamber. The mean value of the particle size distribution is about 600 nm. The interval of the particle size distribution is relatively wide. The aerosol inside the drying chamber contains significant amount of nanoparticles

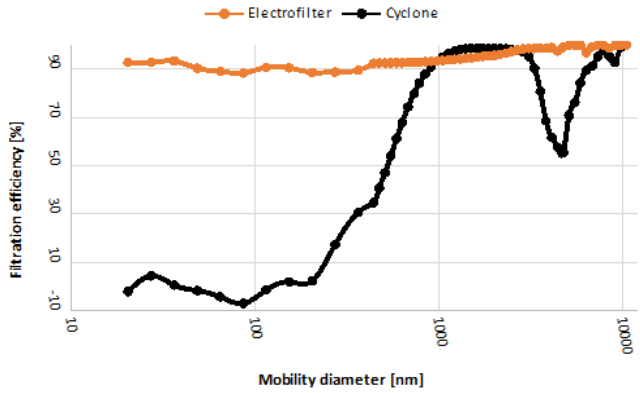

Figure 3: Comparison of filtration efficiencies **o**f the cyclone separator and the electrofilter in dependence on the particle size.

Figure 4: Charging and collecting electrodes of the *AEROFOG KE 1* electrofilter with powdered protein concentrate.

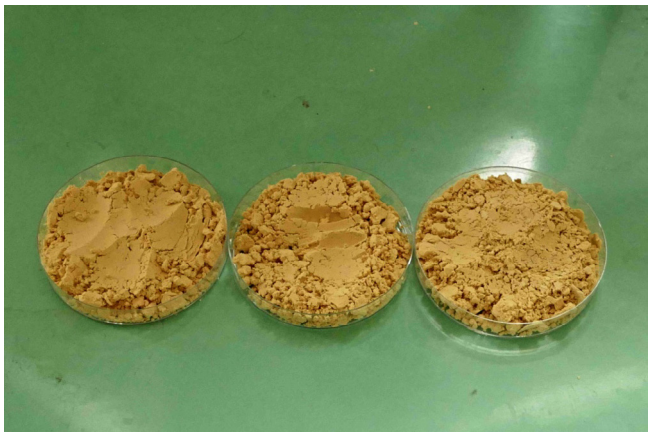

Figure 5: Canola protein concentrate.

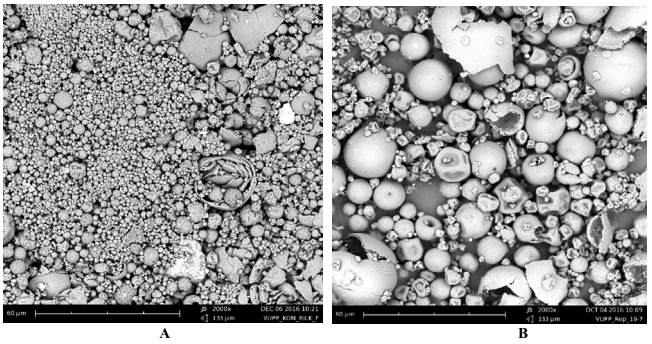

Figure 6: SEM images of hemp protein concentrate dried with the atomizer demonstrator (**A**) and a conventional spray dryer (**B**).

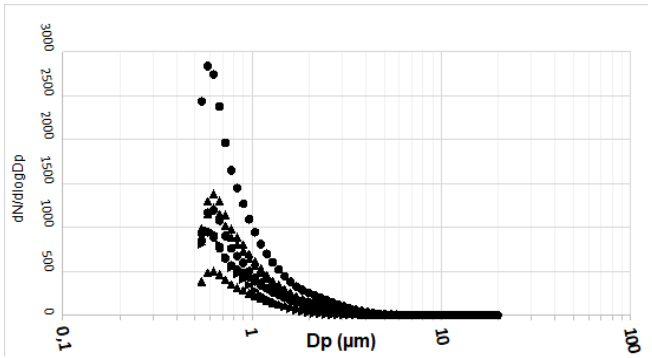

Figure 7: Results of measurement of concentration and particle size distribution in the drying chamber by the ASP aerosol spectrometer (particles >0.5 µm).

smaller than 100 nm, but a small portion of microparticles up to several micrometers, too. However, the dried protein concentrates contained a small fraction even bigger particles, up to 30 µm. The mean value of the particle size distribution of the plant protein concentrates is lower in comparison with the commercial microparticulated whey protein concentrates SIMPLESSE® and LeanCreme™. The SIMPLESSE® products contains microparticles averaging one micron in diameter, with more uniform particle size distribution than our products (www.cpkelco.com). The LeanCreme™ products consist of larger microspheres with the size in the range several micrometers with relatively broad particle size distribution (www.spxflow.com) which is comparable with our products. Results of the determination of the functional properties

of the canola proteins concentrates dried with a conventional spray dryer and the demonstrator ATOMIZER are shown in Figure 9. The microparticulation of the protein concentrates by the CASND process resulted in statistically significant (Student´s T-test, α=0,05) improvement of the foaming capacity and stability, emulsifying activity and emulsion stability. The use of canola / rapeseed concentrates and isolates is often limited by their low solubility and poor functional properties. This is a particular problem in oilseeds, such as rapeseed, because the proteins suffer denaturation during industrial oil extraction that further reduces their solubility. Microparticulation by the CASND technology improves solubility, dispersibility and functional properties of the powdered protein concentrates significantly. The sensory analysis proved a good consumer acceptance of the gluten-free baguettes with addition of the microparticulated canola and hemp protein concentrates (Figures 2 and 10). However, the baguettes with the commercial potato protein concentrates were accepted better than the baguettes with canola and hemp proteins. The difference in the overall perception is statistically significat (Student´s T-test, α =0,05). The baguettes with canola proteins were accepted better, but there is no statistically significant difference in evaluation of the baguettes with hemp and canola proteins. The main reason of the worse acceptance of the baguettes with canola and hemp proteins is a weak typical aftertaste of the protein concentrates.

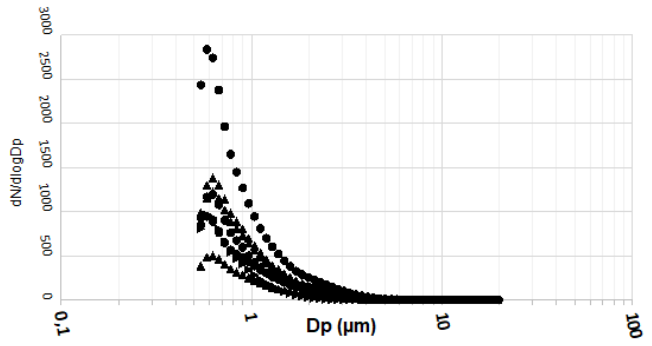

Figure 8: Results of measurement of concentration and particle size distribution in the drying chamber by the SMPS aerosol spectrometer (particles <0.5 µm).

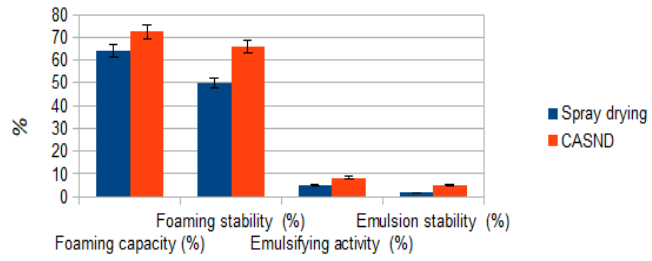

Figure 9: Functional properties of concentrates of canola proteins dried with a conventional spray dryer and the CASND demonstrator atomizer (Aritmetic Average ± Standard Deviation).

Figure 10: Baguettes with the hemp protein concentrate.

Conclusion

The presented results demonstrate a significant industrial potential of the CASND technology. Drying takes place continuously at low energy consumption compared to freeze drying. The operation cost of the ATOMIZER demonstrator is even lower that the operation cost of a conventional spray dryer of the same production capacity, because of lower energy consumption to heat the drying air or gas. The technolgy allows decreasing the powder particle size by an order of magnitude in comparison with the conventional spray drying. Low drying temperatures in the range from 40 to 60°C allow processing of temperature sensitive materials. Gentle drying of enzymes, vaccines, bacterial cultures, amino acids, vitamins and other food or pharmaceutical products are examples of the potential applications. The technology is modular, usable for various types of industries and can be easily scaled-up. This new technology enables production of materials with unique characteristics. New alternative cheap and easy method of plant protein microparticulation by the CASND process without thermal denaturation was described in this study. Microparticulation by the CASND technology improves solubility, dispersibility and functional properties of the powdered protein concentrates significantly. The cyclone separator proved to be highly ineffective to separate the fine submicron particles produced by the CASND process. A new electrostatic powder separator, or a filtration module equipped with a laminated nanofiber membrane highly permeable for the dryig gas or air, has to be developed for the industrial application of the CASND technology.

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