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Protein enriched foods and healthy ageing: Effects of almond flour, soy flour and whey protein fortification on muffin characteristics.

SP Rapport:2016:02

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SP Rapport 2015:2016:02

ISSN 0284-5172
Borås 2015

Content

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1.1 Background

The world's population is continuously getting older. According to the World Health Organization (WHO), the population over 60 years is currently 12 percent, but is expected to double until 2050. During the same period, the number of people over 80 years old will increase with 71 percent. However, the overall health among elderly has not increased in line with the rate of population ageing. In high-income countries, a significant reduction in prevalence of severe disabilities was found, but there was no reduced risk of mild to moderate disabilities (1). Hence, it is important to improve health among elderly in order to increase life quality among this population group and to reduce health care and social care costs (2).

Ageing is associated with several psychosocial and physiological changes. Many body functions are gradually impaired due to lifelong accumulation of cellular and molecular damage. Consequently, vulnerability to environmental challenges is increased, together with an increasing risk of disease and death (2). Some physiological changes have adverse effects on appetite, eating capability and reduced absorption of nutrients, which increases the risk of malnutrition. This includes a decline of sensory functions (e.g. loss of taste or smell), poor oral health, gum inflammation and teeth problem that may affect chewing negatively etc. Furthermore, loss of vision, hearing and impaired musculoskeletal functions can make food shopping and food preparation into challenging tasks that may further reduce food intake within this population group (2, 3). In addition, food intake is affected by disease, psychosocial and environmental factors such as dementia, depression, loneliness, drugs and economical status, which can all affect nutritional status negatively (2, 4).

The prevalence of malnutrition among elderly is uncertain due to many unreported cases and non-standardized detection methods. However, the number is found to increase with age, level of care required and comorbidities, and is considered a serious public health problem (2-4). There is no universally accepted definition of malnutrition, thus it is commonly described as "a state of nutrition in which deficiency, or excess, of energy, protein and micronutrients causes measurable adverse effects on tissue/body form (body shape, size and composition) and functional, and clinical outcome" (4). When ageing, the energy requirement decreases, due to reduced fat-free mass and physical activity (5, 6). In contrast, the need of most other nutrients remains the same or increases, including protein (2, 5). According to the Nordic Nutrition recommendations, the protein intake of elderly should provide around 18 E%, corresponding to approximately 1.2 gram protein per kg bodyweight and day. Proteins are chains of amino acids and there are nine essential amino acids, which cannot be produced in the body.

Thus, these must be obtained from the diet. This is usually no problem for a person who has an adequate intake of energy and eats a balanced diet. However, most vegetable proteins are scarce in one or several essential amino acids, which make it important to combine foods from different protein sources, in order to get adequate amounts of all essential amino acids (6).

Guyonnet et al. (2015) suggests that 5-30 percent of the elderly living at home are protein-energy malnourished, which is in line with the prevalence reported in other studies (3, 4). Protein-energy malnutrition (PEM) is caused by protein-energy deficiency, and is sometimes referred to as starvation. There are other unintentional weight loss syndromes such as sarcopenia and cachexia, but PEM differs from the other two by being reversible when adequate protein and energy requirements are met. However, beneficial effects have been found in patients with sarcopenia who are given adequate amount of protein and energy, together with resistance training (4).

Malnutrition, including PEM, causes loss of muscle and bone mass, which increases frailty and the risk of developing osteoporosis (2, 5). Evidence has proposed that malnutrition significantly increases both morbidity and mortality (3). In addition, malnutrition has adverse effects on cognitive function and ability to care for oneself (2). Research has shown beneficial results on nutritional, clinical and functional outcomes when giving protein-energy malnourished elderly energy and protein rich diets and/or oral supplements (4, 5). In addition, nutritional supplements have improved quality of life in elderly with PEM. Taken this, it is important to provide and produce protein- and energy enriched foods targeting elderly in order to decrease the prevalence of malnutrition among this population group (4).

The project “Active Ageing - individually customized food solutions for health and quality of life among elderly” is a cooperative initiative aiming to maintain life quality and autonomy through individual and personalized meal solutions that meet the requirements and needs of elderly. Nutritious food, tasty food, and food at the right time! The project started in January 2014 and run by several Swedish stakeholders, including Sveriges Tekniska Forskningsinstitut (SP), Findus, ICA, Medirest, Thermoking, BillerudKorsnäs, PRO, Innventia, Högskolan Kristianstad, Sensinet, Krinova, Nine and Kommunförbundet Skåne. Furthermore, the project is funded by Tvärlivsprogrammet. The project comprises several different work packages; of which one involve the development of protein and energy enriched foods.

As a part of this project, the objective of this study was to investigate how the sensory and physical characteristics of muffins change when adding almond flour, soy flour or whey protein powder to a basic muffin formula. Nevertheless, the underlying objective is to enable the development of high-quality protein enriched foods that can be used to improve protein status in elderly.

1.2 Introduction to study

1.2.1.1 Muffins

Muffins are moderate to high sugar-content baked-goods. In addition to sugar, a general muffin contains various quantities of flour, fat and egg, together with baking powder, flavoring and milk (7). The functionality of the different ingredients in cakes, and thus muffins, seems to vary depending on type of baked-goods. The fully process of muffin baking is not yet fully understood. There are speculative and dated data on the functionality of protein and starch, meanwhile the effects of fat and sugar have been studied more recently. Most studies are based on empirism, which gives limited evidence on mechanisms behind observed results (8).

Air bubbles are incorporated in the batter during baking and mixing, resulting in interconnected gas bubbles in the crumb of the final muffins, giving them an aerated structure (7). The air bubbles are stabilized by adsorbed egg protein as well as fat crystals. While mixing, the fat disperses into a protein-stabilized emulsion, giving a stable emulsion of fat and water. When the temperature increases while baking, fat crystals melts and settle as a protective layer of the inside surface of the air bubbles. This prevents the air bubbles from bursting during heat expansion and inhibits coalescence of gas cells (7, 8). The ingredients emulsifying capacities are important for stabilizing the protective layer surrounding the gas cells. In addition, a favorable batter viscosity is crucial for keeping the bubbles within the batter during mixing and baking (7).

When baking, the small air bubbles will increases in size due to gas diffusion of carbon dioxide (from baking powder) and water vapor etc. Consequently, the inner matrix of the final muffins will appear like solid foam. The amounts of air bubbles that are incorporated in the muffin determine the final muffin texture. Sensory quality properties of muffins are mainly dependent of final muffin volume and crumb texture, aiming for a light and airy texture (7). In contrast to bread baking, no full development of gluten happens during mixing. In muffins, gluten is a binder rather than contributing to the muffin structure (8).

When the temperature of the muffin batter rises within the oven, starch will begin to gelatinize and proteins coagulate giving the batter an increased viscosity. As the baking process continues and finalize, the starch granules and protein create the solid structure supporting the muffin shape (7). The gelled starch granulates and continuously network of coagulated egg protein are mainly responsible for the firm structure of the baked muffins (7, 8). Whether other proteins are involved in forming a protein network, in addition to egg proteins, and thus contributing to the muffin form is unclear. Starch has physicochemical properties that allow it to transform liquid batter into a solid structure, including its ability to swell and to bind excess water in the batter. Consequently, starch is the key building block of the muffin crumb. When the batter is exposed to heat, a temperature gradient in the batter is obtained that affects the structure setting (8). Thus, the structure setting is dependent on the gelatinization temperature of starch, which in turn is influenced by the sugar and moisture content of the batter. The final volume of the muffin is dependent on gelatinization temperature, granulates size, and absorbing capacity of starch (7).

When baking in a convection oven, the bottom of the muffins will reach a high temperature before the top and center resulting in an initial firm bottom structure. At this point, the pressure within the gas cells is high, making the cell membrane rupture. This allows air to escape, giving an atmospheric inner pressure of the muffin (8). In addition, baking involves transfer of moisture to the surface of the muffin, and includes water evaporation resulting in water loss. The migration of water is dependent on the temperature and concentration gradient, and is important for the final crumb texture (7). The final moisture content of cake-like products is usually between 18-28 percent, giving them lower moisture content than bread but higher than cookies (8).

1.2.1.2 Almond protein

Almond (Prunus dulcis) is one of the most commonly grown tree nut in the Mediterranean countries, USA, Australia and China. In developed countries, the consumption of almond has increased yearly during the last decades. Almonds have a good nutritional value, with a high content of unsaturated fatty acids, protein and minerals. Its fatty acid profile has been suggested to benefit health. In contrast, almond is a very common food allergen (9).

Almond proteins do not provide all essential amino acids at sufficient levels (low levels of methionine, lysine and threonine), which make it important to combine almond with other protein sources (10). Today, there is little research on the properties and functionality of almond proteins. However, mass spectrometric analysis has identified 434 different proteins, with Amandin being the major one (9). Amandin is a water-soluble storage protein in almond seeds that comprises two sub-units of 20-22 kDa and 38-41 kDa respectively (9, 10). A significant proportion of the almond proteins are considered to be membrane proteins with a hydrophilic-hydrophobic molecular structure, which may offer emulsifying properties to almond products. Consequently, this might be of value for the food industry (9). In addition, almonds have a nutty and sweet flavor (10). There is little or no existing research on the use of almond flour in baked-goods or bread.

1.2.1.3 Soy protein

The cultivation of soybeans had its beginning in ancient Asia for over 3000 years ago, but it was not until the 18th century that soybean cultivation was introduced in Europe. Soybeans are a good vegetable source of protein due to its high protein content and since it provides all essential amino acids (11). In addition, soybeans contain biological active compounds, including isoflavones, and have been associated with reduced risk of developing cardiovascular diseases, some cancers and hyperlipidemia (11, 12). Soy proteins are proposed to have gelling, emulsifying, water binding and oil holding functions, making it valuable for food industry. Soybeans are modified by heat treatment in order to enhance its optimal nutritive and functional properties, give a desirable flavor, and deactivate certain physiological harmful substances, including trypsin inhibitor and hemaglutinin (11).

The soy proteins can be separated into storage globulins, γ-conglycinin, and 9-15.3 percent contaminating soy-based whey proteins, including lipoxygenase,

lectin, β-amylase and Kunitz trypsin inhibitors. In addition, recent evidence has proposed the existence of a lipophilic protein. The storage globulines are divided into four protein categories according to their centrifugal sedimentation coefficients 2S, 7S, 11S and 15 S. β-conglycinin (7S) and glycinin (11S) is the major proteins representing more than 80 percent of the storage globulins. The storage globulins are water-insoulable. However, their solubility is dependent on pH and ion concentration. Glycinin denatures easily at temperatures above 80°C and form insoluble aggregates. In contrast, thermal aggregation of glycinin has been eliminated when heating glycinin and β-conglycinin together (11).
Traditionally, soybeans have been utilized in several different forms including tofu, miso, soy flour etc. (11). Today, soy flour is used in many parts of the world due to its nutritious profile, containing 38-40 percent proteins and 18-20 percent fat, making it a good substitute for wheat flour (12). Soy flour is produced through dehulling, grinding and defatting soy seeds. In similarity with soybeans, soy flour has favorable emulsifying, binding and textural properties (13).

1.2.1.4 Whey protein

Whey protein is a by-product from cheese making, and is a part of the remaining milk serum after separating the coagulum from milk or cream (14). Whey proteins are water-soluble and represent 20 percent of the milk proteins, and include β-lactoglobulin, α-lactalbumin, bovine serum albumin, immunoglobulins, bovine serum albumin, bovine lactoferrin and lactoperoxidase (15). β-lactoglobulin (β-lg) and α-lactalbuminare (α-lac) are the predominating whey proteins, corresponding to 70 percent of the total protein. Thus, these two proteins are mainly responsible for the functional properties of whey protein products (16). Whey proteins are globular molecules that mainly consist of α-helix motifs. Within the α-helix motifs, the hydrophobic/hydrophilic and acidic/basic amino acids are evenly distributed making the molecule rather non-polar. Whey protein is available as whey protein concentrate (35-80% protein) or whey protein isolate that is a purer product (> 90 % protein) (15). Whey proteins have favorable amino acid profiles comprising all essential amino acids at adequate amounts, including high level of branched-chained amino acids such as lysine, isoleucine and valine (16). In addition to the nutritional value of whey protein, research suggests that whey protein have foaming, emulsifying, gelling, solubility and water binding properties, together with increased viscosity development (14, 16).

When processing food, protein functionality is a result of bonds and interactions between protein and other protein molecules, ions, food components and the solvent. One of the most important properties of whey proteins in food processing is its good water solubility over a wide range pH (pH 2 to 9). However, whey proteins are highly heat sensitive and susceptible to changes, such as denaturation, when heated which can limit its use in food development. Heat denaturation is a result of protein unfolding that enables covalent and reversible non-covalent protein-protein interactions, such as disulfide bonds, resulting in protein aggregation. The type and degree of whey protein aggregation is dependent on pH, temperature, ion source and salt concentration. Divalent salts increases the protein aggregation by forming salt bridges between proteins or reducing the inter-protein charge repulsion, which modify the electrostatic charge of proteins at different pH. At the β-lg and α-lac isoelectric points (pH 4.8 to 5.3), the potential of protein-protein interactions reaches its highest level and whey protein aggregates easily. Consequently, by changing the pH the level of protein aggregation can be affected. By having a pH above or under the isoelectric point will result in a net positive or negative charge, giving repulsive forces between the proteins that impede protein interactions, even at heat induction (16).

There are some methods to improve the heat stability of whey proteins, in addition to what has already been mentioned. This includes protein hydrolysis, addition of salt chelators, modified salt composition, sugar addition, enzymatic cross-linking, ultrasonication, molecular chaperons, conjugation with carbohydrates, protein encapsulation etc. These methods improve whey protein aggregation by either reducing the size of the aggregates and/or reducing the level of aggregation. By modifying the heat stability properties of whey protein, whey protein can beneficially be used in wider range food applications in order to develop protein enriched products (16).

1.3 Material and Methods

1.3.1.1 Muffin-making Procedure

A basic muffin formula (Reference) was developed with the following ingredients: 300 g Engelhardt's muffin mix (sugar, wheat flour, whole egg powder, potato starch, baking powder E450 an E500 (equals 0,5% P_2O_5), aromas, NaCl and beta-caroten4), 120 g rapeseed oil, 6 g vanilla sugar, 40 g water (20°C), 100 g quark, together with additional 45 g sugar and 3 g baking powder. The ingredients were stirred in a KitchenAid® artisan for three minutes at highest speed, and thereafter 20 g batter was added to baking forms. Finally, the muffins were baked in a preheated convection oven at 175°C for 11 minutes. The protein content of the reference muffin (REF) was 6.4 g protein per 100 g.

Table 1 Proportions of ingredients in muffins

	REF[1]		**AFM[2]**		**SFM[3]**		**WPM[4]**		**WPM 14.1[5]**	
Ingredients	(g)	(%)	(g)	(%)	(g)	(%)	(g)	(%)	(g)	(%)
Muffin mix	300	48,9	300	35,3	300	42,4	300	41,2	300	34,3
Rapseed oil	120	19,5	120	14,1	120	16,9	120	16,5	120	13,7
Quark	100	16,3	100	11,8	100	14,1	100	13,7	100	11,4
Sugar	45	7,3	45	5,3	45	6,4	45	6,2	45	5,1
Vanilla sugar	6	1,0	6	0,7	6	0,8	6	0,8	6	0,7
Baking powder	3	0,5	3	0,4	3	0,4	3	0,4	3	0,3
Water	40	6,5	90	10,6	70	9,9	120	16,5	200	22,9
Foritfier	0	0,0	185	21,8	64	9,0	35	4,8	100	11,4
Total	614	100	849	100	708	100	729	100	874	100

[1]REF, Reference; [2]AMF, almond flour enriched muffin; [3]SFM, soy flour enriched muffin; [4]WPM, whey protein enriched muffin; [5]WPM 14.1, whey protein enriched muffin 14.1 g protein/100 g

The basic formula was used as a base when developing muffins with three different fortifiers (almond flour, soy flour and whey protein concentrate) and additional water (Table 1). The quantities of almond flour, soy flour, whey protein concentrate and water was chosen in order to give the same protein content per 100 g in all three muffins. In exception, two whey protein enriched muffins were developed of which one had higher protein content. Furthermore, the ratio between the fortifier product and water was chosen to give a similar batter consistency as the reference, but was occasionally adjusted to increase sensory qualities of the muffins.

When developing the almond flour enriched muffins (AFM), the quantity of almond flour was tried out by baking different muffin variants. The variant chosen had the highest content of almond flour still giving adequate muffins, equaling 9.4 g protein per 100 g muffin. In addition to the basic muffin formula, this variant contained 185 g almond flour and 50 g water. Since almond flour has the lowest protein content per 100 g, it was the limiting factor determining the protein content of the three different muffins (Table 2). Consequently, a soy flour enriched muffins (SFM) was developed containing 9.4 gram protein per 100 gram. This was reached by adding 64 g soy flour and 30 g water to the basic formula. For the whey protein enriched muffins (WPM), the same protein content was obtained by adding 35 g whey protein concentrate (soya lecithin instantized whey protein) and 80 g water to the basic formula. In addition, a second whey protein enriched muffin was developed with 14.1 gram protein per 100 g (WPM 14.1). Three batches were baked of each muffin variant and two muffins from each batch were analyzed regarding physical and chemical characteristics.

Table 2 Protein content per 100 g

Fortifier	Protein content per 100 g
Almond flour	21
Soy flour	39
Whey protein	77

1.3.1.2 Physical Characteristics

Weight loss was calculated as the percentage difference between the batter and the final muffins, and was considered to be evaporating water exclusively. Muffin volume was determined by measuring the mean height and width of the muffins, and specific volumes were calculated by dividing the volume by the muffin weight (expressed as cm^3/g). The crust color was determined by using DigiEye colorimeter (DigiEye for food and drink, Vervide, Leicester, United Kingdom). The illuminant used was D65/10, with a shutter speed set to 1/8 and aperture to 8. The instrument was calibrated before each analysis with a color checker chart. DigiEye was used to determine the lightness (L value) of the muffin crust (black = 0, white = 100).

1.3.1.3 Chemical Analysis

The water activity of the muffins was determined by using AquaLab water activity meter (AquaLab, Decagon Devices, Pullman, USA).The instrument was calibrated before each analysis with two standard solutions (a_w = 0.760 and a_w =

0.984). The moisture content was determined by weighing muffin samples before and after being placed overnight in a vacuum oven (900 mbar, 80°C).

1.3.1.4 Sensory Analysis

Sensory characteristics of the four muffins (REF, AFM, SFM and WPM) were discussed in a focus group including seven respondents. The focus group discussion lasted for 1h and 15 min and was recorded. The overall impression, appearance, texture, flavors and acceptance of the muffins was concerned. The respondents described the sensory characteristics of the muffins by using their own words, in addition to some selected common words (Table 3). The common words were chosen to enable comparison of the muffins and to highlight some import sensory characteristics.

Table 3 Sensory characteristics discussed in focus group

Apperance	Texture	Flavor
		Total flavor:
doughy/moist - dry	doughy/moist - dry	intensity
cracks - smooth	hard/compact - soft/fine	swetness
baked - unbaked	soft/smooth - astringent mouthfeel	vanillin
peaky - flat	greasy mouthfeel - non-greasy	off-flavor
glazed - unglazed		
air bubbles: big - small		

1.4 Results and Discussion

1.4.1.1 Overall impression

The focus group got to discuss their overall impression of REF, AFM, SFM and WPM. REF was considered to be the most muffin-like muffin, and the respondents associated it to home-baked muffins. In contrast, WPM looked industry-baked, due to its peaky and glazed outer surface. Both of the muffins had a scent of egg and lemon, which was not perceived for AFM and SFM. AFM was at first associated to scones, since it had a harder, more compact and drier texture. It was also associated to almond paste, because of its nutty flavor and texture, and several of the respondents appreciated the flavor. However, the appearance of AMF was not as appealing as REF and WPM. The appearance of SFM reminded of AFM. Nevertheless, the muffin was thought to be rather tasteless and scentless.

1.4.1.2 Appearance

1.4.1.2.1 Overall appearance

REF was associated to home-baked muffins, due to small cracks covering the crust. The respondents suggested the cracks to be caused by a shape collapse during cooling (Figure 1). In contrast, WPM was considered to appear as artificial, since it barely had any visible cracks. AFM had most cracks, followed by SFM, but the respondents distinguished these cracks from the cracks on REF. Instead, they were considered to be cracks lining the pattern of batter piping, as a result of a too compact batter, which did not distribute evenly in the baking forms. Accordingly, the cracks were deeper and non-appealing. WPM was considered to have the peakiest shape, followed by SFM, meanwhile AFM was flattest. Furthermore, REF and WPM appeared as more glazed and moist (juicy), while AFM and SFM appeared as doughy and under-cooked, in particular SFM. AFM and SFM had a very dry and thick crust in comparison to REF and WPM.

When dividing the muffins, it was obvious that WPM had most and biggest size air bubbles, giving it an aerated structure. The reference was considered to have very small bubbles, making it look porous. The respondents did not observe any visible bubbles of AFM and SFM, thus they appeared as very compact. As mentioned in the introduction, the incorporation of gas bubbles in batter and final muffins is important for the muffin texture.

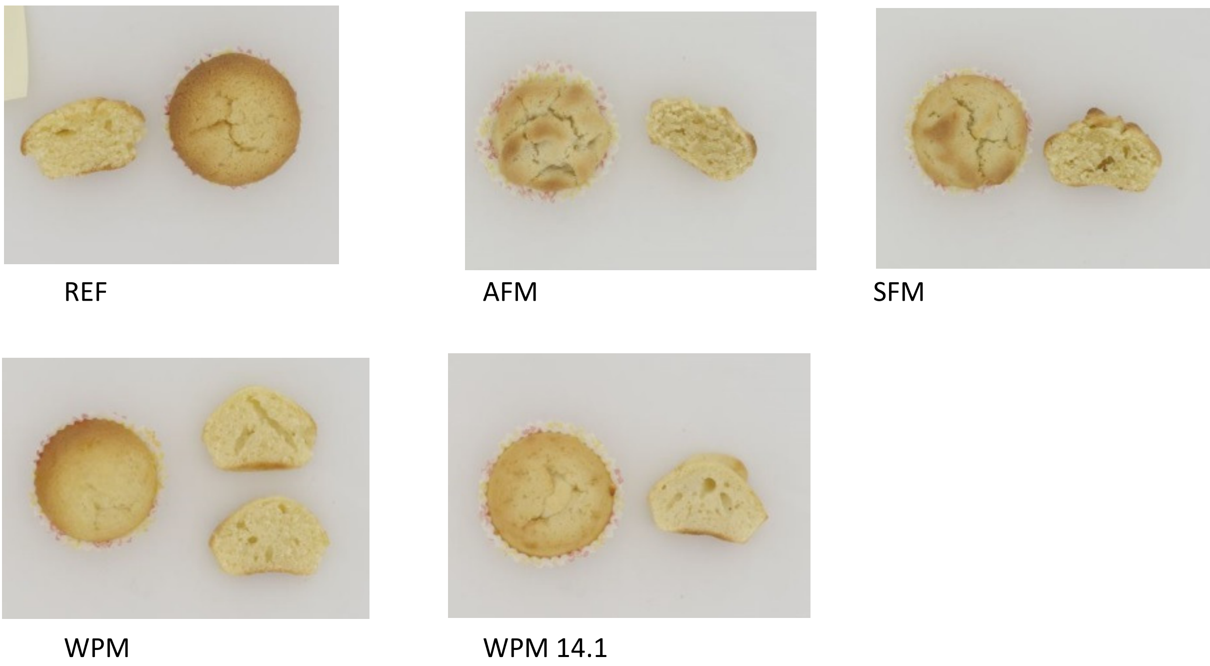

Figure 1. Images of the five muffins baked.
REF, reference; AMF, almond flour enriched muffin; SFM, soybean flour enriched muffin; WPM, whey protein enriched muffin; WPM 14.1, whey protein enriched muffin 14.1 g protein/100 g

In addition, the stabilization of air bubbles are dependent on fat crystal and egg protein, together with batter viscosity and the emulsifying capacity of the ingredients (7, 8). Even if WPM had the smallest proportion of fat, the foaming, emulsifying, viscosity developing and gelling properties of whey protein may allow an increased stabilization of gas bubbles and a more aerated structure (14, 16). However, no conclusion can be drawn since protein functionality was not investigated in this study, making it impossible to compare the different muffins according to these factors. In addition, soy protein and almond protein have not been studied to the same extent as whey protein.

1.4.1.2.2 Color

REF and SFM were considered to have a similar yellow color, both on crust and crumb, with a darker outer surface and a lighter crumb. WPM had a slightly lighter yellow color straight through (golden color), meanwhile AFM had a tint of grey and a darker crumb. The darker color was considered to be a result of a doughy crumb and a high fat content. All muffins were considered to be evenly baked, with exception of AFM that appeared a bit less baked than the rest. However, they had some color differences (from oven-baking), which the respondents explained by the different crust texture. REF was considered to have a porous surface and WPM a glazed surface, while AFM and SFM had dry surfaces.

Evidence shows that lightness/darkness of baked-goods surfaces are obtained as a result of the Maillard reactions between reducing carbohydrates and free amino acid chains (12, 14). The Maillard reaction yields polymerized protein and brown pigment (12). According to the measurements with DigiEye colorimeter REF had the lowest L value, and thus the darkest crust color (Figure 2). In contrast, WPM 14.1 had the lightest color and highest L value, followed by AFM and WPM. SFM had a large standard deviation, which can be explained by the use of a pre-heated oven (used at higher temperatures previous to baking one batch of soy muffins). Thus, this value is not reliable and should be higher. Except for WPM 14.1 and the reference, all muffins have the same protein content but different content of carbohydrates (Table 4). REF had highest carbohydrate content, lowest protein content and was darkest, while WPM 14.1 had the lowest carbohydrate content, highest protein content and lightest crust color. This may be explained by a lower surface temperature of WPM 14.1, which might be due to temperature variation within the oven or higher water content in WPM 14.1. Interestingly, AFM and WPM have a similar L value regardless of the higher carbohydrate content of WPM and the higher level of lysine in whey proteins compared to almond proteins (16, 10). Lysine has been suggested to be an important amino acid in the Maillard reaction (12).

Taken together, there is some inconsistency in which muffin the focus group found to have the most appealing appearance. The majority of the respondents chose REF due to its fluffy and moist (juicy) home-baked look, while other thought WPM looked more appealing considering its muffin-like form and golden color. In contrast, AFM was chosen to have the least appealing appearance due to

b.

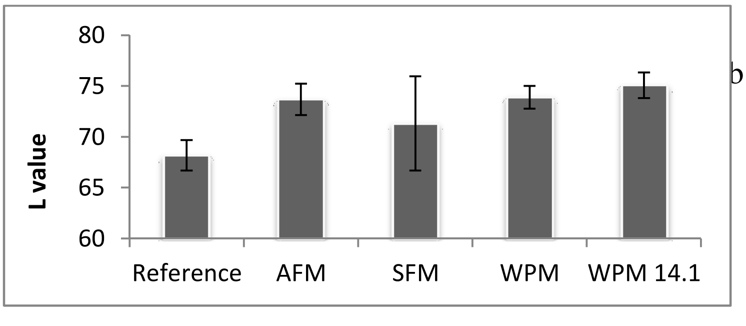

Table 4. Carbohydrate content

	Carbohydrates (g)
Reference	51,18
AMF	38,51
SFM	45,71
WPM	43,15
WPM 14.1	36,59

Figure 2. Influence of almond flour, soy flour and whey protein on muffin lightness/darkness of muffin crust.

REF, reference; AMF, almond flour enriched muffin; SFM, soybean flour enriched muffin; WPM, whey protein enriched muffin; WPM 14.1, whey protein enriched muffin 14.1 g protein/100 g

1.4.1.2.3 Volume

The volumes and specific volumes (SV) of the REF, AFM, SFM, WPM and WPM 14.1 are given in Figure 3 and 4. The largest volume and SV has REF, followed by the whey protein enriched muffins. AFM had the smallest volume and SV. The focus group used words like compact and non-muffin-like to describe the appearance of SFM and AFM. As mentioned above, the pattern after piping the batter was still visible on AFM and SFM, indicating a dense batter. In contrast, a desirable eating quality of most muffins requires a high volume and aery, fine texture (7).

The final volume of muffins is mainly affected by granulates size, absorbing capacity, and gelatinization temperature of starch, of which the latter depends on sugar and moisture content of batter (7). Since starch is an important determinant of muffin volume, the relation between starch and muffin volume were plotted in Figure 5. With the exception of the whey protein enriched muffins, the starch content are positively related to the muffin volume. Nevertheless, there is no linear relationship between the variables, suggesting the involvement of other determinants affecting the final muffin volume.

Furthermore, evidence has proposed that a high water holding capacity of soy flour relative to wheat flours reduces the loaf volume of soy flour enriched breads. In addition, a high ingredient water binding capacity can lower the water activity in food (17). Taken this, the water holding capacity of soy flour may contribute to a smaller muffin volume (13). Contradictory, whey protein had simultaneously a high water binding capacity, the highest water activity and a large volume (Figure 6) (16).

Taken together, it is not possible to compare the different muffins due to the wide variance of affecting determinants. The muffins differ in the proportion of water, starch, sugar, baking powder, salt and egg protein, which can all effect volume and quality of the final muffin, making it impossible to draw any conclusions (7, 16, 18).

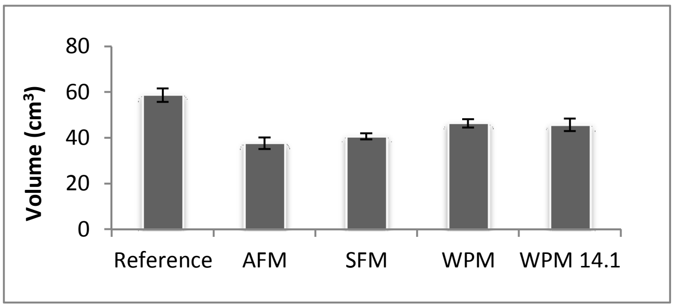

Figure 3. Influence of almond flour, soy flour and whey protein on muffin volume.

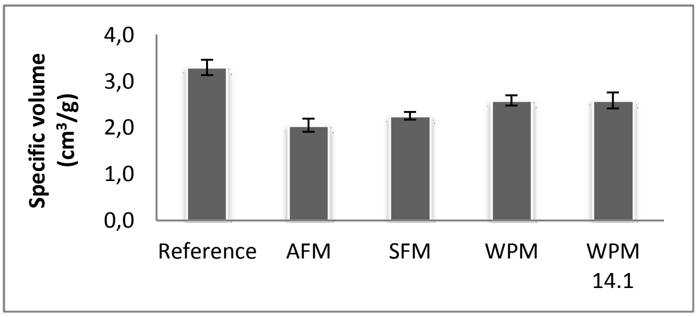

Figure 4. Influence of almond flour, soy flour and whey protein on muffin specific volume.

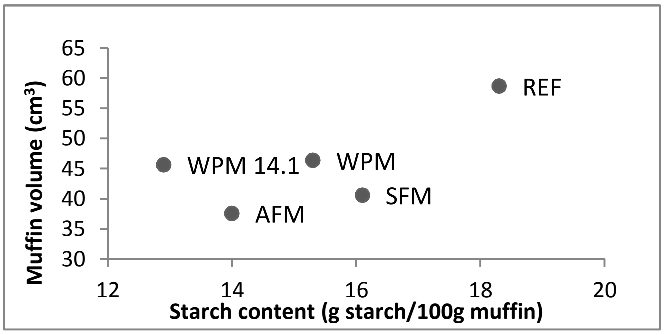

Figure 5. Correlation of starch content and muffin volume.

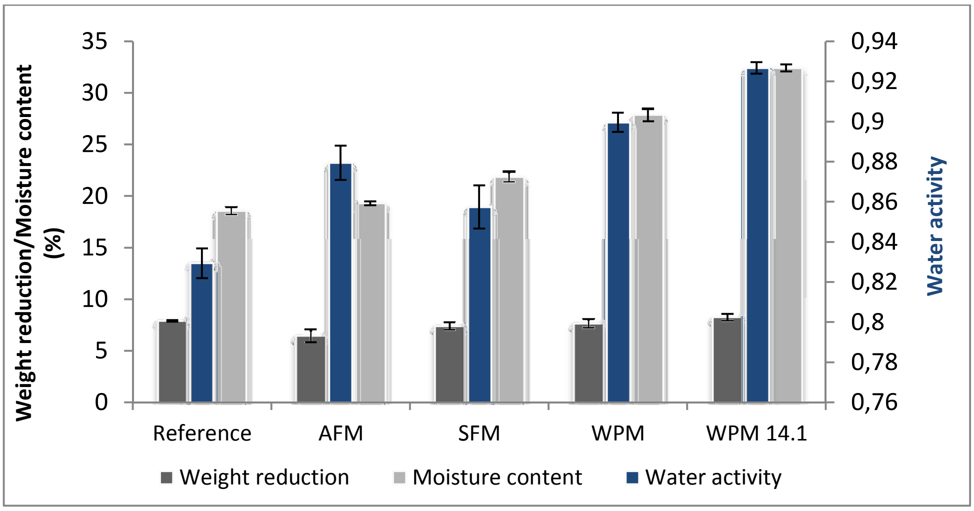

Figure 6. Influence of almond flour, soy flour and whey protein on muffin weight loss (batter-muffin), moisture content and water activity.

REF, reference; AMF, almond flour enriched muffin; SFM, soybean flour enriched muffin; WPM, whey protein enriched muffin; WPM 14.1, whey protein enriched muffin 14.1 g protein/100 g

1.4.1.3 Flavor

REF was described to have a sweet, caramelized crust, with a strong flavor of vanilla and a high sweetness. REF had the highest flavor, which can be explained by a dilutive effect of the added fortifiers and water in the other muffins. Some of the respondents discerned a flavor of lemon, however there is no added lemon flavoring. In addition, the respondents perceived an off-flavor of baking powder. There were some respondents that considered REF to have the most appealing flavor, however, some considered it to be too sweet.

The majority of the respondents thought that AFM had a pleasant, nutty flavor that reminded them of almond paste. However, it was not considered to have a typical muffins taste. AFM was thought to be rather heavy and filling, making it undesirable to eat a whole, normal-size muffin. Some respondents experienced an atypical almond flavor, suggesting the almond flavor to origin from something else. It was considered to be less sweet than REF, a sweetness of nuts rather than sugar was perceived. There was difficult to discern a flavor of vanilla, however, the respondents suggested an underlying hint of it. Furthermore, a bitter off-flavor of baking powder or bitter almond was perceived after swallowing. Some of the respondents thought that AFM had the best flavor due to the nutty flavor, but as a muffin the reference was to prefer.

For SFM, it took long time before the respondents felt any flavors, and the flavor was considered weaker than in REF. The flavor was described as neutral and insipid. However, there was a slightly stronger vanilla flavor than in AFM, and some higher sweetness. The respondents felt a dryness in SFM mixed with a sense of butteriness/moisture, giving it an unbaked feeling. In addition, some associated SFM to bread. Off-flavors like egg and sourness was brought up, however, no consensus was reached. Previous research has suggested a possible undesirable beany flavor that is caused of unfavorable flavor compounds formed during soybean processing (13). The majority of the respondents considered SFM to have the least appealing flavor. In contrast, two respondents chose it as their favorite, appreciating the lower sweetness.

The focus group described the flavor of WPM as tasty and similar to REF, however the flavors were weaker. They felt a touch of vanilla, together with a high sweetness. The majority of the respondents tasted an off-flavor of egg and baking powder, and agreed upon distinctive mouth dryness after eating WPM. It was considered to have most off-flavors of all muffins, making some respondents think it had the least appealing flavor. The perceived mouth-dryness is in line with Burrington (2012), who reports an association of whey protein and increased astringency (19). The level of astringency is depending on pH and protein concentration, where astringency is increased at low pH ($pH < 3.5$). The mechanism behind is suggested to be interactions between whey protein and saliva proteins. Furthermore, lipid oxidation is proposed to be the major cause of whey off-flavors and several sensory attributes related to whey have been listed, including soapy, brothy, bitter, cucumber, cooked/milky flavors, sweet aromatic, cardboard etc. In contrast, no egg flavor was related to whey protein. Some flavors have been suggested to successfully mask the whey flavor and aroma, such as tropical flavors (pineapple, mango and coconut), apple, peach and citrus. On the contrary, berry flavors have not been found to cover the whey flavor (19).

1.4.1.4 Texture

The muffin texture was considered by cutting the muffin with a cutlery knife and discussing how the muffins felt with the fingers and in the mouth. The crust of REF was a bit crispy and sticky to get through with the knife. When through the crust, the crumb fell apart with no resistance. The respondents shared a feeling of pressing through the muffin rather than cutting. With the fingers, REF felt very moist, fluffy, elastic, porous and sticky. It had a tendency to give sticky fingers. In addition, it perceived sticky in the mouth since it slightly stuck to the teeth and palate. However, the muffin melted fast in the mouth, spreading out, and making it easy to swallow without the need of a drink. It gave a homogenous feeling in the mouth, and left a greasy feeling on the tongue. Overall, it felt smooth in the mouth. The majority of the respondents thought that REF had the most appealing texture due to its smoothness.

AFM had a hard crust that took some effort to get through with the knife and the crumb had a relatively tough consistency. The respondents associated it to cutting almond paste. When cutting, it fell apart in bigger pieces, following the crack structure. With fingers, the crust felt crunchy and hard, not elastic as REF. The crumb crumbled as soon it was touch, and felt moist and compact. When pressing the crumb with the fingers, it did not go back to its initial position but stayed pressed. In mouth, AFM felt a bit astringent and was chewier than REF, together with some graininess. There was some different opinions whether it stuck to the palate or not, however, REF was considered more sticky. AFM was a bit doughy and did not melt on the tongue, thus the respondents considered it to be less smooth than REF. However, it was considered to be easy to swallow, but it left a dryness/astringent feeling after swallowing.

SFM had a thick and hard crust, however, it was easier to cut through than AFM. For both AFM and SFM, they had a distinct texture difference of the crust to the crumb. Previous studies have shown soy flour addition to give bread a harder crust and crumb, together with a more compact structure (12). SFM had a high crumbliness and fell apart most and easiest of all muffins. With fingers, SFM had a dry and though crust, with a moist crumb. However, the crumb felt drier than the rest of the muffins. The respondents considered SFM to have a dry/moisture gradient throughout the muffin. It was considered to be compact, but with a dry substance compared to AFM. In mouth, SFM was very dry and together with water it became gluey. SFM was thought to be chewy, sticky, astringent and doughy, making hard to swallow without something to drink. It did not melt in mouth or give a greasy mouth feeling. The majority of the respondents considered SFM to have the least appealing texture, due to it being doughy and hard to swallow.

WPM required most effort to get through with knife, since the muffin structure held together best of all muffins. It was possible to cut neat slices with no crumbles. It did not crumble at all, making the respondents refer to a foam rubber ball. It was very elastic; the crust followed the knife before the crust ruptured. With fingers, the crust of WPM felt sticky like REF, with a less moist crumb. However, the crumb was not dry, but rather hard and elastic straight through. The respondents did not consider WPM to be compact since it had the most aerated structure with big gas bubbles. It had a high sponginess. In mouth, WPM was difficult to swallow, since it did not melt by itself, making it very chewy. In contrast, some respondents thought the firm structure made it easy to swallow since it did not stuck to teeth and palate, and left no crumbs to choke on. WPM was considered to be quite smooth, and there were no greasy mouth feeling after eating the muffin. However, it left an astringent feeling.

WPM was thought to have the highest springiness, followed by the REF. AFM and SFM had a firmer and more compact texture. Evidence suggests that the springiness of cakes is positively associated with protein aggregation (20). This is in line with the results of this study, since whey proteins are very heat-sensitive and aggregates easily (16). In a study by Singh et al. (2015) navy bean protein did not significantly affect the springiness of the cakes, in contrast of gluten-containing cookies. Therefore, navy bean proteins were suggested to lack the ability to form the network with gelatinized starch as gluten does (20). Since soybeans are related to navy beans this might explain the lack of sponginess in SFM. Furthermore, a weakness with this study is that no pH measurements were made. PH affect protein aggregation, which makes it important to develop muffins with different pH in order to recognize the effect pH has on muffin texture and flavor. As mentioned above, there are some different treatments that can be used to improve the heat stability of whey that may be used to improve WPM texture (16).

Furthermore, the perceived moistness of the muffins where not related to the moisture content of the muffins, neither the water activity nor the proportion added water. The respondents considered REF to be the moistest muffin, followed by AFM. These muffins had the lowest moisture content of all. In addition, REF had the lowest water activity and proportion added water.

1.4.1.5 Acceptance

In general, all respondents accepted REF since it lived up to common expectations of muffins. It was considered to be a traditional muffin, and the focus group agreed that they were willing to eat it again and buy it themselves. They also felt positive about serving REF to an older relative. One of the respondents thought it was a bit too sweet, but most muffins in stores are probably as sweet.

The majority of the respondents would like to eat AFM again, due to its nutty flavor. However, they were more hesitant to buy it considering its non-muffin-like appearance. At a first glance, AFM does not look very appealing. It would probably be better to sell AFM as something else than a muffin, such as an almond paste cake. Since the majority of the respondents liked AMF, they would not mind serving it to older relatives.

The majority of the respondents did not want to eat SFM again, because of its insipid flavor and non-appealing texture. . This is in line with a study by Sabanis et al. (2009) who found soy flour enriched bread to have low acceptability due to non-desirable flavor and increased hardness (12). Consequently, the respondents would not buy it again nor serve it to older relatives, because of it being difficult to swallow and tastelessness. However, two of the respondents chose SFM as their favorite since it was not as sweet as REF and had a heavier structure.

WPM was neither considered to be tasteful or bad tasting. It was none of the respondents' favorite and the majority would not buy it in store. Even if the taste was acceptable, the texture was too elastic/spongy. The respondents would bring it to an older relative if they knew that it was a healthy choice, but also since it was perceived as easy to swallow.

1.4.1.6 Limitations

One weakness with the chosen study design is the use of a focus group for the sensory analysis. When discussing, the respondents influenced each other's opinions of the muffins, which resulted in a more uniform description of the sensory characteristics. It was also difficult to get all respondents to share their opinions to the same extent. Consequently, some respondents' opinions became more dominant. It would be valuable to do a more comprehensive evaluation of the sensory characteristics by including additional respondents that separately got to answer a questionnaire.

Another weakness with chosen study design is the limited protein content. By aiming to keep the protein content constant for all fortified muffins (except for WPM 14.1), it only allowed us to reach a level of 9.4 g protein per 100g muffin, equaling 47 percent more protein than in REF. In Sweden, in order for a muffin to be labeled "protein source" there is a requirement of more than 12 E% protein. In addition, the labeling "rich in protein" requires more than 20 E% protein (21). None of the produced muffins (AFM, SFM and WPM) reached these thresholds, which would be desirable in order to market the muffins as "good protein sources". Moreover, higher protein content would benefit the target group and make the muffins a more valuable protein source. However, these muffins are still to prefer to normal muffins that contains less protein. Furthermore, it is deceptive to only take account to E% protein since it depends on the amount of carbohydrates and fat in foods, and not only the total gram protein. For elderly with PEM, it is beneficial to have muffins that contain high amounts of both fat and protein, which consequently lower the E% protein.

Since almond flour have the lowest protein content per 100 gram, it was the limiting factor when determining the protein content of SFM and WPM. Consequently, the proportion of almond flour became very dominating. Even if the taste of AFM was appreciated, its texture and appearance was affected by the high proportion almond flour and reduced proportion of the other ingredients. Thus, it is not favorable to increase the protein content in AFM by only using more almond flour. Furthermore, SFM had a low acceptability and it is likely that the sensory characteristics would be more negatively affected by adding additional soy flour. However, it would be valuable to investigate the effect on muffin quality when alternating the proportion of baking powder and flavoring etc. In contrast, the proportion of whey protein concentrate in WPM is relatively low (4.8 %), which enables more whey protein to be added. Even if none of the respondents chose WPM as their favorite, none of them ranked it last. The appearance of WPM was ranked high and the flavor was acceptable, except for the mouth dryness that was perceived after eating WPM. However, the texture was not considered desirable due to its sponginess. As mentioned above, evidence shows possibility to reduce sponginess and improve flavor. By overcoming these problems, whey protein offers a great ability for protein fortification due to its high protein content.

Taken together, since the proportion of the fortifiers differs, together with the quantities of the other ingredients, it is problematic to use the same basic formula for all muffins. It would probably be better to adjust the muffin formula for each muffin variant, or use the same proportion of the different ingredients, in order to avoid big differences in used quantities of baking powder, sugar, starch, flavoring etc. However, this would make it impossible to have the same protein content in all muffins, and thus make it difficult to compare the effect of the different proteins.

The major limitation of this study is the different proportions of all ingredients in the four muffins (REF, AFM, SFM, WPM). There are several different determinants that affect every parameter of muffin quality. E.g. muffin volume is affected by the quantity of water, starch, baking powder, sugar and egg protein etc., together with type and amount of other proteins (7, 16, 18, 22). As soon as one ingredient is changed, the proportions of all the other ingredients change as well. Furthermore, most parameters are dependent of functional properties of proteins, including water holding capacity, gelling, foam forming, emulsifying properties etc. In turn, these are affected by pH, ionic strength, moisture content, temperature and several other factors (7, 8, 16, 22). Consequently, it is impossible to distinguish the effects of the different ingredients and fortifiers on the final muffin quality.

1.5 Conclusion

Except for REF, there were different opinions regarding the protein-enriched muffins. Interestingly, several of the respondents preferred AFM to REF considering its taste, meanwhile two of the respondents thought SFM was to prefer. Nevertheless, the majority of the respondents found SFM distasteful. WPM was ranked somewhere in the middle, and was considered to have the best appearance of all the fortified muffins, but a non-favorable texture. Consequently, all fortifiers affected the sensory characteristics of the muffins negatively in one or several ways, showing the complexity of adding different protein sources to a basic muffin formula.

Future studies will need to investigate all possible determinants of muffin quality separately, and for each muffin variant alone. Some possible determinants include pH, batter viscosity, starch content and protein functionality etc. It would be valuable to investigate what role pH have on muffin texture and flavor, since it has been recognized to effect whey protein aggregation, together with level of astringency. In addition, pH has effect on some protein functionalities, including emulsifying, viscosity, water holding capacity, foam forming and gelling properties etc. In turn, these properties affect the final muffin quality regarding shape forming, gas bubble incorporation, volume and texture etc. Therefore, it would be desirable to bake several batches of the same muffin variant and remain everything constant except for pH, in order to discern the effect of pH alone. Furthermore, it would be interesting to measure batter viscosity and emulsifying capacity of the used proteins, and investigate what role they have in air bubble incorporation. However, I don't know if it's feasible to change these determinants alone, without changing the proportion of other ingredients in the muffin formula. Moreover, starch content is one of the most important determinants for muffin volume, making it interesting to investigate how muffin volume varies when adding different proportion of starch to one muffin variant. Taken together, it is required to investigate determinants of muffin quality separately in order to draw any conclusion and to be able to distinguish what determinants that cause which differences in sensory and physical muffin characteristics. Furthermore, the protein content of muffins can be improved further by adding protein-rich muffin fillings.

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SP Rapport 2015:2016:02
ISBN
ISSN 0284-5172

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